Université de Montréal

CYTOCHROME P450 IID6 ACTIVITY IN HIV+/AIDS PATIENTS.

par

Antonietta Di Girolamo
Département de Pharmacologie
Faculté de Médecine

Mémoire présenté à la Faculté des études supérieures
en vue de l'obtention du grade de
Maître ès sciences (M.Sc.)
en Pharmacologie

Janvier 1998

Page d'identification du jury

Université de Montréal

Faculté des études supérieures

Ce mémoire intitulé:

CYTOCHROME P450 IID6 ACTIVITY IN HIV+/AIDS PATIENTS.

Présenté par:

Antonietta Di Girolamo

a été évalué par un jury composé des personnes suivantes:

Mémoire accepté le:

SUMMARY

The aim of this study was to determine whether or not disease progression in HIV+ patients affects the expressed activity of the cytochrome P450 enzyme CYP2D6. The cytochrome P450 enzyme CYP2D6 genotypes were compared to the expressed CYP2D6 phenotypes in a cross-sectional study of a predominantly Caucasian and male cohort of 42 HIV+ patients. The individual CYP2D6 genotypes, "extensive metabolizer" (EM) or "poor metabolizer" (PM), were determined by PCR-based amplification followed by restriction fragment length analysis. The patients were also probe-drug phenotyped with dextromethorphan (DM) which is *O*-demethylated by the CYP2D6 isoform. The expressed enzyme activity for each patient was determined by urinary molar ratios of dextromethorphan (DM) / dextrorphan (DT) as measured by high performance liquid chromatography (HPLC). Each patient was designated as having either a CYP2D6 EM phenotype or a CYP2D6 PM phenotype.

The results demonstrated that forty patients (95%) possessed an *EM* genotype which is consistent with previously observed distributions in predominantly male Caucasian populations. In healthy seronegative populations genotype and phenotype have been shown to be essentially interchangeable measures of CYP2D6 activity. However, in this cohort, 24 of the 40 patients with an *EM* genotype expressed a PM phenotype. The shift from *EM* genotype to PM phenotype could not be attributed to AIDS-defining illnesses or current clinical status as measured by CD4 count, body weight, body mass index (BMI) and biochemical parameters such as liver function

tests and albumin. The shift from *EM* genotype to PM phenotype attributable to metabolic drug interactions was identified in only 5 patients.

The data demonstrates that changes in expressed CYP2D6 activity occurred within this HIV+ population with the movement from EM to PM phenotypes irrespective of disease state. The results suggest that clinical status and disease state do not correlate with CYP2D6 metabolic capacity within a HIV+/AIDS population. The data also suggests that a basic marker for drug metabolism such as probe-drug phenotyping may prove to be a more accurate tool in determining individual drug response in a non-healthy population *i.e.* HIV+ population. Probe-drug phenotyping for CYP2D6 activity performed prior to the initiation of treatment with drugs whose metabolism is mediated by this enzyme may prove to be a useful tool for the clinician in determining drug response and consequently, optimizing treatment.

Résumé

Le cytochrome P450 (CYPs) est une classe importante d'enzymes hépatiques responsables pour la biotransformation de la majorité des médicaments. L'expression de l'activité de cette classe d'enzymes exhibe une variabilité inter-individuelle importante. Ces variations ont été relié aux différences inter-individuelles dans l'efficacité et toxicité des médicaments.

Certains CYPs ont un polymorphisme génétique *i.e.* deux populations distinctes: l'une dont le métabolisme est plus lent relativement à la moyenne (métaboliseurs lents, ML) et l'autre dont le métabolisme est plus rapide (métaboliseurs rapides, MR).

La base génétique pour le polymorphisme des enzymes CYP2D6, CYP2A6, CYP2C9 et CYP2C19 a été identifiée. Pour les enzymes non polymorphiques tel que le CYP3A, le phénotype par sonde métabolique demeure la seule façon de déterminer l'activité des ces enzymes. Cette approche est basée sur l'identification et la quantification de "patterns" de métabolites spécifiques produits par la sonde métabolique.

Même si le génotype semble gouverner l'expression de l'activité des enzymes polymorphiques, des changements relatifs peuvent être apportés par des facteurs non-génétiques tels que: les interactions médicamenteuses, facteurs environnementaux (tabagisme, alcoolisme, polluants, *etc.*), maladies et malnutrition. Donc, une personne normalement en santé peut réagir différemment à un

médicament lorsque sa santé est compromise.

L'effet sur l'activité des CYPs par la progression de la maladie chez les patients atteints du VIH n'est pas connu. Des études antécédentes ont démontré que la progression de maladie associée au VIH peut influencer le métabolisme des médicaments. Ce phénomène a été démontré avec l'enzyme de phase II, le N-acetyltransférase II (NAT2) où la progression de la maladie entraîne un changement dans l'activité enzymatique et peut augmenter la toxicité des médicaments métabolisés par cet enzyme.

Avec l'approbation de la nouvelle classe de médicaments, les inhibiteurs de protéase (PIs), dans le traitement du VIH, les interactions médicamenteuses est désormais un problème important dans l'usage clinique. Cette nouvelle classe de médicaments possède un profil cinétique caractérisé par une courte demi-vie, un métabolisme de premier passage important et une liaison aux protéines plasmatiques élévée.

Le but de cette étude a été de déterminer si la progression de la maladie chez les patients séropositifs affecte ou non l'activité exprimée de l'enzyme CYP2D6 du cytochrome P450. Cette étude rapporte les résultats préliminaires à cet effet en utilisant l'enzyme CYP2D6.

Dans une étude transversale d'une cohorte de 42 patients séropositifs mâles, majoritairement Caucasiens, les génotypes de l'enzyme CYP2D6 du cytochrome P450 ont étés comparés à leurs phénotypes exprimés. Les génotypes individuels de l'enzyme CYP2D6 ont été déterminés par une réaction d'amplification en chaîne par

la polymérase (PCR) suivi par l'amplification d'un fragment restreint. Ces méthodes ont permis d'identifier trois allèles: *CYP2D6(A), CYP2D6(B)* et *CYP2D6(D)*, qui comptent pour 95.4% des mutations associées à un métabolisme lent. D'autres mutations existent, mais elles sont rares et n'ont donc pas été considérées dans cette étude. Les génotypes individuels déterminés pour chaque patient ont permis de les classifier comme métaboliseur rapide (MR) ou comme métaboliseur lent (ML).

Le phénotype pour les patients a été établi à l'aide du dextrométhorphane (DM) comme sonde métabolique, qui est O-démethylé par l'isoforme CYP2D6 du cytochrome P450. L'activité exprimée de l'enzyme CYP2D6 pour chacun des patients a été déterminée par les ratios molaires urinaires du dextrométhorphane (DM) / dextrorphane (DT) tels que mesurés par "high performance liquid chromatography" (HPLC). Un phénotype CYP2D6 MR ou CYP2D6 ML a alors été attribué à chaque patient.

Les résultats ont montré que 40 patients (95%) possèdent un génotype MR , ce qui concorde avec les distributions précédemment observées chez les populations mâles, majoritairement Caucasiens. Dans les populations séronégatives, le génotype et le phénotype ont été démontrés comme étant des mesures essentiellement interchangeables de l'activité de l'enzyme CYP2D6. Pourtant, dans cette cohorte, 24 des 40 patients avec un génotype MR ont exprimé un phénotype ML. Ce basculement d'un génotype MR à un phénotype ML n'a pas pu être attribuable à une maladie opportuniste ni à l'état de santé courant tel qu'indiqué par le décompte lymphocytaire de CD4, le poids, l'indice de la masse corporelle ou par les paramètres

biochimiques tels que les tests de fonction hépatique et l'albumine. Le basculement d'un génotype MR à un phénotype ML attribuable à des interactions médicamenteuses n'a été identifié que dans cinq patients.

Les données indiquent que des changements dans l'activité exprimée par l'enzyme CYP2D6 se sont effectuées à l'intérieur de cette population séropositive par la transition de phénotypes MR à ML indépendemment de l'état de santé du patient. Les résultats suggèrent qu'il n y a pas de correlation entre l'état clinique et/ou la maladie, et la capacité métabolique de l'enzyme CYP2D6 dans une population atteinte du VIH+/SIDA. Les données suggèrent aussi qu'un marqueur de base pour le métabolisme des médicaments tel que le phénotype par sonde métabolique peut être un meilleur outil pour déterminer la réponse individuelle aux médicaments chez une population dont la santé est compromise, telle qu'une population séropositive. La détermination du phénotype de l'enzyme CYP2D6 par sonde métabolique, effectué avant le début d'un traitement peut se révéler comme étant un outil valable pour le clinicien. Le phénotype permettrait de déterminer la réponse individuelle aux médicaments dont le métabolisme est dépendant de cet enzyme, et conséquemment permettrait d'optimiser le traitement.

Table of Contents

List of Appendices

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS

AA:	African American
ABV chemo:	combination chemotherapy of adriamycin, bleomycin, vincristine, decadron
AIDS:	Acquired Immune Deficiency Sydrome
ALS:	amytrophic lateral sclerosis
ARC	AIDS-related complex
AS:	Asian
AZT:	zidovudine
B_{12}:	vitamin B_{12}
BLQ:	below level of quantitation
BMI:	body mass index
BSA:	bovine serum albumin
CD4:	T helper cells which express the CD4 molecule on their surface
CDC:	Centers for Disease Control
CMV:	cytomegalovirus
CNS:	central nervous system
CYPs:	cytochrome P450
CYP2D6:	cytochrome P450 isoform IID6
CYP3A:	cytochrome P450 isoform IIIA
d4T:	stavudine
ddC:	zalcitabine
ddI:	didanosine
DM:	dextromethorphan
DT:	dextrorphan
DTP:	diphtheria, tetanus toxoid, pertussis vaccine
EDTA:	ethylenediaminetetraacetic acid
E.E.:	extraction efficiency

EM:	extensive metabolizer
FTD:	frontotemporal dementia
GI:	gastrointestinal
GM-CSF:	granulocyte-macrphage colony-stimulating factor
HDL:	high density lipoprotein
HIV:	human immunodeficiency virus
HIV+:	HIV positive
HPLC:	high performance liquid chromatography
HSV:	herpes simplex virus
IDTC:	Immune Deficiency Treatment Center
IFN:	interferon
IFN α:	interferon alpha
IFN γ:	interferon gamma
IL-1:	interleukin 1
IL-1 α:	interleukin 1 alpha
IL-1 β:	interleukin 1 beta
IL-2:	interleukin 2
IL-4:	interleukin 4
IL-6:	interleukin 6
K.S.:	Kaposi's sarcoma
Lev:	levallorphan
LDL:	low density lipoprotein
LPS:	lipopolysaccharide
MAC:	mycobacterium avium complex
MGH:	Montreal General Hospital
MFO:	mixed function oxidation
mRNA:	messenger RNA
NAT2:	N-acetyltransferase II
NNRTI:	non-nucleoside reverse transcriptase inhibitor

OHL:	oral hairy leukoplakia
OI:	opportunistic infection
PAH:	polycyclic aromatic hydrocarbons
PCP:	*pneumocystis carinii* pneumonia
PCR:	polymerase chain reaction
PI:	protease inhibitor
PM:	poor metabolizer
PML:	progressive multifocal leukoencephalopathy
QC:	quality control
r:	correlation coefficient
r^2:	regression coefficient
RTI:	reverse transcriptase inhibitor
S.D.:	standard deviation
T4 lymphocytes:	T helper cells
TNF:	tumor necrosis factor
TNF α:	tumor necrosis factor alpha
USP:	United States Pharmacopeia
VBV chemo:	combination chemotherapy of vinblastine, bleomycin, vincristine, decadron
VLDL:	very low density lipoprotein
W:	Caucasian
WI:	West Indian
3HM:	3-hydroxymorphinan
3MM:	3-methoxymorphinan
3TC:	lamivudine

Acknowlegements

First and foremost, I would like to acknowledge and thank my co-director, Dr. Irving W. Wainer without whose guidance, help and support over the last few years, this work would not have been possible. I thank him for having been my mentor, teacher and a constant support despite his busy schedule. I would also like to thank him for always having shown confidence in my abilities, even when I doubted myself. I am grateful to him for having provided me with a very rich and educational experience.

I am also very grateful for the invaluable contribution from my director, Dr. Patrick du Souich, whose guidance and direction made it possible for the work done over the last three years, to crystallize and come together.

I would like to thank Karen Fried, lab manager, for having taught me the "essence" of good science and the "art" of chromatography. I would also like to thank my friend and colleague, Isabel Anacleto for keeping the work atmosphere light and interesting, even when times seemed their darkest.

Last, but not least, I would like to thank the resident "computer whiz", Marianna Di Girolamo, for her computer knowledge and support in bringing this body of work to its completion.

To my parents Venturino and Mariannina,

who have always sacrificed their lives so that mine could be better;

To my nieces Marianna, Angela, Marilou, and Vanessa;

and to my nephew Rino,

who have always managed to keep my life interesting;

To my brothers, Giovanni and Angelo

for their encouragement and support.

CHAPTER I: INTRODUCTION

1. Drug Metabolism and Cytochrome P450

1.1 The Cytochrome P450 Monooxygenase System:

The cytochrome P450 (CYPs) constitute a key class of xenobiotic metabolizing enzymes and catalyze the majority of drug biotransformations [1, 2]. This superfamily of enzymes includes a wide variety of enzymes which are involved in the metabolism of steroids, vitamins, fatty acids and xenobiotics [2]. The xenobiotic metabolizing enzymes are divided into three main families: CYP1, CYP2 and CYP3. These families are further divided into subfamilies: CYP1A, CYP2B, CYP2C, CYP2D, CYP2E, CYP3A which contain a variety of isoforms *i.e.* CYP2C9, CYP2C19. These isoforms primarily mediate the metabolic conversions of phase I oxidative transformations.

Sites of biotransformation: The main site of drug biotransformation is the liver. Enzymes involved in phase I oxidative reactions are found in the endoplasmic reticulum, while phase II conjugation enzymes are found mainly in the cytosol. In addition to the liver, drugs can be subjected, to some extent, to extrahepatic biotransformation. Other organs which demonstrate significant metabolic capacity include the kidneys, gastrointestinal tract, skin, lungs and brain [3].

1.2 Structure of Cytochrome P450:

Cytochrome P450 is the terminal oxidase component of an electron transfer

system, existing in multiple forms of monomeric molecular weight of approximately 45,000 - 55,000 Da, embedded in the lipid bilayer of the endoplasmic reticulum [4]. It is classified as a heme-containing enzyme (hemoprotein) with iron protoporphyrin IX as the prosthetic group (Figure 1).

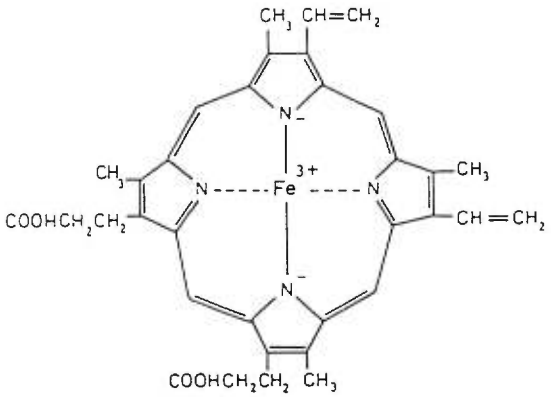


Figure 1: Structure of ferric protoporphyrin IX, the prosthetic group of cytochrome P450

1.3 Role of Cytochrome P450 in Drug Metabolism:

The role of the CYPs is to execute the metabolism of lipophilic xenobiotic compounds by the insertion of a single atom of molecular oxygen into a substrate. This reaction modifies the substrate through oxidation by the introduction of a functional group (-OH, $-NH_2$, -SH), or by unmasking a functional group through dealkylation, thereby rendering the substrate more polar (phase I reaction). Further metabolic processing is facilitated following this phase I reaction by subsequent reactions with phase II enzymes in which an endogenous substrate such as

glucuronic acid, glutathione or sulphate moieties combines with the newly established functional group, to form a highly polar conjugate which is readily excreted. In addition to catalyzing drug biotransformation, cytochrome P450 mediates oxidation reactions which may activate foreign compounds to toxic and mutagenic products. These reactions usually represent the initiating events in subsequent drug toxicity and carcinogenesis [5-6]. Hence, understanding the function of the CYPs system is vital to health and well-being.

The most predominant and most intensively studied reaction catalyzed by the cytochrome P450 system is the mixed-function oxidation (MFO) reaction, which catalyzes the hydroxylation of hundreds of structurally diverse drugs and chemicals. The MFO reaction conforms to the following:

$$NADPH + H^{-} + O_2 + RH \xrightarrow{\text{CYP 450}} NADP^{+} + H_2O + ROH$$

where RH represents an oxidizable drug substrate and ROH the hydroxylated metabolite. Reducing equivalents derived from NADPH are passed to the membrane-bound cytochromes P450 via the flavoprotein NADPH- cytochrome P450 reductase. These reducing equivalents are used to reduce molecular oxygen, one atom being incorporated into the substrate and the other being reduced to water. In addition to hydroxylation reactions, cytochrome P450 catalyses the *N*-, *O*- and *S*-dealkylation reactions as well as one- or two-electron reduction reactions [4].

2. INTERINDIVIDUAL VARIABILITY

2.1 Genetic Polymorphism and Drug Metabolism

Cytochrome P450 enzymes exhibit a large degree of interindividual variability in their levels of expression and these variations have been related to interpatient differences in drug efficacy and toxicity [1]. Much of the interindividual variations result from the variability in the expression of different CYP enzymes in the liver and extrahepatic tissues [7].

In most cases, interindividual differences in the activity of CYP isoforms are quantitative as enzymatic activity appears unimodally distributed. Some CYPs are genetically polymorphic (polymorphism defines the existence of more than one monogenic trait in a normal population which produce at least two distinct phenotypes) [7]. For these enzymes, the activity usually falls into two clearly defined and qualitatively different populations: individuals whose rate and extent of metabolism is lower relative to the mean ("poor" metabolizers, PM) and those who have a faster or more extensive metabolism ("extensive" metabolizers, EM).

The genetic basis for the polymorphism of CYP2A6, CYP2C9, CYP2C19 and CYP2D6 have been identified. Historically, most polymorphisms had been identified on a clinical level, based on impaired or reduced metabolic capacity of the individual [2]. Following the administration of standard doses of certain drugs, patients who experienced adverse drug reactions were identified as "poor metabolizers" whereas those with regular patterns of metabolism who did not experience adverse drug reactions were identified as "extensive metabolizers" [7].

However, with the advent of gene cloning, which encodes the xenobiotic-metabolizing enzyme, and with the availability of new techniques which allow for the identification of allelic variants, polymorphism can now be identified and understood at the DNA level.

The metabolism of debrisoquine 4-hydroxylation represents one of the earlier and most extensively studied examples of metabolic polymorphism [8, 9]. Metabolic phenotyping with debrisoquine and other CYP2D6 substrates such as sparteine and dextromethorphan have demonstrated a bimodal distribution of EMs and PMs. The PM phenotype occurs at a frequency of 7% in Caucasian populations [10-12] and 1% in Chinese populations [10]. This suggests that there is an ethnic variation in the frequency of the PM phenotype.

The debrisoquine-sparteine polymorphism is caused by a monogenic autosomal recessive defect as demonstrated by various family studies [13-16]. Several mutant *CYP2D6* alleles associated with poor metabolism of debrisoquine have been identified [2]. The three most common mutant alleles are identified as *CYP2D6A, CYP2D6B* and *CYP2D6D*, and describe almost all of the inactivating mutations [17]. These alleles are known in the newer nomenclature as *CYP2D6*3, CYP2D6*4* and *CYP2D6*5* respectively [18]. According to Sachse [18], the identification of these three mutant alleles accounts for 95.4% of the alleles associated with poor metabolism.

*CYP2D6A (CYP2D6*3)* contains a single base pair deletion in exon 5 which causes a frameshift [19]. *CYP2D6B (CYP2D6*4)* mutation leads to altered RNA

splicing resulting from a base change of G to A at the intron 3/exon 4 boundary. This also results in a frameshift [20]. *CYP2D6D (CYP2D6*5)* mutation is generated by the complete deletion of the coding region of CYP2D6 [21].

*CYP2D6A (CYP2D6*3)* and *CYP2D6B (CYP2D6*4)* mutations can be identified by restriction fragment length genotyping. The *CYP2D6D (CYP2D6*5)* mutation can be identified by Southern blotting and by a long polymerase chain reaction (PCR) technique [22] When both methods are combined, it allows for the identification of at least 95% of poor metabolizers.

*CYP2D6A (CYP2D6*3)*, *CYP2D6B (CYP2D6*4)* and *CYP2D6D (CYP2D6*5)* alleles are associated with poor debrisoquine metabolism. Other mutations associated with impaired debrisoquine metabolism have been identified. These include the *CYP2D6C* allele, in which deletion of a single lysine residue has occurred [23]. This is the only allele capable of producing an intact protein, although at markedly reduced levels. The other type of anomaly known consists of a large insertion upstream of CYP2D6 in some individuals with the *CYP2D6B* allele [24]. This large insertion upstream of CYP2D6 is also common among Chinese subjects, although the CYP2D6B-associated mutations are not present and may account for the average slower metabolism seen in Oriental races [25].

2.2 Clinical Implications of Genetic Polymorphism

Among the metabolizing populations, polymorphic distribution is the cause of two important clinical consequences: 1) one phenotype may experience a different

drug efficacy-toxicity profile and 2) one population may have increased susceptibility to diseases such as cancer.

The clinical significance of genetic polymorphism and drug response depends on whether the pharmacological activity resides in the affected substrate or in the metabolite and on the importance of the pathway to the overall biotransformation of the drug [7]. Thus if the parent drug is the active compound, PMs have a decreased capacity to metabolize the drug and eliminate it (unless alternate pathways involving other enzymes exist). In general, the limited metabolism seen in the PM is responsible for severe adverse drug reactions (toxicity) when receiving standard doses of drugs which have a narrow therapeutic window. By the same token, if the metabolite is the active compound, as is the case of many drugs, the metabolic activation is decreased in PMs. The PMs will form smaller amounts, if any, of the active compound necessary to produce an effect and drug response is diminished. A classic example of this phenomenon is the metabolism of codeine in poor metabolizers of CYP2D6 [26]. Codeine is O-demethylated via CYP2D6 to morphine which provides the analgesia. In PMs, smaller amounts of morphine are formed from codeine and the analgesic response to the drug is diminished or lost [27]. Therefore, in PMs, codeine may prove to be ineffective in providing analgesia.

2.2.1 Clinical significance of CYP2D6 polymorphism

The clinical significance of CYP2D6 polymorphism has become apparent by the fact that several important drugs with narrow therapeutic range (*i.e.* antiarrythmics, antidepressants and neuroleptics) are metabolized by this enzyme [9,

12]. To date at least thirty (30) therapeutically important drugs are metabolized primarily or in part by CYP2D6.

While the PM phenotype in CYP2D6 polymorphism is a risk factor for drug-related toxicity of CYP2D6 substrates, the EM phenotype carries a different risk associated with disease processes. The extensive metabolizer phenotype has been associated with increased susceptibility to developing various malignancies, particularly lung cancer in smokers [28, 29]. The hypotheses are that either the CYP2D6 gene is linked with another gene predisposing to lung cancer or that extensive activity of CYP2D6 is associated with extensive conversion of procarcinogenic substances in tobacco smoke to carcinogens [30, 31]. Results from various studies into this effect have been controversial and the hypotheses remain to be confirmed.

Several studies have been conducted to determine whether a person with an EM phenotype was susceptible to certain diseases. The earliest study of this type was conducted by Ayesh *et al.* [28] in which the debrisoquine phenotypes of 245 bronchogenic carcinoma patients and 234 smoking controls were compared to assess susceptibility to lung cancer. Findings from this study suggested that PMs were less susceptible to develop lung cancer by comparison to EMs. While a number of similar studies [29, 32] confirmed the findings of Ayesh *et al.*, other studies showed no significant difference between the two phenotypes [33-35]. In a recent study conducted by Christensen *et al.*[36], a random effects model was used to assess the odds ratio for the risk of lung cancer; the findings showed no association between

CYP2D6 polymorphism and lung cancer risk.

In addition to lung cancer susceptibility, CYP2D6 distribution has been studied in relation to other diseases. Kaisary *et al.* [37] used metabolic phenotyping to detect an association between aggressive tumors in bladder cancer and the EM phenotype. In another study, Fleming *et al.* [38] suggested that recurrence of non-aggressive tumors was less likely in patients with a PM phenotype than in patients with an EM phenotype.

Another disease which has received considerable attention in relation to CYP2D6 polymorphism is Parkinson's disease. In a study originally conducted by Barbeau *et al.* [39] with a group of patients with Parkinson's disease, it was suggested that among these patients there was an increase in the frequency of the PM phenotype, and that the onset of disease occurred at an earlier age in PMs than the EMs. In a subsequent report [40] the original findings from Barbeau *et al.* were attributed to drug interferences in the phenotyping procedure. Subsequent studies failed to reproduce the results reported by Barbeau *et al.* and have generally found no association between CYP2D6 phenotype and Parkinson's disease susceptibility except for a study conducted by Benitez *et al.* [41] in which there was evidence of a direct relationship between age of onset of disease and rate of debrisoquine oxidation. A possible biological basis for an association between increased susceptibility to Parkinson's disease and the PM phenotype has emerged from the findings that neurotoxins known to induce parkinsonism in certain animal species are thought to be CYP2D6 substrates [42-43]. The suspected neurotoxins are:

1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine and 1,2,3,4-tetrahydroisoquinoline.

In another study by Armstrong *et al.* [44] no differences in overall genotype frequencies between Parkinson's disease cases and controls were observed, however an increased frequency of the *CYP2D6B* allele was observed in the Parkinson's disease group. The increased frequency of the *CYP2D6B* allele represented a relative risk of 2.7 for the development of the disease in subjects heterozygous for the *CYP2D6B* allele. A similar phenomenon was observed in a recent study [45] on amyotrophic lateral sclerosis (ALS) susceptibility and gene polymorphism. This study examined the frequencies of variant CYP2D6 alleles in patients with ALS, as compared to patients with frontotemporal dementia (FTD) and patients with Alzheimer's disease, and various combinations of the three disorders. In this study, there was a significant increase in the frequency of the *CYP2D6B* allele in the ALS group, although no difference was observed in the overall PM genotype distribution among the different groups. These findings suggest that possession of a *CYP2D6B* allele may be a risk factor for the development of ALS.

2.3 Interindividual Variability in Drug Metabolism (Non-Genetic Factors)

Interindividual variation in drug metabolism refers to the differences in responsiveness to therapeutic agents observed among individuals. While genetic polymorphisms are of primordial importance in determining the activity of drug-metabolizing enzymes, other non-genetic factors also play an important role in modulating the activity of these enzymes, thus influencing individual drug responses.

These non-genetic factors include: enzyme induction and inhibition, metabolic drug interactions, exposure to environmental polluants, disease state, age and gender [46-47].

Enzyme induction refers to the increased synthesis of *de novo* cytochrome P450 protein often associated with repeated exposure to certain chemical substrates and environmental polluants. Induction results in an acceleration of metabolism and corresponding decrease in the pharmacologic action of the inducer and of coadministered drugs. However, for drugs that are metabolized to reactive metabolites, enzyme induction may be associated with increased metabolite-mediated tissue toxicity [46]. In some cases, a substrate can induce both the biotransformation of other compounds as well as its own metabolism. A well-characterized example of this autoinduction occurs with the anticonvulsant, carbamazepine [47]. Various substrates appear to induce several isoforms of the cytochrome P450 family. The most extensively studied CYP isoforms which are subjected to induction are: CYP2B1 which is induced by phenobarbital in experimental animals, and CYP1A1 which is induced by polycyclic aromatic hydrocarbons (PAHs), of which 3-methylcholanthrene is a prototype, altering rates of drug metabolism in both experimental animals and in humans. Exposure to PAHs from industrial polluants, cigarette smoke and charcoal-broiled meats can result in dramatic induction of CYP1A1 both in the liver and extrahepatically. Other prototype inducers of cytochrome P450 enzymes include glucocorticoids, macrolide antibiotics, anticonvulsants and some steroids for CYP3A4, and isoniazid, acetone

and chronic ethanol consumption for CYP2E1. Clofibrate, a hypolipidaemic drug, has a specificity for inducing other distinct isoforms, CYP4As, that do not readily metabolize drugs but are responsible for the omega-hydroxylation of several fatty acids, leukotrienes and prostaglandins [46]. In general, inducers are relatively non-specific in that they cause a general proliferation of the hepatic endoplasmic reticulum or of the drug-metabolizing enzymes [4]. Many inducers of cytochrome P450 also induce phase II enzymes such as glucuronosyltransferases and glutathione transferases [46-47].

Enzyme inhibition results in elevated levels of the parent drug, prolonged pharmacological effects and an increased incidence of drug-related toxicity. Inhibition of drug metabolism can arise by several mechanisms including the destruction of pre-existing enzymes, competitive inhibition and inactivation of the enzyme through complexation and subsequent inhibition [4]. Many therapeutic drugs and xenobiotics possess the ability to destroy the cytochrome P450 in the liver. A well-known mechanism has been identified for olefinic (C=C) and acetylenic (C≡C) derivatives which are metabolized by CYP450 to form alkylated or substrate-heme adducts, reactive intermediates, which attack the heme moiety of the enzyme.

These "suicide substrates" which result in cytochrome P450 heme destruction include several classes of commonly used therapeutic agents. Among these are the synthetic steroids ethinyl estradiol and norethindrone, the barbiturates secobarbital and allobarbital, the thyroid hormone antagonist propylthiouracil, the anesthetic

agent fluroxene, and the analgesic sedatives allylisopropylacetylurea, diethylpentenamide, and ethchlorvynol [4, 46]. A major consequence of the heme destruction entailed by these compounds is a significant and sustained drop in the levels of functional cytochrome P450, resulting in a reduction in the overall capacity of the liver to metabolize drugs. Since olefinic and acetylenic groups are found in many therapeutic agents in use, many drug-drug interactions can be rationalized at the level of cytochrome P450 destruction.

Competitive inhibition results when two or more substrates compete for the active site of the same enzyme. The substrate that is present in higher concentrations or for which the affinity of the enzyme is higher will bind to the cytochrome P450, and as a result, the metabolism of the other substrate will be decreased. One mechanism by which drug metabolism is inhibited is through the complexation to the cytochrome P450, rendering it inactive and preventing its further participation in the metabolism of other substrates. A classic example of this type of competitive inhibition is illustrated by imidazole-containing drugs such as cimetidine and ketoconazole which bind tightly to the heme-iron of cytochrome P450, forming an inactive complex and effectively reducing the metabolism of endogenous substrates (*i.e.* testosterone) or coadministered drugs [46]. In the case of macrolide antibiotics such as troleandomycin and erythromycin, the metabolites resulting fom the cytochrome P450 demethylation and subsequent oxidation of the parent drug, form a stable complex with the heme-iron and render it catalytically inactive [4, 46].

It has long been recognized that the very young, particularly the newborn,

and old individuals have an increased susceptibility to pharmacologic and toxic drug activity as compared to young adults. Studies have indicated that the increased sensitivity of neonates to drugs may be related to very low or, at times, unmeasurable drug-metabolizing capacity which subsequently increases to adult levels of enzyme activity. The pattern of development is variable for the different enzymes and is dependent upon individual differences in drug metabolism and gender [4, 47]. Conversely, as the individual ages, the drug-metabolizing capacity decreases. This diminished abilitity to metabolize drugs may reflect changes brought about by disease processes, or may be due to reduced amount and/or activity of metabolic enzymes, and/or reduced availability of essential endogenous cofactors [46, 47].

Sex-dependent variations in drug metabolism have been well-documented in rats where the differences were clearly associated with androgenic hormones whereby young adult male rats metabolized drugs much faster than mature female rats or prepubertal male rats [46]. Limited clinical reports suggest that similar trends exist in humans *i.e.* decreased oxidation of estrogens and benzodiazepines in females relative to the males [47]. However, incontrovertible evidence of gender-specific differences in drug metabolism among humans is yet to be obtained.

Many disease states are known to directly or indirectly affect drug metabolism. The most prominent of these disease states are the acute and chronic conditions leading to the destruction of liver tissue, with impairment of liver function and hepatic drug metabolism [46, 47]. Such conditions include: cirrhosis of the liver, alcoholic liver disease [46, 48] fatty liver disease, biliary cirrhosis, hemochromatosis

[46], chronic active hepatitis [48] and viral hepatitis [49, 50]. Depending on their severity, these conditions can lead to the impairment of hepatic biotransformation, particularly microsomal oxidases, and thereby markedly affect drug elimination [46]. The major mechanism by which the liver's ability to metabolize drugs is impaired results from the destruction of functional hepatocytes precipitated by the various above-mentioned conditions, and resulting in decreased metabolic capacity. Other mechanisms which impair the liver's ability to metabolize drugs include decreased hepatic blood flow, which decreases the clearance of flow-dependent drugs, and hypoalbuminemia which leads to decreased plasma binding and increased clearance of selected drugs [4]. Decreased hepatic blood flow can result from conditions such as cardiac insufficiency [46, 47] or it can be a compensatory mechanism in liver damage where blood flow is shunted past the damaged tissue [4].

Other non-hepatic diseases which could potentially influence drug metabolism hve been investigated in experimental animal models. These include thyroid dysfunction, pituitary dysfunction, adrenal dysfunction and gonadal dysfunction [46]. Hypothyroidism has been shown to increase the half-life of antipyrine, digoxin, methimazole and practolol, whereas hyperthyroidism decreases the half-life of these drugs [46]. Dysfunction of the pituitary, adrenal cortex and gonads have been shown to markedly impair hepatic drug metabolism in rats [46]. Although the effects of endocrine dysfunction have been well-explored in experimental animal models, there is limited clinical corresponding data in humans. Therefore, extrapolation of the findings in animal models is tentative.

Recently, it has become apparent that general infectious diseases can affect drug metabolism. It has been observed that viral infections are followed by a drop in enzyme content, resulting from cytochrome P450 degradation [4]. This phenomenon has been associated with the production of cytokines activated by the host immune response and with the production of nitric oxide ellicited by the immunocompetent cells of the liver [4]. The role that cytokines play in modulating cytochrome P450 activity will be discussed in further detail in Section 3.

While non-genetic, environmental factors have been reported to modulate the activity of certain drug-metabolizing enzymes *i.e.* CYP1A1, CYP2B1, CYP2E1, CYP3A, and glucuronidation *etc.*, the genetic polymorphism of CYP2D6 predominantly governs the activity of this enzyme and is largely responsible for the interindividual variability observed. CYP2D6 is known to be a "solid" enzyme which does not appear to be inducible.

3. Cytokines and Drug Metabolism

The role of cytokines on cytochrome P450 mediated drug metabolism has been investigated by different groups. Both *in vitro* and *in vivo* studies have been conducted in animal models and in humans, lending support to the down-regulation of CYPs by various cytokines. The cytokines mostly investigated are: Interleukin-1 (IL-1) α, β; Interleukin-2 (IL-2); Interleukin-6 (IL-6); tumor necrosis factor (TNF) α and Interferon (IFN) α, γ.

Animal studies: IL-1 has been shown to reduce CYP activities in mice and rats [51-53]. Administration of recombinant IL-1 or lipopolysaccharide (LPS)-induced inflammation resulted in a reduction of the metabolic activities of CYP1A1, 2B1, 2C11, 2D1, 2E2 and 3A *in vitro*. The decrease in CYP2D1 activity in rat liver microsomes isolated from IL-α treated animals was associated with a decrease in the level of CYP2D mRNA, suggesting that IL-α down-regulated CYP2D activity by reducing the mRNA encoding the enzyme [53]. Bertini *et al.* [54] investigated the effects of several cytokines on mouse liver *in vivo*, and reported a decrease in the liver CYP 450-dependent drug metabolism by IL-1 α/β, TNF-α, lymphotoxin and IFN-α/γ, while IL-2 had no effect. When tested *in vitro* on isolated hepatocytes, only IL-1 depressed CYP 450-dependent drug metabolism while other cytokines were inactive, suggesting that the effect of the cytokines on the liver *in vivo* is not a direct effect, but is mediated by other factors. In a study conducted by Nadin *et al.* [55], the effects of recombinant murine TNF-α on the expression of specific CYPs in the male rat liver were investigated. The findings showed that TNF-α down-regulates CYP2C11 and CYP3A2 at the pre-translational level, whereas CYP2A1 and CYP2C6 seemed refractory to TNF-α.

Fantuzzi *et al.* [56] showed that inhibitors of cytochrome P450 activity such as metyrapone and SKF525A, suppressed the production of TNF in rats that were pre-treated with endotoxins, suggesting that this inhibition might be related to either the inhibition of lipoxygenase inhibitors or a cytochrome P450 enzyme that is

implicated in the oxidation of endogenous substrates involved in the inflammatory response.

Thal *et al.* [57] investigated the effect of IL-2 on the expression of CYP1A1, 1A2, 2B1, 2C11, 2D1 and 3A in rats, following the administration of human recombinant IL-2. A dose and time dependent decrease in CYP enzymes was demonstrated. All CYPs investigated revealed an important decrease in their expression except for CYP1A1. CYP3A expression was particularly decreased by IL-2 treatment, showing a 70% reduction for rats non-induced and induced by phenobarbital [57]. Cytochrome P450 inhibitors have also been shown to decrease IL-2 synthesis *in vitro* [58]. Contrary to previous results on the effects of IL-2 on CYPs expression, Kurokohchi *et al.* [59] demonstrated that *in vivo* injection of IL-2 to rats induced an increase in the amounts of immunoreactive CYP2D protein and its mRNA, suggesting that the enzymatic activities were up-regulated at the mRNA level. This result is unexpected and it is interesting considering that the analogous enzyme in humans is CYP2D6 which has never been shown to be inducible by any compound [60, 61]. In a study conducted by Ansher *et al.* [62], mice were administered LPS and DTP (diphteria, tetanus toxoid, pertussis) vaccine. The findings showed an association between DTP vaccine and LPS administration and a reduction in CYP1A2 and CYP2E1 mRNA expression. This modulation of hepatic mRNA expression was seen concomitantly with a significant rise in circulating levels of IL-1, IL-6 and TNF-α.

Human studies: Using cultures of primary human hepatocytes, several groups were able to demonstrate a depression in the activity of selected CYPs and a decrease in the mRNA levels encoding for these enzymes. Abdel-Razzak *et al.* [63] demonstrated that IL-1, IL-4, IL-6, TNF-α and IFN-γ significantly affected the expression of major P450 genes. Following three days of treatment with IL-1, IL-6 and TNF-α, it was shown that IL-1 decreased P450 mRNA levels encoding for CYP1A2 and CYP3A by more than 50% and 80 % respectively. IL-6 and TNF-α were associated with an approximate 50% decrease in P450 mRNA levels for CYP1A2 and CYP3A. Nifedipine oxidation, which is a primary reflection of CYP3A activity was also decreased by approximately 50% following IL-1, IL-6 and TNF-α treatment. Abdel-Razzak *et al.* also reported a large variation between human liver samples.

The work undertaken by Li *et al.* [64] demonstrated that TNF-α induced inhibition of *Cyp 17* gene expression was not due to a change in the mRNA half-life, but rather due to repression of *Cyp 17* transcription. TNF-α is more active than IL-6 at repressing the inducibility of CYP1A in primary human hepatocyte cultures, while IL-6 had a more prominent effect on repressing CYP3A than TNF-α.

3.1 Relationship between cytokines and HIV disease

There is a strong relationship between cytokine levels and HIV disease. HIV infection and the subsequent activation of the monocyte/macrophage system increases the production of TNF-α, IL-1, IL-6, granulocyte-macrophage

colony-stimulating factor (GM-CSF) and IFN-α [65, 66]. Cytokines which mediate the inflammatory response such as TNF-α and IL-1β have been found in high levels in the sera or in culture supernatants in monocytes from AIDS patients [67-69]. Il-6 levels are elevated in HIV-positive (HIV+) patients and even more so in patients with Kaposi's sarcoma [70]. IL-2 which plays an important role in the production of CD4 cells, is decreased in HIV+ patients who also possess a defect in its receptor expression [71]. Plasma concentrations of TNF, also known as cachectin, have been shown to be increased in HIV+ patients. Soluble TNF receptors, which are also increased in HIV+ patients, appear to correlate with worsening immune function [72-74] and this has led to the hypothesis that high TNF-α levels are predictors of disease progression and worsening clinical outcome [72]. Changes in cytokine levels during HIV disease may result from HIV expression itself, or may be derived from the immune activation in response to the HIV infection, or may be elicited from opportunistic infections occuring in AIDS patients. The relationship between HIV-induced changes in cytokines and potential repercussions on drug metabolism cannot be excluded.

4. HIV Disease and Drug Metabolism

4.1 Overview of the Pathophysiology of HIV Disease

HIV is a retrovirus associated to the subfamily of lentiviruses. HIV more precisely consists of two viruses, HIV-1 and HIV-2, which are considered to be the

etiological agents of HIV disease. HIV-1 is globally distributed while HIV-2 is found predominantly in West Africa. The major characteristics of the HIV-1 virus are a long incubation period, a wide genetic variability and the ability to mutate as it replicates, giving rise to "resistant strains".

HIV infects target cells - T4 lymphocytes which express the CD4 molecule on their surface. Once the HIV virus has penetrated into the T4 lymphocyte, the envelope enclosing the virus is broken down and viral RNA is released. The viral RNA is transcribed into DNA which is integrated into the nucleus of the T4 lymphocyte. At this stage two outcomes are possible: 1) the virus may remain dormant and simply be transmitted to daughter cells with each mitosis. The infected T4 cells of this person can be transmitted to others and cause infection. The HIV virus can also chronically infect other white blood cells and macrophages [75].

2) The virus may become active and reproduce itself which results in cell death and release of a large number of virions (infectious viral particles) that go on to infect other T4 lymphocytes [75]. When a large number of the body's T4 cells have been destroyed directly or indirectly by the virus, the body's immune defences are reduced (immunosuppression) and the risk of developing AIDS is considerably advanced.

4.2 Clinical course of HIV disease

The course of HIV disease is highly variable for any particular individual. In the majority of individuals, acute primary infection is followed by a long period of asymptomatic HIV infection. The time between primary HIV infection and significant immunosuppression is long and variable, ranging from 2 to 10 years [76].

The median time from initial infection to the development of AIDS is approximately 10 years although the rate of disease progression varies substantially among patients [77]. During this time the HIV virus is replicating within the lymph nodes and there is a slow gradual reduction in the number of T4 lymphocytes, averaging a loss of 60 - 100 cells/year.

Prior to the advent of viral load testing, CD4 lymphocyte count and p24 antigenemia were the surrogate markers available to the clinician to monitor HIV infection and predict the relative risk for disease progression. A CD4 cell count gives a "crude" measure of the individual's immune status and has been associated with a certain degree of variability [78-81]. This variability ranges from diurnal fluctuations in patients to laboratory variability arising from specimen manipulation and testing [77]. With the measurement of viral load to complement CD4 cell count, the clinician is now better equipped to monitor disease progression. While CD4 counts are known to fluctuate to a great extent in the same patient, viral load measurements are fairly stable over time [82].

Early HIV-1 disease: Early stage HIV disease is defined by a CD4 cell count of greater than 500 cells/mm^3, with most HIV-infected patients being asymptomatic. A period of persistent generalized lymphadenopathy may accompany this "asymptomatic" phase. Other clinical manifestations of early HIV disease may include aphthous ulcerations of the oral mucosa and dermatological abnormalities *i.e.* seborrheic dermatitis, exacerbation of pre-existing psoriasis and eosinophilic

folliculitis [83-85].

Intermediate stage of HIV disease: The intermediate stage of HIV disease is defined by a CD4 count between 200 and 500 cells/mm^3. Although the risk of developing opportunistic infections (OI) is higher in this stage as compared to the early stage, most HIV-infected patients remain asymptomatic or demonstrate only mild disease manifestations [77].

This stage is characterized by constitutional symptoms such as headache, fatigue, malaise, myalgias and arthralgias. The frequency of skin and oral lesions may worsen throughout the stage [83, 84]. A variety of more clinically evident symptoms such as intermittent fever, recurrent diarrhea, oropharyngeal candidiasis, recurrent HSV (herpes simplex virus) infection *etc.* may manifest themselves during this stage of the illness (see Appendix I). This symptoms complex, referred to as ARC (AIDS-related complex) in the older nomenclature, usually indicates a higher likelihood for progression to AIDS [77]. Historically, the initiation of antiretroviral therapy was indicated when CD4 cell count dropped below 500 cells/mm^3. With the availability of viral load measurements and a better understanding of the kinetics of viral replication and turnover of HIV-infected cells, this guideline has come under scrutiny and HIV-opinion leaders are now favoring the "hit hard, hit early" approach which encourages the initiation of combination antiretroviral therapy in the early stages of the disease.

Late-stage HIV disease: This stage is defined by a CD4 cell count between 50 and

200 cells/mm^3 [77]. Patients with late stage disease are at a substantial risk for developing AIDS-defining OI such as toxoplasmosis, PCP (*pneumocystis carinii* pneumonia), esophageal candidiasis, tuberculosis and AIDS-related malignancies such as Kaposi's sarcoma and lymphoma. In 1993 the CDC (Center for Disease Control) classification system (Appendix I) was changed to include all patients with CD4 count < 200 cells/mm^3 to be categorized as having AIDS even in the absence of AIDS-defining OI. This change was implemented to reflect the increased risk of developing new AIDS-defining illnesses when CD4 count is less than 200 cells/mm^3. In addition to developing opportunistic infections, other physiological abnormalities may manifest themselves at this stage. These include hematologic disturbances (anemia, neutropenia, thrombocytopenia), hormonal disturbances (hypogonadism in men, menstrual irregularities in women) [86] and rarely endocrine dysfunction (hyperthyroidism, hypothyroidism, adrenal insufficiency) [87]. In addition to continuing combination antiretroviral therapy, the initiation of prophylactic therapy to prevent opportunistic infections (*i.e.* PCP prophylaxis) is mandated when CD4 count drops to below 200 cells/mm^3.

When CD4 count drops to below 50 cells/mm^3, the risk of developing certain OIs which are associated with more profound immunosuppression, becomes significantly higher [88-90]. At this advanced stage of HIV disease, patients may develop coexisting opportunistic infections which may disseminate, and which tend to relapse after initial successful therapy. Patients may also develop CNS (central

nervous system) complications such as PML (progressive multifocal leukoencephalopathy) and HIV-associated dementia. Another manifestation of advanced HIV disease is wasting syndrome, characterized by involuntary weight loss with no obvious underlying pathology. Continuation of antiretroviral therapy with primary and secondary prophylaxis play a major role in prolonging survival in this population [77].

4.3 Impact of HIV on drug metabolism

The impact of HIV disease on drug metabolism is two-fold: 1) disease progression in HIV can affect drug metabolism and toxicity as demonstrated by altered activity of the enzyme N-acetyltransferase II (NAT2) [91-93], resulting in increased adverse drug reactions; and 2) the approval of the new class of protease inhibitors in the treatment of HIV disease increases the potential of drug-drug interactions in clinical use.

Treatment for HIV disease and associated opportunistic infections has progressed substantially over the last ten years. Today clinicians have at their disposal an arsenal of antiretroviral agents including reverse transcriptase inhibitors (RTIs), nucleoside analogues, non-nucleoside reverse transcriptase inhibitors (NNRTIs) and more recently, the addition of a new class of drugs, the peptidomimetic protease inhibitors (PIs). As treatment for HIV infection moves towards combination therapy (*i.e.* two nucleoside RTIs + one PI) in the earlier stages of HIV infection compounded by the addition of prophylaxis in the later stages of the disease, drug-drug interactions are an inescapable complication of polypharmacy.

Drug-drug interactions have not been a major problem in the clinical setting due to the pharmacokinetic profiles of the nucleoside analogues. Systemic bioavailability of orally administered nucleoside analogues is generally high ranging from 60 - 90% [77]. Absorption of most nucleoside analogues, with the exception of ddI, is not affected by food [77]. Plasma protein binding is quite low and elimination of most nucleoside analogues, with the exception of zidovudine (AZT), is achieved through renal clearance. Hepatic glucuronidation is the primary pathway responsible for the metabolism of AZT, accounting for 75% of its elimination [77]. Lamivudine (3TC), which is phosphorylated, does not alter the metabolism of other nucleoside analogues [77].

The newer class of antiretrovirals, the PIs, have a different kinetic profile than the nucleoside analogues characterized by a relatively short half-life and high first pass metabolism; they are highly bound to plasma proteins and are extensively metabolized by the liver [94]. *In vitro* studies suggest that the CYP isoform that is the most important for the metabolism of PIs is CYP3A [94-96] with minor contributions of CYP2D6 and CYP2C9 [94]. In this new class of drugs three PIs have been approved for the treatment of HIV (saquinavir, ritonavir and indinavir). Saquinavir's kinetic profile is characterized by reduced absorption, high first pass metabolism and poor overall bioavailability [94]. Ritonavir is highly bound to plasma proteins (> 98%) and binds with a high affinity to CYP3A. As well, ritonavir may displace other PIs from the plasma proteins and inhibit their metabolism. Renal elimination is not significant, with less than 5% of ritonavir and saquinavir excreted as unchanged drug [94]. Indinavir is less tightly bound to plasma proteins

(approximately 60%) and less than 20% is excreted in urine as unchanged form. While drug-drug interactions did not appear to be a concern when treatment of HIV was limited to the use of nucleoside analogues, the approval and use of PIs has changed this aspect dramatically.

As combination drug regimens proliferate, the HIV-infected patient is likely to take multiple prolonged drug regimens resulting in potentially important drug interactions. While CYP2D6 represents a secondary pathway in the metabolism of PIs and the primary pathway in the metabolism of other drugs commonly used in the clinical setting (*i.e.* fluoxetine, codeine) , the measurement of its activity can provide an index of overall metabolic capacity in a patient population whose metabolism is potentially compromised.

CHAPTER II: OBJECTIVE AND RATIONALE

1. Objective

The objective of this study is to determine if disease progression in HIV positive (HIV+) patients affects the expressed activity of the cytochrome P450 (CYP) enzyme CYP2D6.

2. Rationale for Using CYP2D6 as the Marker Enzyme

The effect of disease progression on the activity of CYPs in HIV+ patients is unknown. This study aims to document whether the progression of the disease in HIV+ patients affects CYP2D6 expression. CYP2D6 was chosen as a marker for the following reasons:

1) The genetics of CYP2D6 have been extensively studied, and individual genotypes can be rapidly determined [9, 97]. Humans can be divided into EM and PM genotypes with a population-based distribution which can range from only 7 PMs out of 695 Chinese subjects (1.01%)[10] to a PM frequency of 7% in Caucasian populations [10-12].

2) The activity of expressed CYP2D6 can be measured by determining the extent of the O-demethylation of the probe drug dextromethorphan (DM). CYP2D6 probe drug phenotypes correlate with the genotype [61, 98, 99, 100], and large population studies and clinical protocols [10, 30, 101] have used DM metabolism as the determinant of PM and EM CYP2D6 activity.

3) The metabolic phenotypes determined using DM metabolism have been shown to be reproducible over time in healthy populations: *i.e.* PMs remained PMs and EMs remained EMs [100]. DM is metabolized by two pathways: CYP2D6 mediated O-demethylation and CYP3A mediated N-demethylation. The coadministration of CYP3A inhibitors with DM does not appear to modify the O-demethylation [100], nor affect the determined genotype of CYP2D6, *i.e.* CYP2D6 EMs do not convert to PMs through CYP3A inhibition. This suggests that the two pathways are independent.

4) CYP2D6 mediates the biotransformation of several classes of drugs that are of major therapeutic value including cardiovascular drugs [9, 60], psychotropic agents [102-104], codeine [26], fluoxetine [98, 105] and ritonavir [106]. In addition, individuals who have a *CYP2D6* PM genotype (or phenotype) are at increased risk for developing drug-related toxicities when drugs metabolized by this pathway are administered [107]. Thus, a disease induced shift from an EM to a PM phenotype could produce previously unobserved toxicities.

CHAPTER III: EXPERIMENTAL DESIGN

1. STUDY DESIGN

This is a cross-sectional, descriptive study in which individual genotypes for CYP2D6 were compared to the expressed CYP2D6 phenotype in a predominantly Caucasian and male cohort of 42 HIV+ patients.

1.1 Study Site

All subjects recruited into the study are actively followed at the Montreal General Hospital (MGH) Immunodeficiency Treatment Centre (IDTC). A total of one-hundred and twenty-five (125) HIV+ patients were recruited during routine clinic visits to undergo various metabolic phenotyping studies including glucuronidation, sulphation, and N-acetyltransferase II (NAT2). Regulatory approval from the MGH Ethics Committee had been obtained prior to the start of the study.

1.2 Patient Selection

A subgroup of forty-two (42) patients underwent genotyping and phenotyping studies for cytochrome P450 isoform, CYP2D6. This subgroup was comprised of patients at different stages of the disease, ranging from asymptomatic HIV infection to advanced disease progression to AIDS. Written informed consent (Appendix III) was obtained from each subject prior to the initiation of the phenotyping/genotyping studies. Clinical data including a detailed HIV history,

concomitant medications, past illnesses and current clinical status was obtained at time of enrollment (Appendix V). The phenotyping protocol allowed for the continuation of current drug therapy.

2. Protocol Development

2.1 Metabolic Phenotyping

This approach utilizes the identification and quantification of specific metabolite patterns produced by test compounds or "probe-drugs". Dextromethorphan is a probe drug used in previous studies with healthy volunteers, to assess the enzymatic activity of CYP2D6 and CYP3A4. Dextromethorphan (DM) is O-demethylated into dextrorphan (DT) via CYP2D6 and N-demethylated into 3-methoxymorphinan (3MM) via CYP3As (Figure 2). The identification and quantification of dextromethorphan and its metabolite dextrorphan provide a relative measure of CYP2D6 hepatic enzyme activity, while the N-demethylated metabolite 3MM reflects CYP3A4 enzyme activity in the liver and the gut [100].

Dextromethorphan was chosen as the probe drug for the phenotyping studies because it is safe, inocuous and readily available. The phenotyping protocol itself is simple, user-friendly and non-invasive.

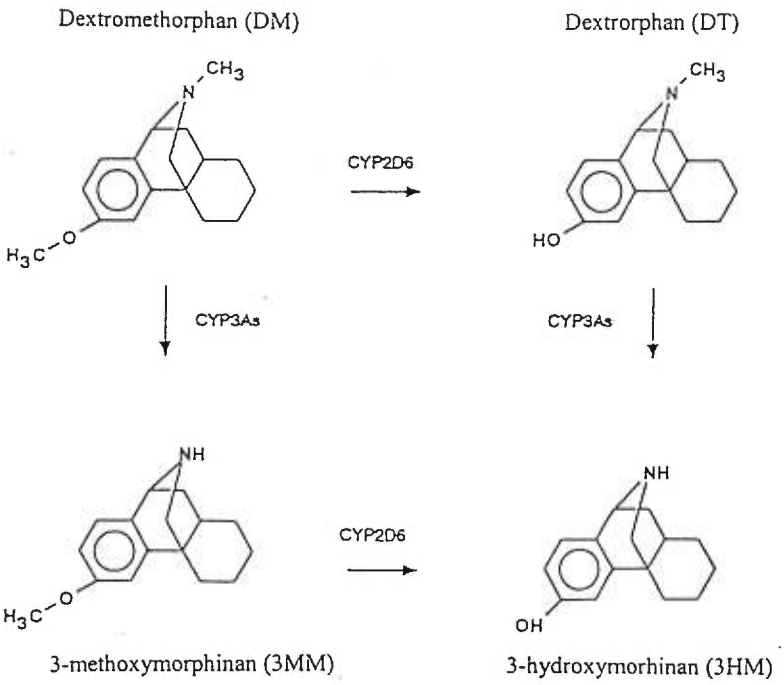


Figure 2: Chemical structures of DM and of its *O*- and *N*- demethylated metabolites

2.2 Probe-Drug Phenotyping Protocol

Each of the forty-two participants received a single oral dose of 30 mg of dextromethorphan (10 mL of Robitussin DM®, donated by Whitehall-Robins Inc., Mississauga, ON, Canada). A kit containing 10 mL of Robitussin DM® and two urine specimen containers properly labeled were given to the participants along with instructions for dosing and storing of samples. Prior to the ingestion of the probe-drug, a pre-dose blank urine (20-40 mL) sample was obtained. Following the ingestion of dextromethorphan, a 4-hour spot urine (20-40 mL) was obtained. The urine was frozen at -20 °C until the samples were returned to the Pharmacokinetics/

Pharmacogenetics laboratory at the Montreal General Hospital. Once the samples were returned to the laboratory, they were thawed and pipetted into four polypyrene tubes (4 mL), each containing an aliquot of 3 mL of urine. The samples were heat-treated at 60 °C for 60 min providing for the inactivation of any virus and then stored at -20 °C until analysis. The recovery of DM and DT was not affected by the heat treatment; the recovery of the analytes being equivalent in both the heated and unheated urine samples. The subject was also given a questionnaire to complete as part of the phenotyping protocol (Appendix IV).

3. Experimental Method for Phenotyping

3.1 4-Hour Spot Urine Procedure

The objective of this study was to design a protocol which could be used in a population in which it was anticipated that there would be a large inter-subject variability in urinary output. Thus a spot urine sample could not be used to compare absolute urinary excretion of the analytes, DM and DT. However, urinary molar ratios of the parent compound (DM) over the metabolite (DT) was deemed to be a reliable index when comparing inter-subject variability.

Previous studies determining urinary molar ratios of DM/DT to assess CYP2D6 phenotype have utilized 0-8 h urinary samples [100, 101]. In the study conducted by Ducharme *et al.* [100] four healthy subjects were phenotyped with a single oral dose of 30 mg of DM. Urine samples were collected at 0 h and at 2, 4, 6,

8 h post dosing. The urinary molar ratios of DM/DT were determined for each sample and for the 0-8 h period. The results indicated that the DM/DT molar ratios in the 4-hour urine sample were in agreement with those determined for the 0-8 h period. In further studies by Ducharme *et al.* [100], the 4-hour post dosing urine was used to assess inter-day variations in eight healthy volunteers. The results showed no significant changes in DM/DT molar ratios over a 50 and 90-day period. All subjects were classified as extensive CYP2D6 metabolizers and retained their classification over time. Hence, the utilization of a 4-hour spot urine to determine DM/DT molar ratios is a reliable measurement of CYP2D6 activity, is reproducible over time and is used as a parameter in the phenotyping protocol of this study.

Pre-dose and 4-hour post-dose urine samples were collected as per protocol and frozen at -20° C until analysis.

The urinary molar concentrations of dextromethorphan (DM) and its O-demethylated metabolite, dextrorphan (DT), were determined using a reverse-phase high performance liquid chromatography (HPLC) method described by Hou *et al.* [107] and modified by the use of a Chem Elut solid-phase extraction cartridge.

3.2 Materials

Dextromethorphan was purchased from Sigma Chemical Company (St. Louis, MO, USA) and dextrophan was purchased from Hoffman-LaRoche (Mississauga, ON, Canada). The internal standard levallorphan (Lev) was obtained

from the USP Reference Standard. Chem-Elut extraction columns purchased from Varian Canada Ltd. (Mississauga, ON, Canada) were used for the solid-phase extraction.

3.2.1 Standard Stock Solutions

Stock solutions of 1 mg/mL of DM and DT were prepared by dissolving the standards in 0.01 N HCl. These stock solutions were serially diluted with 0.01 N HCl to give standard concentrations of 0.0, 10, 50, 75 100 and 250 µg/mL. Quality controls for DM and DT were prepared from the stock solutions to give standard concentrations of 0.0, 7.5, 30 and 100 µg/mL. A 1 mg/mL stock solution of Lev was prepared. One ml of this stock was mixed with 9.0 mL of H_2O to give a 100 µg/mL internal standard stock solution. This was further diluted with the sample to give a final concentration of 0.5 µg/mL of Lev.

3.2.2 Standard curves and quality controls

Calibration curves were prepared in triplicate by adding 100 µL of each stock solution to 9800 µL of saline to give final concentrations over the tested range of 0.10 - 1.00 µg/mL for DM and 0.10 - 2.50 µg/mL for DT. Quality controls (QC) for DM and DT were prepared in triplicate by adding 100 µL of each stock solution to 9800 µl of saline to give final concentrations of 0.0, 0.075, 0.30 and 1.0 µg/mL.

3.2.3 Sample Extraction

DM and DT are secondary amines and at physiologic pH, they exist as

hydrochloride salts which are not soluble in organic solvents. The urine is alkalinized in order to generate the respective free bases and is passed over a Chem Elut column which adsorbs the sample as a thin aqueous film on a hydrophilic support. Butanol/hexane is then added to the column which extracts the DM and DT free bases from the aqueous film. The compounds are back-extracted into 0.1 N HCl which forces the formation of the hydrochloride salts and favors solubility in the aqueous layer.

This solid-phase extraction was accomplished as follows: after thawing the urine samples, 10.0 mL of each sample was pipetted into duplicate, labeled, snap-cap centrifuge tubes. To these, 250 μL of 5 N NaOH and 50 μL of Lev were added. The centrifuge tubes were capped and vortex-mixed. Following this, the samples were decanted into Chem Elut extraction columns, placed in a Baker extraction block. The samples were allowed to elute for 5 min. An aliquot of 10 mL butanol/hexane (10:90, v/v) was then added and eluted for 3 min. Following this, a second aliquot of 5 mL of butanol/hexane (10:90, v/v) was added. When the eluent stopped flowing, vacuum was applied to the block. A 300 μL aliquot of 0.1 N HCl was added to the samples. The collection tubes were capped and the samples placed in an Eberbach mechanical shaker for 20 min. The samples were centrifuged at 3000 rpm in a Beckman J-6M centrifuge with a JS rotor for 5 min. The organic layer was discarded and a 50 μL sample was injected onto the HPLC system.

3.3 Separation Chromatography

3.3.1 HPLC System

The reverse phase chromatographic system was composed of a Waters Associates pump Model 510 and a Waters Associates autosampler Model 712B equipped with a 50 μL sample loop and a Spectrovision flourescence detector Model FD300. A Waters Associates Millennium integrator, version 2.0 connected to a 486 DX33 computer using the software Millennium Chromatography Manager 2010, version 2.0 was used for electronic data collection. DM and DT were separated by HPLC with the use of DuPont Zorbax SB-Phenyl column (150 cm X 4.6 mm I.D.).

3.3.2 HPLC Conditions

The mobile phase was composed of 100 mM KH_2PO_4 buffer (pH 4.0), acetonitrile, methanol [57:23:20, v/v/v], modified with 0.1% triethylamine. The chromatography was carried out using a solvent flow rate of 0.5 mL/min at ambient temperature. The detection of the compounds of interest was done by fluorescence set at an excitation wavelength of 200 nm and emission wavelength of 304 nm. Under these chromatographic conditions, dextrophan was eluted at 4.7 min, levallorphan was eluted at 6.3 min and dextromethorphan was eluted at 9.1 min.

3.4 Assay Validation

3.4.1 Accuracy and Intra-Day Precision

Standard calibration curves were run in triplicate over three days to determine

accuracy. Response was determined and used to calculate the slope and intercept from each set of calibration standards. These regression parameters were used to determine back calculated concentrations with % bias (see Table I). For concentrations above 0.1 μg/mL the back calculated value was within 15% bias of the expected concentration. Due to variability in extraction efficiency (E.E.) near to the lower limit of quantification (0.1 μg/mL), the expected back calculated concentrations were 87% bias of the expected.

The intra-day variability was determined by calculating the coefficient of variation (C.V.) between triplicates. The intra-day variability for DM was less than 10.50%, and for DT less than 11.50% (see Table II).

3.4.2 Reproducibility and Inter-Day Precision

The reproducibility and inter-day precision was determined by the average response for each standard on separate days and by the determination of the C.V. from these data. The C.V. was less than 7.50% for both DM and DT (see Figure 3).

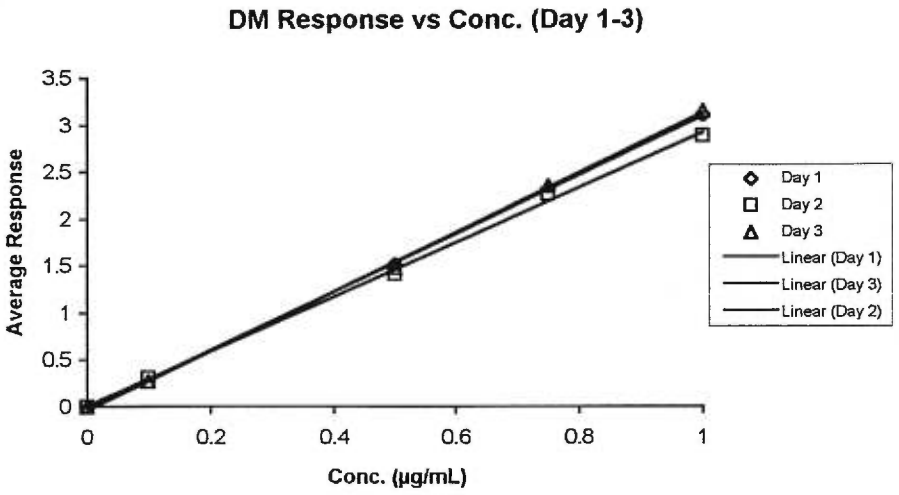
3.4.3. Absolute Recovery

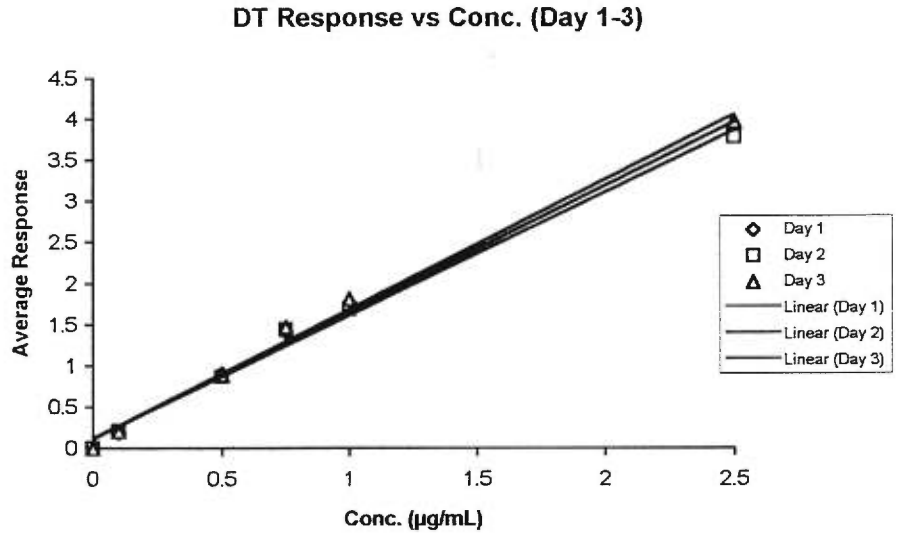
The absolute recovery was determined by comparing the area measured from the extracted samples to the area of direct injections to determine the extraction efficiency of each sample. The overall extraction efficiency for DM was 74.15% and 82.83% for DT (see Table III).

3.4.4 Linearity

A linear correlation response was found over a range of 0.10 -1.0 μg/mL for DM and 0.10 - 2.5 μg/mL for DT. The correlation coefficient (r) ranged from 0.9987

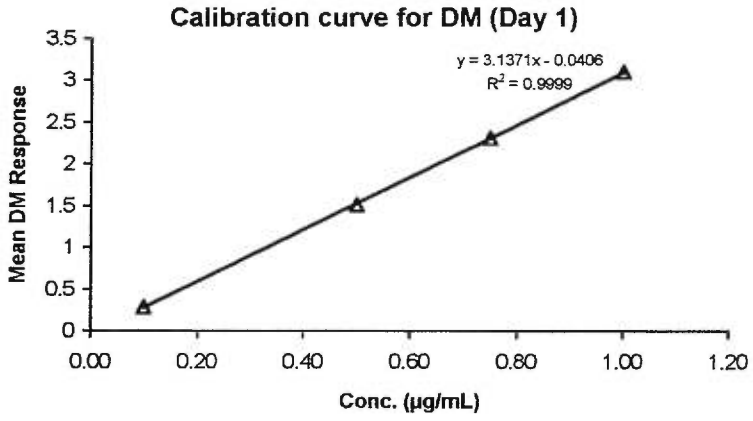
to 1.0000 for DM and from 0.9967 to 0.9971 for DT. The regression coefficients (r^2) ranged from 0.9975 to 0.9999 for DM and from 0.9925 to 0.9942 for DT (see Figure 4-5).

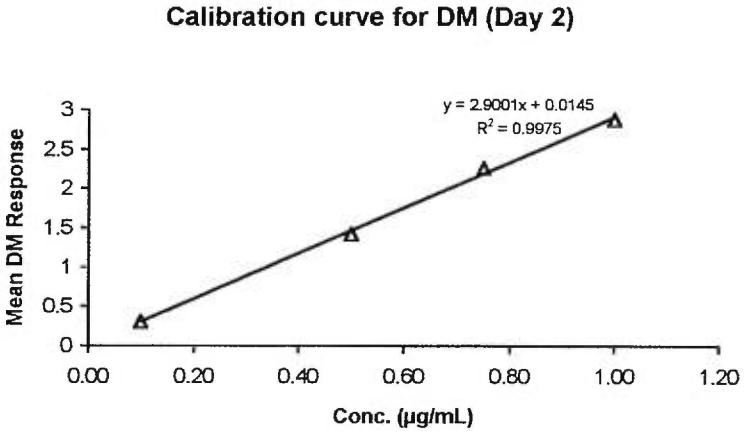




Conc. (µg/mL)	0.1	0.5	0.75	1	2.5
% C.V. (DM)	7.73%	3.44%	1.85%	4.70%	N/A
5 C.V. (DT)	6.63%	2.36%	1.75%	3.57%	2.38%

Figure 3: Response *vs* Concentrations for DM and DT on Validation Days 1 ,2, 3





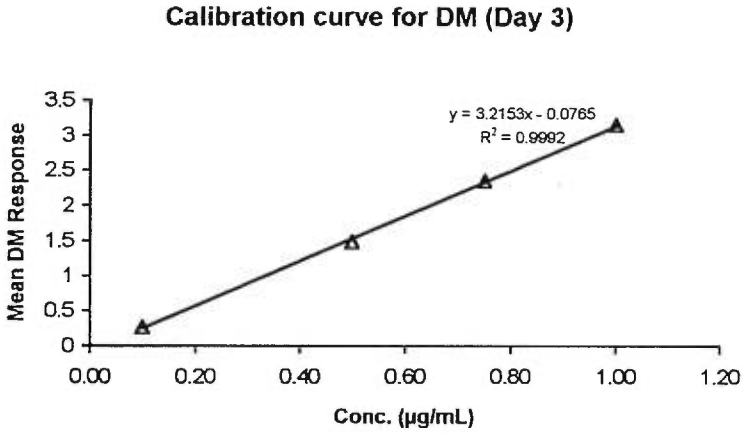
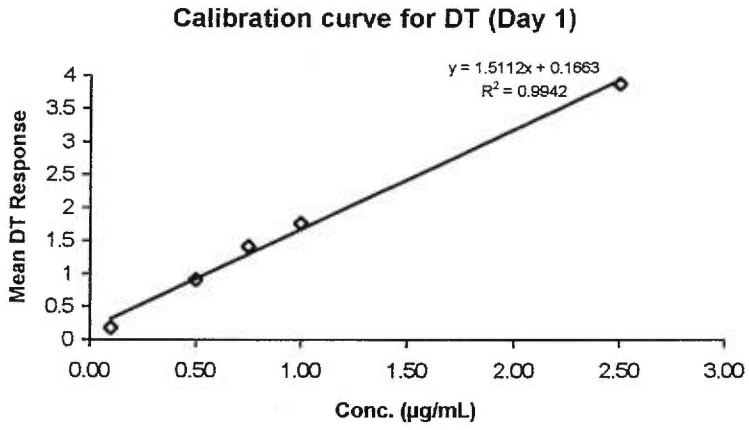
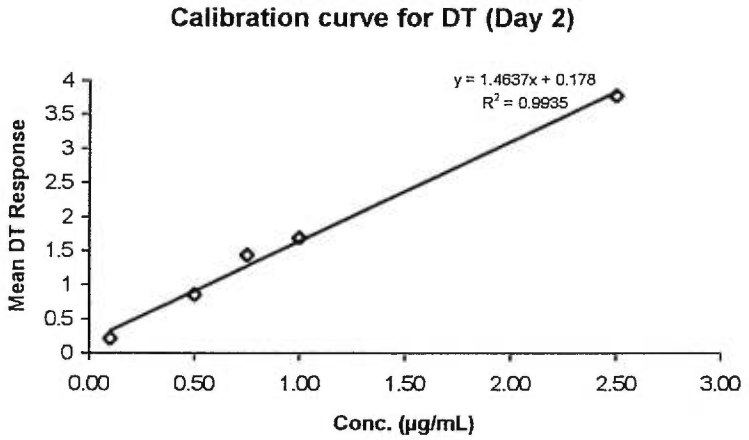


Figure 4: Calibration Curves for DM on Validation Days 1, 2, 3





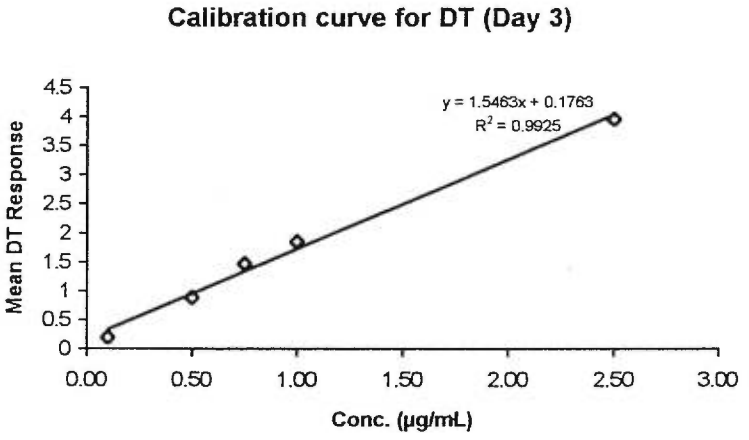


Figure 5: Calibration Curves for DT on Validation Days 1, 2, 3

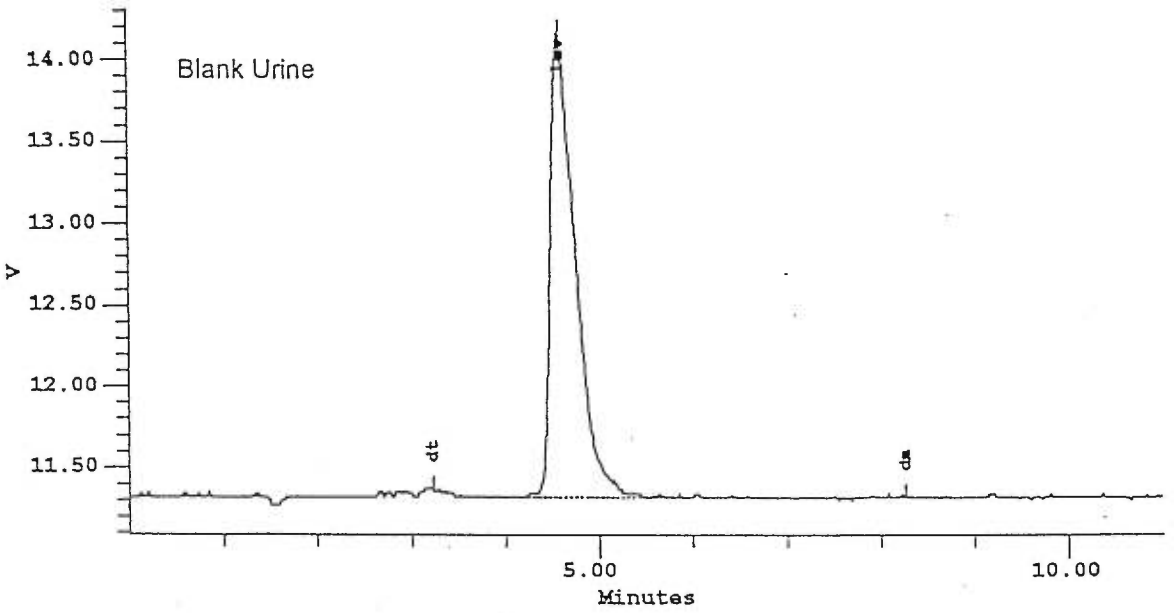


Figure 6a: Sample chromatogram of a blank urine

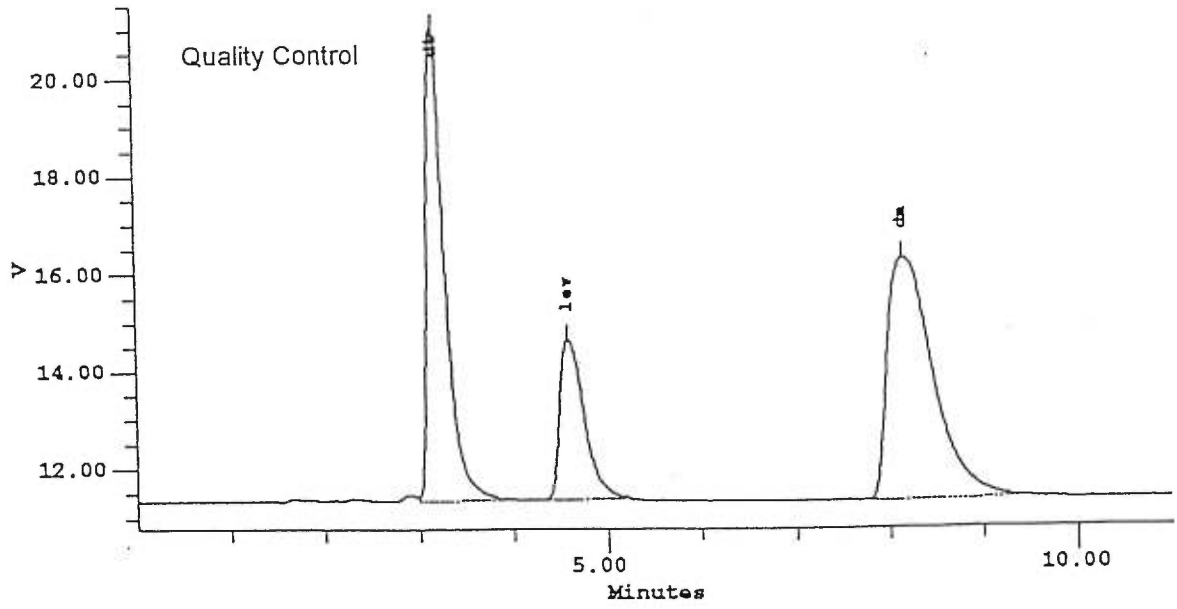


Figure 6b: Sample chromatogram of a spiked urine (QC)

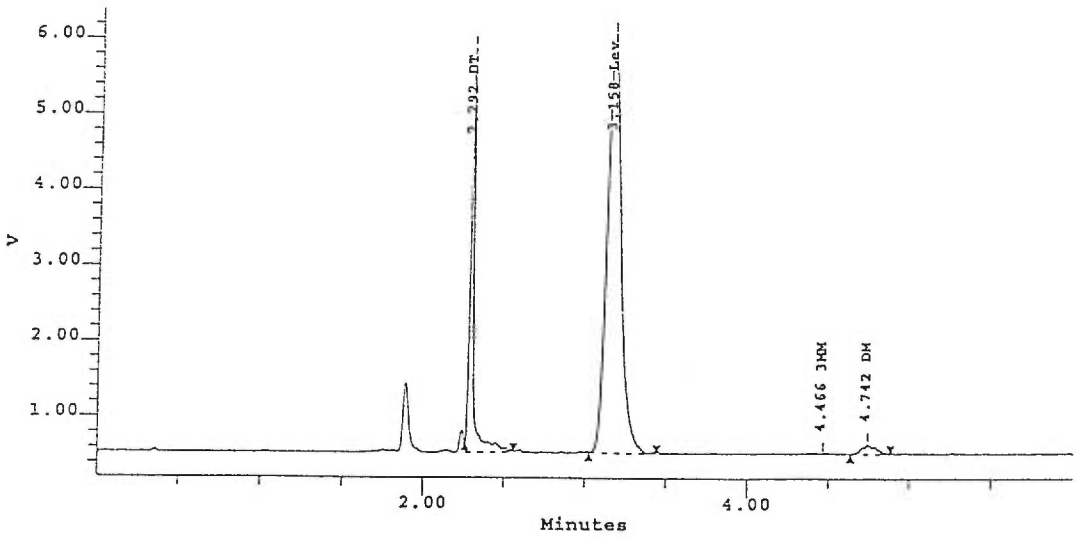


Figure 7a: Sample chromatogram of a patient sample which represents an "extensive" metabolizer (EM)

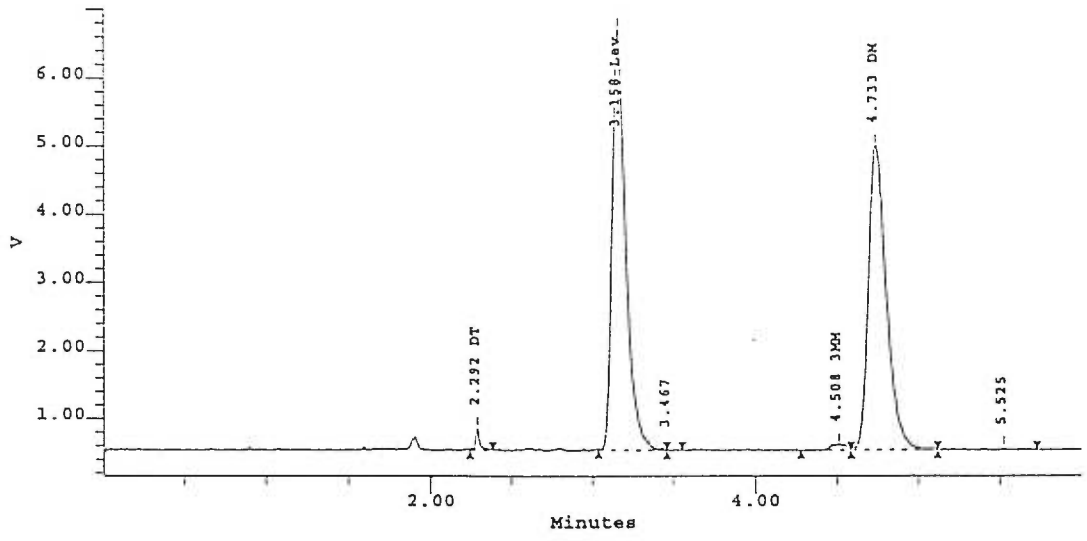


Figure 7b: Sample chromatogram of a patient sample which represents a "poor" metabolizer (PM)

Table I: Accuracy of Dextromethorphan and Dextrorphan Extraction

Back calculated concentration of extracted samples with % Bias Day 1 (n=3)			
Compound	Theoretical Conc.	Back Calculated Conc. **(Mean ± S.D.)**	% Bias*
DM	0.075 µg/mL **(QC 1)**	0.091 ± 0.008	21.90
	0.300 µg/mL **(QC 2)**	0.313 ± 0.009	4.41
	1.000 µg/mL **(QC 3)**	1.001 ± 0.071	0.06
	0.100 µg/mL	0.102 ± 0.004	2.23
	0.500 µg/mL	0.496 ± 0.059	0.79
	0.750 µg/mL	0.750 ± 0.130	0.01
	1.000 µg/mL	1.002 ± 0.126	0.18
DT	0.075 µg/mL **(QC 1)**	0.026 ± 0.023	65.56
	0.300 µg/mL **(QC 2)**	0.315 ± 0.043	4.86
	1.000 µg/mL **(QC 3)**	1.229 ± 0.054	22.93
	0.100 µg/mL	0.013 ± 0.004	86.94
	0.500 µg/mL	0.489 ± 0.040	2.22
	0.750 µg/mL	0.830 ± 0.047	10.68
	1.000 µg/mL	1.060 ± 0.161	6.04
	2.500 µg/mL	2.46 ± 0.221	1.70

* % Bias = [(Calculated - Theoretical) / Theoretical] x 100

Table I: *continued*

Back calculated concentration of extracted samples with % Bias Day 2 (n=3)			
Compound	Theoretical Conc.	Back Calculated Conc. **(Mean ± S.D.)**	% Bias*
DM	0.075 µg/mL **(QC 1)**	0.080 ± 0.007	3.48
	0.300 µg/mL **(QC 2)**	0.840 ± 0.073	5.26
	1.000 µg/mL **(QC 3)**	1.000 ± 0.296	0.12
	0.100 µg/mL	0.102 ± 0.018	2.25
	0.500 µg/mL	0.483 ± 0.056	3.43
	0.750 µg/mL	0.776 ± 0.063	3.49
	1.000 µg/mL	0.989 ± 0.058	1.13
DT	0.075 µg/mL **(QC 1)**	0.000 ± 0.007	93.51
	0.300 µg/mL **(QC 2)**	0.385 ± 0.028	28.45
	1.000 µg/mL **(QC 3)**	1.490 ± 0.105	48.52
	0.100 µg/mL	0.234 ± 0.012	76.58
	0.500 µg/mL	0.469 ± 0.026	6.28
	0.750 µg/mL	0.862 ± 0.030	14.94
	1.000 µg/mL	1.034 ± 0.112	3.37
	2.500 µg/mL	2.460 ± 0.045	1.51

* % Bias = [(Calculated - Theoretical) / Theoretical] x 100

Table I: *continued*

Back calculated concentration of extracted samples with % Bias Day 3 (n=3)			
Compound	Theoretical Conc.	Back Calculated Conc. **(Mean ± S.D.)**	% Bias*
DM	0.075 µg/mL **(QC 1)**	0.100 ± 0.014	30.42
	0.300 µg/mL **(QC 2)**	0.307 ± 0.041	2.18
	1.000 µg/mL **(QC 3)**	0.990 ± 0.106	0.63
	0.100 µg/mL	0.107 ± 0.006	7.45
	0.500 µg/mL	0.484 ± 0.113	3.18
	0.750 µg/mL	0.755 ± 0.097	0.66
	1.000 µg/mL	1.003 ± 0.131	0.35
DT	0.075 µg/mL **(QC 1)**	0.080 ± 0.018	0.87
	0.300 µg/mL **(QC 2)**	0.436 ± 0.063	45.31
	1.000 µg/mL **(QC 3)**	1.630 ± 0.161	62.82
	0.100 µg/mL	0.021 ± 0.020	79.40
	0.500 µg/mL	0.467 ± 0.026	6.68
	0.750 µg/mL	0.842 ± 0.007	12.22
	1.000 µg/mL	1.065 ± 0.095	6.47
	2.500 µg/mL	2.460 ± 0.148	1.74

* % Bias = [(Calculated - Theoretical) / Theoretical] x 100

Table II: Intra- and Inter-Day Variability of DM and DT Extraction

Compound	Theoretical Conc.	C.V. (%) Day 1	C.V. (%) Day 2	C.V. (%) Day 3
DM	0.075 µg/mL (QC 1)	3.27	3.02	5.91
	0.300 µg/mL (QC 2)	0.94	8.68	4.56
	1.000 µg/mL (QC 3)	2.28	10.17	3.39
	0.100 µg/mL	1.42	5.91	2.23
	0.500 µg/mL	3.90	3.95	7.62
	0.750 µg/mL	5.64	2.80	4.13
	1.000 µg/mL	4.07	2.00	4.17
DT	0.075 µg/mL (QC 1)	11.05	4.03	6.11
	0.300 µg/mL (QC 2)	6.69	3.74	7.42
	1.000 µg/mL (QC 3)	2.67	4.46	6.00
	0.100 µg/mL	2.26	5.47	9.98
	0.500µg/mL	4.47	3.05	2.90
	0.750 µg/mL	3.29	2.07	0.44
	1.000 µg/mL	9.11	6.63	5.22
	2.500 µg/mL	5.69	1.19	3.73

C.V. = (Standard Deviation / Mean) x 100

Table III: Absolute Urinary Recovery for DM and DT

Compound	Theoretical Conc.	% Recovery Day 1	% Recovery Day 2	% Recovery Day 3
DM	0.10 µg/mL	74.74	83.75	72.51
	0.50 µg/mL	64.15	59.93	62.70
	0.75 µg/mL	85.06	83.35	86.50
	1.00 µg/mL	73.74	68.48	74.86
Average		74.42	73.88	74.14
DT	0.10 µg/mL	97.72	104.72	99.48
	0.50 µg/mL	71.17	67.93	70.06
	0.75 µg/mL	91.87	93.11	95.10
	1.00 µg/mL	76.77	73.41	78.81
	2.50 µg/mL	76.16	74.24	77.86
Average		81.54	82.68	84.26

4. Experimental Method for Genotyping

4.1 *CYP2D6* Genotyping Studies:

During a routine clinic visit, blood was drawn from each patient participating in the study, for determination of *CYP2D6* genotype. A single 7 mL venous blood sample was collected into a tube containing ethylenediaminetetraacetic acid (EDTA) and stored at -20 °C until analysis.

4.2 Materials

All restriction buffers, bovine serum albumin (BSA), and restriction enzymes were from New England Biolabs Ltd (Mississauga, ON, Canada). All primers were synthesized by Sheldon Biotechnology Centre (Montreal, Qc, Canada).

4.3 DNA Isolation

DNA was isolated from whole blood using a commercial kit (Puregene DNA Isolation kit, Gentra Systems, Minneapolis, MN, USA). Reagents supplied in the kit include: RBC Lysis Solution, Cell Lysis Solution, Protein Precipitation Solution, DNA Hydration Solution and RNase A Solution.

Three-hundred (300) µL of whole blood was added to 900 µL of RBC Lysis Solution in a microfuge tube. The additive and the solution were mixed by inversion and incubated at room temperature for 10 minutes, inverting again during the incubation period. The mixture was then centrifuged for 20 seconds at 13 000-16 000 g. The supernant was drawn up with a micropipette, and the visible white cell pellet was left behind along with 10-20 µL of residual liquid. The microfuge tube was vortexed vigorously to resuspend the white cells in the residual supernatant.

Following this, 300 µL of the Cell Lysis Solution was added to the microfuge tube,

pipetting in an upward and downward fashion in order to lyse the cells. The cell lysate was incubated at 60 °C for 1 h. The introduction of a heating step (60 °C for 1 h) during the cell lysis stage provides for the inactivation of any virus. The next step was the addition of 1.5 μL of RNase A Solution to the cell lysate. This was then mixed by inversion 25 times and incubated at 37 °C for 15 min. The sample was allowed to cool to room temperature and 100 μL of Protein Precipitation Solution was added. The sample was vortex-mixed vigorously at high speed for 20 sec and then centrifuged at 13 000-16 000 g for 3 min. Following centrifugation, the supernatant was transferred to a clean 1.5 mL tube containing 300 μL of isopropanol. The tube was inverted gently 50 times to mix. The tube was centrifuged again at 13 000-16 000 g for 1 min to allow for pellet formation of the DNA. The supernatant was removed and the tube was drained on clean absorbent paper. An aliquot of 300 μL of 70% ethanol was added to the tube. The tube was inverted several times to wash the DNA pellet. Following this step, the sample was centrifuged at 13 000-16 000 g for 1 min. The ethanol was removed and discarded. The tube was drained on clean absorbent paper and the sample allowed to air dry for 15 min. To this sample 100 μL of DNA Hydration Solution was added and the DNA was allowed to rehydrate overnight at room temperature. The sample was stored at 2-8 °C until analysis. The DNA isolated from 1 x 10^6 lymphocytes or 300 μl was dissolved in 100 μl of the supplied DNA resuspension buffer.

The identification of the *CYP2D6(A)* (del A2637; *CYP2D6*3*) and *CYP2D6(B)* (G1934A; *CYP2D6*4*) mutations was performed by restriction genotyping using a modification of the method of Douglas *et al.* [109]. Reactions

were carried out in a volume of 50 μL containing 5 μL (~0.3 mg) of DNA; deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxythymidine triphosphate and deoxyguanosine triphosphate, each at 0.2 mmol/L; 12.5 pmol of primer 2D6-1827 (5' CGCCTTCGCCAACCACTCCG 3'); 12.5 pmol of primer 2D6-2662 (5' GGCTGGGCTGGGTCCCAGGTCATAC 3'); and 5 mL of 10 x PCR reaction buffer (100 mmol/L Tris HCl, pH 9.0, 500 mmol/L KCl, 15 mmol/L $MgCl_2$, 2 mg/mL gelatin, 1% Triton X100) (Vector Biosystems, Toronto, ON, Canada).

The DNA in the reaction mixture was first denatured for 10 min at 96 °C and then cooled to 4° C. One unit of *Taq* polymerase (Vector Biosystems) was then added to each sample. Each sample was reheated for 2 min at 96 °C and then subjected to 30 cycles in a thermal cycler with each cycle consisting of a 10 sec denaturation at 96 °C, 30 sec annealing at 60 °, and 1 min extension at 65 °C.

To identify the *CYP2D6(A)* mutation, 10 μL of the amplified material was mixed with 5 μL of the following: 1.5 μL of NEBuffer R; 0.2 μL 10 mg/mL BSA; 0.33 μL of *Tai* I (6 units/mL); and 2.97 mL of water. The restriction mixture was overlaid with mineral oil and incubated overnight at 65 °C.

To identify the *CYP2D6(B)* mutation, 10 μL of the amplified material was mixed with 5 μL of the following: 1.5 μL of NEBuffer 2; 0.2 μL 10 mg/mL BSA; 1 μL of *Bst*N I (10 units/mL); and 2.3 mL of water. The restriction mixture was overlaid with mineral oil and incubated overnight at 60 °C.

The identification of the *CYP2D6(D)* (CYP2D6 deleted; *CYP2D6*5*) was performed by a "long PCR" technique using the Expand™ Long Template PCR System (Boehringer Mannheim Canada, Laval, Qc, Canada) [110]. Reactions were

carried out in a volume of 50 μL containing 5 μL (~0.3 mg) of DNA; deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxythymidine triphosphate and deoxyguanosine triphosphate, each at 0.2 mmol/L; 12.5 pmol of primer CYP-13 (5' ACCGGGCACCTGTACTCCTCA 3'); 12.5 pmol of primer CYP-24 (5' GCATGAGCTAAGGCACCCAGAC 3'); 5 μL of 10 x Expand™ buffer 1; and 0.75 μL (2.5 units) of Expand™ Long Template enzyme mixture.

Each sample was heated for 1 min at 93 °C and then subjected to 30 cycles in a thermal cycler with each cycle consisting of a 1 min denaturation at 93 °C, 2 min annealing at 65 °C, and 10 min extension at 68 °C. The heating and cooling rates for all steps were 1°C/sec except for cooling from 93 °C to 65 °C where the rate was 0.5°/sec.

4.4 Gel Electrophoresis

The restriction products were analysed by electrophoresis of 10 μL of the reaction mixture on a 10% polyacrylamide gel containing TBE buffer (89 mmol/L Tris base, 89 mmol/L boric acid, 2 mmol/L EDTA) for 2.3 h at 70 V. The gel was then stained for 45 min in 0.5 μg/mL ethidium bromide, photographed under UV light, and the banding profile identified on the basis of molecular weight standards.

The products from the "long PCR" reaction were analysed by electrophoresis of 10 μL of the reaction mixture on a 0.8% agarose gel containing TPE buffer (80 mmol/L Tris-phosphate, 2 mmol/L EDTA) and 0.156 μg/ml ethidium bromide for 1 h at 68 V. The gel was then photographed under UV light and the banding profile identified on the basis of molecular weight standards.

CHAPTER IV: RESULTS

1. Patient Characteristics:

The age of the 42 unrelated, HIV+ patients ranged from 26-65 (median, 41 years). Thirty-seven (37) of the 42 patients (88%) were male and 5 (12%) were females. Ninety percent of the study population was Caucasian, 5% was African-American and the remaining 5% accounted for other ethnic origin. The CD4 count of the patients ranged from 2 cells/mm^3 to 900 cells/mm^3 (average CD4 count of 216 cells/mm^3), 14 (33%) had AIDS and 10 (24%) had active AIDS-defining opportunistic infections at the time of testing (see Table IV). Renal profiles were within normal range for all patients. Liver enzymes were either normal or within a grade 1 elevation for all patients except one patient who had a hyperbilirubinemia four times the upper limit of normal. Alkaline phosphatase and liver transaminase for this patient were within normal limits.

2. Genotypes:

Forty patients (95%) possessed an extensive *CYP2D6* metabolizer {*EM*} genotype, while two patients (5%) possessed a poor *CYP2D6* metabolizer {*PM*} genotype (see Table IV). These results correlated with previously observed data in a predominantly male Caucasian population [61, 99, 100].

The use of restriction genotyping [109] and "long PCR" [110] allowed the

identification of the following three PM-associated alleles with their respective frequencies: *CYP2D6(A)* (0.047), *CYP2D6(B)* (0.33) and *CYP2D6(D)* (0.047). The estimated allele frequency in the Caucasian population reported in the literature is as follows: *CYP2D6(A)* (0.020), *CYP2D6(B)* (0.207) and *CYP2D6(D)* (0.0195) [18]. Other PM-associated alleles are known, but are relatively infrequent in the population, for example, *CYP2D6*6* (0.0093) and *CYP2D6*15* (0.0008) [18, 111]. Genotyping alone for the *CYP2D6(A)*, *CYP2D6(B)* and *CYP2D6(D)* mutations allows for the identification of 95% of the PM-associated alleles, 88% of genotypes where both alleles are of the slow type, and 98% of genotypes where one allele is *EM* type and the other *PM* type based on a recent study [18]. Thus, as the PM-associated alleles act recessively, genotyping for the 3 mutations mentioned above allows for the correct classification in the large majority of cases into extensive metabolizer (*EM*) and poor metabolizer (*PM*) genotypes.

Of the two patients classified as PMs, one was homozygous for the *CYP2D6(B)* mutation, while the other carried both the *CYP2D6(B)* and *CYP2D6(D)* mutation , Table IV. Of the forty patients classified as EMs, 60% were homozygotes for the wild-type allele, 32 % were heterozygotes for the *CYP2D6(B)* mutation and 5% were heterozygotes for the *CYP2D6(A)* mutation, Table IV. The most frequently observed mutant allele was the *CYP2D6(B)* mutation, present in both phenotypes. These results are in agreement with previously reported values for Caucasian populations [112-114].

Table IV: Characteristics of 42 HIV+ patients genotyped and phenotyped for CYP2D6

PATIENT NO	GENOTYPE[1]	PREDICTED PHENOTYPE	[DM]/[DT] RATIO	OBSERVED PHENOTYPE	AGE	ETHNIC ORIGIN	GENDER
1	*AABB*	EM	0.06	EM	30	W	F
2	*AABB*	EM	1.8	PM	42	W	M
3	*AABB*	EM	0.15	EM	39	W	M
4	*AABB*	EM	3.8	PM	41	AA	M
5	*AABb*	EM	0.72	PM	35	W	F
6	*AABb*	EM	3.03	PM	32	W	M
7	*AABb*	EM	0.36	PM	46	W	M
8	*AABb*	EM	0.05	EM	36	W	M
9	*AABb*	EM	0.77	PM	59	W	M
10	*AABB*	EM	0.15	EM	32	W	M
11	*AABB*	EM	0.75	PM	32	W	F
12	*AABb*	EM	7.25	PM	43	W	M
13	*AABB*	EM	0.47	PM	40	AA	M
14	*AABB*	EM	BLQ (DM)	EM	40	W	M
15	*AABB*	EM	0.13	EM	33	W	M
16	*AABB*	EM	BLQ (DM)	EM	47	W	M
17	*AABb*	EM	1.21	PM	44	W	M
18	*AABb*	EM	1.17	PM	45	W	M
19	*AABb*	EM	BLQ (DM)	EM	54	W	M
20	*AABB*	EM	BLQ (DM)	EM	26	W	M
21	*AaBB*	EM	2.92	PM	30	W	F
	AaBB	EM	0.98	PM			
22	*AABB*	EM	0.59	PM	43	W	M
23	*AABB*	EM	BLQ (DM)	EM	33	W	M
24	*AABB*	EM	0.07	EM	41	W	F
25	*AABb*	EM	0.46	PM	39	W	M
26	*AABB*	EM	3.37	PM	40	W	M
27	*AaBB*	EM	12.63	PM	60	W	M
28	*AABB*	EM	4.6	PM	47	WI	M
29	*AABB*	EM	2.51	PM	54	W	M
30	*AABB*	EM	0.31	EM	41	W	M
31	*AABb*	EM	0.85	PM	32	W	M
32	*AABB*	EM	0.09	EM	41	W	M
33	*AABB*	EM	1.02	PM	42	W	M
34	*AABb*	EM	6.15	PM	44	W	M
35	*AABb*	EM	6.01	PM	31	W	M
	AABb	EM	5.72	PM			
36	*AABB*	EM	BLQ (DM)	EM	41	W	M
37	*AABB*	EM	0.28	EM	38	W	M
38	*AB/del*	EM	5.96	PM	46	AA + AS	M
	AB/del	EM	19.03	PM			
39	*AABB*	EM	BLQ (DM)	EM	65	W	M
40	*AABB*	EM	2.27	PM	40	W	M
41	*AAbb*	PM	71.11	PM	65	W	M
	AAbb	PM	1.12	PM			
42	*Ab/del*	PM	0.65	PM	34	W	M

Abbreviations: W: Caucasian, WI: West Indian, AA: African American, AS: Asian; BLQ(DM) Below the limit of quantitation for dextromehtorphan (DM), DT: dextrorphan; EM: Extensive metabolizer, PM: Poor metabolizer.

[1]The small "*a*" indicates the subject carries the *CYP2D6(A)* mutation; the small "*b*" indicates the subject carries the *CYP2D6(B)* mutation and "*del*" the subject carries the *CYP2D6(D)* mutation. A capital letter indicates the wild-type form is present.

PM-associated alleles were assumed to act recessively when predicting the phenotype to be observed.

3. Phenotypes:

A DM/DT ratio of 0.3 was established as the point in which there is a segregation (antimode) between EM and PM CYP2D6 phenotypes. This antimode had been previously determined in a cohort of 65 healthy HIV negative volunteers whose demographics were comparable to the patients included in the Ducharme study [100]. The volunteers were unrelated, non-smoking Caucasians, 57 male and 8 female, average age 36 ± 19 years. Using the same DM phenotyping protocol, 4 of the 65 volunteers (9%) were classified as poor metabolizers.

The antimode of 0.3 in the Ducharme study [100] was determined using a probit analysis. Probit analysis consists of plotting the log ratios of DM/DT *vs* the probit (difference from the mean, in standard deviations, + k, where k is an integer yielding a positive probit). Exponential curves were fitted to suspected independent subpopulations to determine the antimode [15]. The antimode was defined by the point at which the curves cross to yield a clear break between PM and EM CYP2D6 metabolizers, thereby giving two distinct populations with no overlap. The same 0.3 antimode for the division between EM and PM CYP2D6 phenotypes has been found by other investigators using healthy seronegative populations [11, 100].

The data from this study is presented as a frequency histogram in Figure 8a. The corresponding probit plot, shown in Figure 8b, produced a single curve; the results could not be fitted to two intersecting curves. Thus, the analysis failed to show the existence of an antimode, nor, therefore two distinct populations of

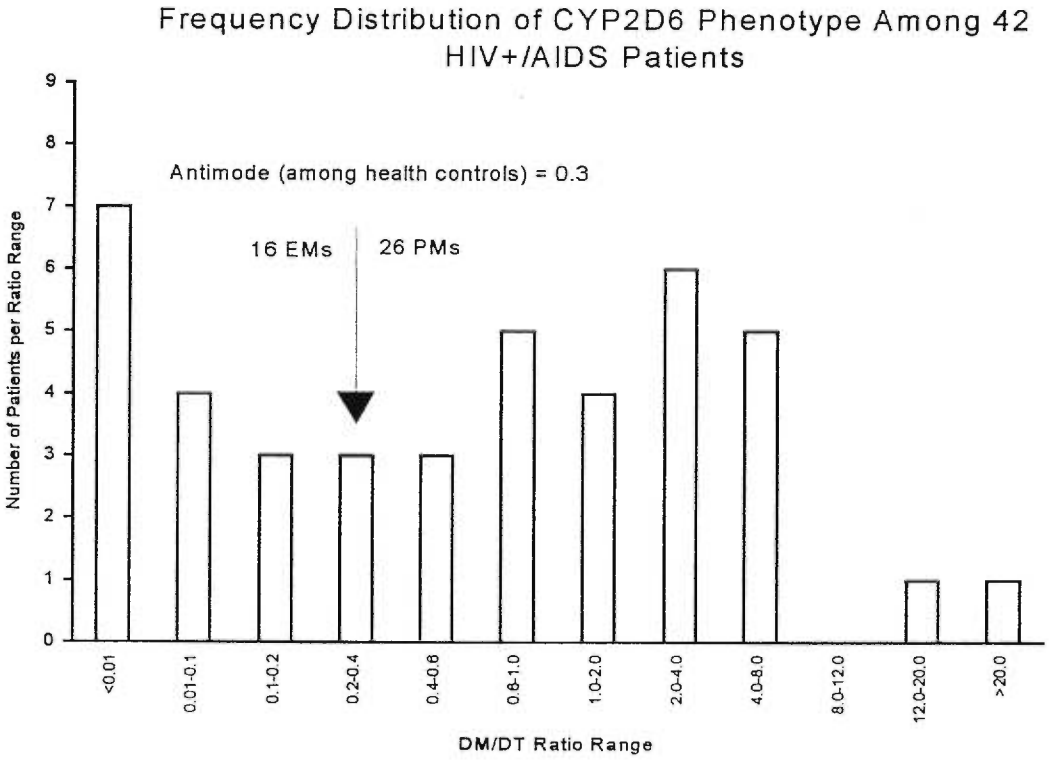


Figure 8a: Frequency distribution of CYP2D6 phenotype among the 42 patients which were phenotyped.

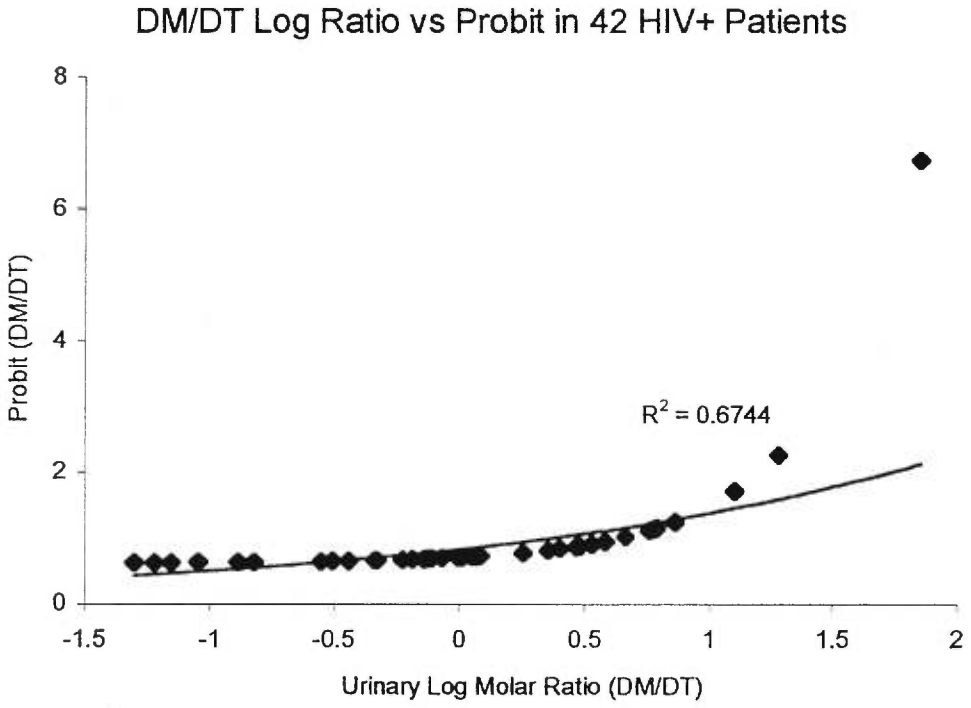


Figure 8b: Log urinary molar ratio vs Probit analysis does not demonstrate two distinct and different populations between EMs and PMs.

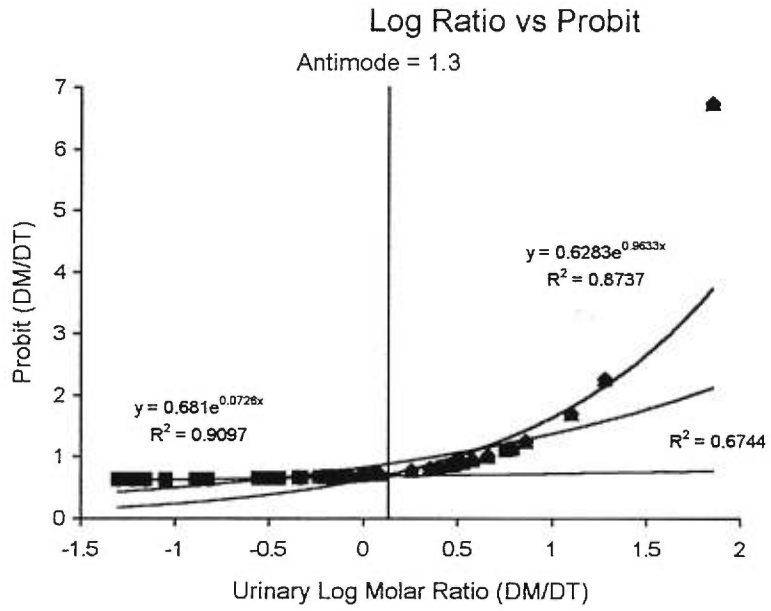


Figure 9a: Log urinary molar ratio vs Probit analysis does not demonstrate two distinct and different populations using an antimode of 1.3.

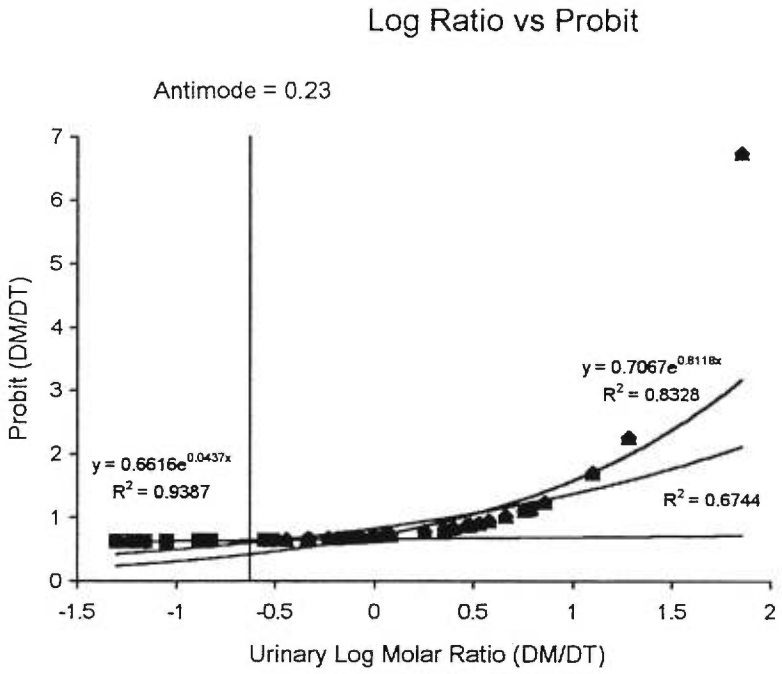


Figure 9b: Log urinary molar ratio vs Probit analysis does not demonstrate two distinct and different populations using an antimode of 0.23.

metabolizers, (see Figure 9).

However, even though two clear phenotypic populations were not observed, it is unlikely that the distinction between EM and PM CYP2D6 metabolizers has disappeared. The shape of the histogram suggests that the separation of the population into EM and PM phenotypes has been blurred by a conversion of genotypic EMs to phenotypic PMs. In order to analyze the data with stable terms of reference, it was examined using the established antimode of 0.3. When using this antimode, 26 of the 42 patients (62%) had a PM phenotype while the remaining 16 (38%) were classified as EM, Table IV. This is a 7-fold increase in the PM frequency as compared to the 9% reported in the Ducharme study [100].

4. Genotype *vs.* Phenotype:

A comparison of genotype *vs.* assigned phenotype showed that the two patients who were genotypically *PM* were also phenotypically PM and 16 of the 40 patients (40%) who were genotypically *EM* were also phenotypically EM, Table V. However, 24 of the 40 patients (60%) who were genotypically *EM* expressed a PM phenotype, Table V.

Among the 24 patients who had an *EM* genotype but expressed a PM phenotype (*EM*→PM group), 8 patients (33%) had AIDS including 6 whose AIDS-defining illnesses were active at the time of phenotyping, Table VI. Five additional patients in the *EM*→PM group were taking medications known to inhibit

Table V: Distribution of CYP2D6 genotype/phenotype among 42 HIV+/AIDS patients.

		PHENOTYPE		
		EXTENSIVE	**POOR**	**total**
genotype	**extensive**	16	24	40
	poor	0	2	2
	TOTAL	16	26	42

Table VI: Incidence of Disease Progression among 42 HIV+/AIDS patients.

	INCIDENCE OF DISEASE PROGRESSION		
	ACTIVE AIDS-DEFINING ILLNESSES	PAST AIDS-DEFINING ILLNESSES	HEPATITIS
EM ⟶ PM GROUP (n=24)	6 (25%)	2 (8%)	4 (17%)
EM/EM GROUP (n=16)	4 (25%)	2 (13%)	2 (12%)

CYP2D6 activity at the time of phenotyping; 4 of these had no active disease and one had stable cutaneous Kaposi's Sarcoma, Table VIIa. All patients in the *EM*→PM group had normal or grade 1 elevation in liver enzymes except for one (Patient #6) who demonstrated a 4-fold elevation in bilirubin level (direct and indirect bilirubin) with normal transaminase and alkaline phosphatase levels.

In the 16 patients who had an *EM* genotype and expressed an EM phenotype (*EM*/EM group), 6 patients (38%) had AIDS including 4 whose AIDS-defining illnesses were active at the time of phenotyping, Table VIIa. None of the *EM*/EM patients were taking medication known to inhibit CYP2D6 activity, (Table VIIb) and all of the patients had renal and liver function tests within normal limits.

Table VIIa: Medication, disease status and history of 24 patients with observed PM phenotype despite *EM* genotype

PATIENT NO	MEDICATIONS	ACTIVE DISEASE	HISTORY
2	fluconazole, diazepam, oxazepam, terbutyline, budesonide, Empracet®, dimenhydrinate, metoclopramide, cisapride, quinine	oral candidiasis	hepatitis, weight loss
4	AZT/3TC/loviride (double blind)	none	herpes zoster
5	3TC	none	none
6	nystatin, rifampin, erythromycin, Factor VIII	***PC*P**, Wasting****, oral candidiasis, rhodococcus, hyperbilirubinemia	***PC*P****, OHL, lymphadenopathy, thrombocytopenia, neutropenia
7	AZT, ddI, Septra®	oro-genital herpes	***PC*P****, oro-genital herpes
9	albuterol	thrombocytopenia	oral candidiasis, herpes zoster
11	AZT/ddC/saquinavir (double blind)	none	none
12	AZT vs indinavir* (double blind)	none	none
13	AZT, 3TC, Septra®	**KS****	oro-genital herpes
17	albuterol , nystatin, ibuprofen, nicotine patch	none	oral candidiasis
18	Septra®, AZT	none	OHL, oro-genital herpes, constitutional symptoms
21	ddI, dapsone, fluconazole	cervical dysplasia	***PC*P****, condyloma, neutropenia
	pentamidine	**CMV disease****, liver failure	**Esophageal candidiasis****, thrombocytopenia
22	Empracet®	none	bacterial pneumonia, oro-genital herpes, GI intolerance to AZT
25	AZT, 3TC, saquinavir, Septra®, amoxicillin, Empracet®, levothyroxine	none	oral candidiasis, hepatitis, oro-genital herpes, herpes zoster
26	ddC, cimetidine*, methadone*, quinine	none	oro-genital herpes
27	AZT, 3TC, Septra®, acyclovir	oro-genital herpes	***PC*P****, oral candidiasis, weight loss, constitutional symptoms
28	AZT, 3TC	none	oral candidiasis, OHL, lymphadenopathy, hepatitis
29	fluoxetine*, pindolol	none	oral candidiasis
31	pentamidine	none	oro-genital herpes
33	acyclovir	none	oro-genital herpes
34	d4T vs indinavir* (double blind)	**KS****, oro-genital herpes, peripheral neuropathy	oral candidiasis, lymphadenopathy, condyloma, constitutional symptoms
35	AZT, 3TC, ddC, oxazepam, VBV chemo	**KS****, molluscum	lymphadenopathy
	AZT, 3TC, saquinavir, oxazepam, CD8+ infusions/IL-2	**KS****	molluscum
38	naproxen, cimetidine*, hydrochlorothiazide, allopurinol	B12 deficiency	lymphadenopathy, hepatitis, oro-genital herpes, herpes zoster
	AZT/ddC/saquinavir (double blind), Septra®, acyclovir, cimetidine*, fluoxetine*, hydrochlorothiazide, allopurinol	none	lymphadenopathy, hepatitis, oro-genital herpes, herpes zoster, B12 deficiency
40	Septra®, clonazepam	**KS**, mycobacterium****, oral candidiasis	oro-genital herpes

* Medications known to inhibit CYP2D6 activity

**AIDS-defining illnesses

Abbreviations: VBV chemo: combination chemotherapy of vinblastine, bleomycin, vincristine and decadron; IL: interleukin; OHL: oral hairy leukoplakia; KS: Kaposi's sarcoma; CMV: cytomegalovirus; *PC* P: *pneumocystis carinii* pneumonia.

Table VIIb: Medication, disease status and history of 16 patients whose observed EM phenotype matched their genotype

PATIENT NO	MEDICATIONS	ACTIVE DISEASE	HISTORY
1	none	lymphadenopathy, onychomycosis	condyloma, oro-genital herpes,constitutional symptoms, vaginal candidiasis
3	AZT, atovaquone	none	*PC* P**, lymphadenopathy, oro-genital herpes
8	AZT, ddC, Septra®, acyclovir	none	lymphadenopathy, condyloma, hepatitis, oro-genital herpes
10	ddC, dapsone, ABV chemo	KS**, disseminated *MA* C**, oral candidiasis, lymphadenopathy	oro-genital herpes
14	AZT, 3TC, Septra®, zopiclone	none	OHL, lymphadenopathy
15	AZT/ddC/saquinavir (double blind), Septra®, ketoconazole	oral candidiasis	OHL, lymphadenopathy, oro-genital herpes, herpes zoster
16	AZT, 3TC, Septra®	none	OHL, oro-genital herpes
19	omeprazole, sulfasalazine, CD8+ infusions	**KS**, gastric lymphoma****	**Non-Hodgkin's lymphoma of parotid**, herpes zoster**
20	acyclovir	**CMV disease****	oro-genital herpes
23	acyclovir	none	herpes zoster
24	pentamidine, Excedrin®	**Wasting****	OHL, hepatitis, liver failure
30	Septra®	none	OHL, oro-genital herpes, herpes zoster
32	AZT/ ddC /saquinavir (double blind)	none	none
36	3TC, AZT, clindamycin, ketoconazole, acyclovir, Septra® desensitization	none	*PC* P**
37	AZT, 3TC, fluconazole, metoprolol, enalapril, glyburide	none	oral candidiasis, myopathy
39	acyclovir, 3TC, dapsone, trimethoprim, AZT	none	neutropenia, peripheral neuropathy

**AIDS-defining illnesses

Abbreviations: ABV chemo: combination chemotherapy of adriamycin, bleomycin, vincristine and decadron; OHL: oral hairy leukoplakia; KS: Kaposi's sarcoma; CMV: cytomegalovirus; *PC* P: *pneumocystis carinii* pneumonia; *MA* C: *mycobacterium avium* complex

5. Statistical analysis

Statistical analysis for correlation was conducted using GraphPad Instat software. Pearson correlation was used to determine the linearity of the association between the CYP2D6 phenotype, as determined by DM/DT ratio, and clinical parameters (CD4 count, BMI, weight and albumin levels) was carried out. Using this statistical test, no correlation was found between the DM/DT ratio and the various clinical parameters (see Table VIII).

A univariate analysis using *SAS/STAT* software was used to determine the normality of distribution of the parameters measured and the equality of the variance between the EM and PM group. Weight , BMI and albumin were normally distributed in this sample population whereas the DM/DT ratio and CD4 count showed a non-normal distribution. A T-Test procedure was used to determine the equality of variance between the EM group and the PM group for each parameter. Depending on the variance, Student's T-Test or Welch's approximate T-test was used to determine whether the means of the EM and PM groups are equal (see Table IX).

Table VIII: Pearson correlation for DM/DT ratio *vs* clinical parameters (CD4, BMI, weight, and albumin)

Parameter	Correlation coefficient	95% Confidence interval	Two-tailed *p*-value
DM/DT ratio vs CD4	0.1087	-0.2187 to 0.4140	0.5161
DM/DT ratio vs BMI	0.1032	-0.2435 to 0.4266	0.5613
DM/DT ratio vs weight	0.1531	-0.1952 to 0.4672	0.3873
DM/DT ratio vs albumin	-0.1968	-0.5016 to 0.1516	0.2647

Table IX: Unpaired T-Test , taking into consideration variances between the two phenotypes.

Parameter	Mean Difference	Variance	Statistical test	95%Confidence interval	Two-tailed *p* value
DM/DT ratio	5.662	unequal	Welch's	0.6366 to 10.688	0.0286*
CD4 count (cells/mm^3)	64.287	unequal	Welch's	-21.579 to 150.15	0.1381
BMI	2.201	unequal	Welch's	-0.5765 to 4.979	0.1165
weight (kg)	6.357	unequal	Welch's	-2.169 to 14.882	0.1319
albumin (g/L)	0.0644	equal	unpaired T-Test	-2.767 to 2.896	0.9635

* considered significant

An unpaired t-test was conducted on the DM/DT ratio and the four clinical parameters (BMI, CD4 count, weight and albumin level) to determine whether there was a difference between the two phenotypes. Notably, only the difference in the DM/DT ratio between the EM and PM phenotypes was considered significant.

Due to the fact that DM/DT ratio and CD4 count was not normally distributed in the sample population, a nonparametric analysis of variance was undertaken. Using the Wilcoxon signed rank test to determine variance, a very significant difference was observed in the DM/DT ratio between the EM and PM phenotypes ($p < 0.0001$). Using the same statistical test, no significant difference was observed in the CD4 count between the EM and PM phenotypes ($p = 0.2676$).

To examine the association between CYP2D6 phenotype and the risk of

having a BMI< 21, an odds ratio analysis was carried out. No difference between EM and PM phenotype with regard to BMI < 21 was observed (odds ratio 0.9375; 95% confidence interval: 0.1631 to 5.389; p = 1.000) (Fisher's Exact Test). The same analysis was used to determine whether phenotype was associated with a CD4 count of < 200 cells/mm^3 . Once again the odds ratio analysis showed no difference between EM and PM phenotype (odds ratio 3.792; 95% confidence interval: 0.9726 to 14.782; p = 0.0637).

CHAPTER V: DISCUSSION

In the 42 patients included in this study, the distribution of *EM* and *PM* genotypes was consistent with the distribution found in healthy, seronegative populations. The data suggest that conversion to a seropositive status and progression to AIDS has no relationship to metabolic genotype. This is consistent with previous studies on the distribution of N-acetyltransferase II (NAT2) genotypes, in which no differences in genotypic distribution were found in matched cohorts of healthy, seronegative populations and AIDS patients [91, 92].

However, in the patient population involved in this study, the expression of the CYP2D6 genotype is modified. This is an interesting finding given that in general, there is concordance between phenotype and assigned genotype. In healthy populations, direct correlations have been established between metabolic genotypes and probe drug phenotypes [98, 115]. Unlike the studies in healthy seronegative populations, probe drug phenotyping with DM did not produce DM/DT ratios which displayed a bimodal pattern and probit analysis of these ratios could not divide the patients into EM and PM phenotypes. Exponential curves were tested for goodness of fit at two different antimodes, 0.23 and 1.3, but both these antimodes failed to yield a clear break between PM and EM CYP2D6 metabolizers without overlap (Figure 9a and 9b).

No evident reason could be discerned for what appears to be a unimodal distribution (Figure 8a) of the DM/DT ratios. When examining the data presented in

the frequency histogram (Figure 8a), a valley exists between the metabolic ratios of 0.2 and 0.6. This valley reflects the fact that a distribution still exists between the EM and PM phenotypes. However, the shift of EM to PM phenotypes is probably responsible for the loss of the clear antimode. In order to compare the DM phenotyping results with the established CYP2D6 genotypes and to examine the data for factors affecting CYP2D6 enzyme expression, the DM/DT ratios were analyzed using an antimode of 0.3. This antimode had previously been determined in similar seronegative populations using DM as the probe drug for phenotyping for CYP2D6 activity [11, 30, 99, 100].

Based on the genotyping results and previous studies in healthy, seronegative volunteers, the expected distribution of the EM:PM phenotypes in the HIV+ patients included in this study was 40:2. The 0.3 antimode gave an EM:PM phenotype distribution of 16:26; 24 of the patients had a PM CYP2D6 phenotype and an EM *CYP2D6* genotype. Clearly the results of this study indicate that genotype and phenotype don't correlate in the patient population studied.

In order to explain the observed discrepancy between CYP2D6 genotype and phenotype, the parameters most commonly used to assess clinical status in this patient population were examined. These include CD4 count, BMI, weight, albumin, liver function and renal profile. Renal profiles were found to be within normal range for all patients phenotyped, therefore it is unlikely that altered DM renal clearance is considered as a possible source of discrepancy between genotype and observed phenotype.

When examining liver function, one patient (# 6) had a 4-fold elevation in bilirubin level although the transaminases and alkaline phosphatase were within normal range. Patient # 21 was diagnosed with liver failure at the time of phenotyping. Conflicting results have been reported on liver diseases and drug metabolism [48-50]. According to Paintaud *et al.* [116] drug metabolism not only depends on liver involvement but may also depend on histological changes in the liver (acute or chronic hepatitis, cirrhosis) and on the etiology (viral infection, drug-induced toxicity or autoimmune changes in the liver). From the results of this study, the role that liver disease plays in altering CYP2D6 activity is not clear. Hepatitis has been reported in both EMs and PMs, however, the etiology underlying the hepatitis (whether it is viral, toxic or auto-immune) is not known.

When examining the other clinical parameters, no correlation was found between DM/DT ratios and the CD4 count, BMI, weight and albumin. Additional analyses were undertaken in which the patients were divided into EMs and PMs based on DM/DT ratio. The major clinical parameters, CD4 count, BMI, weight and albumin, were then compared between the two metabolic groups using the unpaired T-test. No significant difference was observed between the EM and PM phenotypes.

The mean CD4 count for was 172 cells/mm3 for the phenotypic EMs *vs* 236 cells/mm3 for the phenotypic PMs ($p = 0.1381$). CD4 count has been previously used as a surrogate marker for disease progression and a CD4 count of less than 200 cells/mm3 has been historically associated with advanced disease. Thus, if disease progression were to play a role in altering metabolic phenotype, as in NAT2, then

one would expect the PMs to have a lower CD4 count. This suggests that although a decreased CD4 count may reflect a deterioration in immune status, it does not necessarily reflect an altered CYP2D6 metabolizing capacity.

A similar conclusion can be derived when looking at BMI and weight for both phenotypes. The mean BMI for EMs was 23.6 ± 4.15 *vs* 25.8 ± 4.23 for PMs ($p = 0.1165$); the mean weight was 71.4 ± 11.2 kg for EMs *vs* 77.8 ± 14.6 kg for PMs ($p = 0.1391$). Although not statistically significant, the PMs have generally a higher BMI and body weight when compared to the EMs. This may be explained by the fact that PMs may have a higher fat content than the EMs, although this was not measured.

Lipid disturbances may also have an effect on altered metabolic activity. It is important to note that HIV disease is characterized by disturbances in lipid metabolism [87, 117, 118]. These disturbances include increased triglycerides, increased very low density lipoprotein (VLDL) and increased plasma free fatty acids late in the disease. Cholesterol levels and high density lipoprotein (HDL) are decreased early on in the disease and low density lipoprotein (LDL) is decreased in the middle to late stages of the disease [87]. These HIV-induced lipid disturbances are a part of the phenomenon that is produced in AIDS patients who experience a rapid weight loss following a secondary opportunistic infection which results in a hypermetabolic state. Following the resolution of the infection, weight is usually regained when caloric intake is increased. However, the weight gain is manifested predominantly as increased adipose tissue with little increase in lean body mass

[119-121]. These changes in lipid metabolism could also influence hepatic drug metabolizing enzyme rates, without being reflected in actual weight or BMI. This suggests that a measure of lean body mass through bioelectrical impedence may be a more accurate marker to correlate clinical status with drug metabolizing capacity as opposed to the conventional markers such as body weight and BMI.

Nutritional status is another factor that may account for the variability in expressed CYP2D6 phenotype. When examining the data, two patients had a diagnosis of wasting at the time of phenotyping (patient # 6 and 24). Patient # 6 from the *EM*→PM group also had active opportunistic infection and liver disease (hyperbilirubinemia) at the time of phenotyping. Patient # 24 from the *EM*/EM group had wasting as the only active disease at the time of phenotyping. From this data, the relationship between a PM phenotype and wasting is not clear.

Malabsorption is usually characterized by hypoalbuminemia. In this study, hypoalbuminemia was not found in any of the patients, in either phenotypic group. Thus malabsorption does not appear to be a possible source for the discrepancy between CYP2D6 genotype and expressed phenotype.

These results suggest that clinical status as measured by CD4 count, body weight, BMI, albumin and nutritional status do not necessarily correlate with altered drug metabolizing capacity. In this study, these conventional clinical markers were not reliable in postulating an association between hepatic metabolic changes and HIV disease progression. This leads to the suggestion that other mechanisms are involved

in altering the metabolic activity of the CYP2D6 isoform in this patient population.

One mechanism by which a metabolic phenotype can be altered is through drug-drug interactions. Observed drug metabolism can be reduced by administration of specific enzyme inhibitors or by co-administration of drugs which compete for the same metabolizing enzyme [46]. Drug-drug interactions with most nucleoside analogs has not been a pressing issue for clinicians for several reasons: 1) most nucleoside analogs are rapidly cleared from the plasma, with elimination half-lives averaging one hour; 2) most nucleoside analogs, with the exception of AZT, are cleared renally; and 3) AZT is cleared mainly via hepatic glucuronidation which accounts for 75% of its elimination [77]. In this study, nucleoside analogues do not appear to affect the EM and PM distribution. For protease inhibitors which undergo extensive hepatic phase I biotransformation, drug-drug interactions are an important clinical question. Indinavir has been shown *in vitro* to inhibit CYP3A4 activity and to a lesser extent CYP2D6 activity [96]. *In vitro* studies have also shown ritonavir to be a potent inhibitor of CYP3A-mediated biotransformations [106, 122, 123]. Ritonavir has also been found to be an inhibitor of CYP2D6 activity [106]. In this study, six patients were being treated with protease inhibitors. In the *EM*→PM group, 4 patients (# 11, 25, 35 and 38) were taking saquinavir and 2 patients (# 12 and 24) were taking indinavir, Table VIIa. While it is possible that saquinavir may inhibit microsomal CYP2D6 activity, there is no evidence in the literature supporting this possibility. Patient # 38 was also taking fluoxetine and cimetidine which are

known to be CYP2D6 inhibitors and which are more likely to be responsible for the decreased CYP2D6 activity in this patient. Furthermore, it is noteworthy that in the *EM*/EM group, 2 patients (# 15 and 22) were taking saquinavir, (Table VIIb) and yet the genotype and phenotype correlated.

Patients # 12 and # 34 were taking the protease inhibitor indinavir, which has been shown *in vitro*, to inhibity CTP3A4 activity and to a lesser extent CYP2D6 activity [96]. When examining the clinical data for these two patients, no other factor other than the indinavir, seems to explain the discrepancy between EM genotype and PM phenotype. Thus, the medication appears to have had an inhibitory effect on CYP2D6 activity of these two patients.

Examination of the data reveals that only 5 of the 24 *EM*→PM patients (#12, 26, 29, 34, 38) were taking medications which have been shown *in vitro*, to inhibit CYP2D6, Table VIIa. It is noteworthy that when patient # 38 was phenotyped for a second time, a four-fold increase in the DM/DT ratio was observed, Table IV. This increase in DM/DT ratio, which reflects a decrease in CYP2D6 metabolic activity, coincided with the addition of a second CYP2D6 inhibitor (fluoxetine) to his treatment regimen which contained cimetidine.

A second mechanism by which phenotype can be altered is disease progression. This has been observed for the enzyme NAT2 in two studies involving AIDS patients [91, 93]. Lee *et al.* [91] reported an increased prevalence of slow acetylator phenotype (93%) among AIDS patients with acute illness. The study also

reported no difference in acetylator phenotype in stable AIDS patients and asymptomatic HIV+ patients when compared to seronegative controls. Lee *et al.* [91] suggested that acute illness in AIDS patients and not HIV infection per se, may play a role in the modifying the ability of the patients to metabolize drugs via NAT2 pathway. In another study, O'Neil *et al.* [93] observed that the distribution of NAT2 phenotype was unimodal and skewed towards the slow acetylators, suggesting a conversion from *fast* acetylator phenotype to *slow* acetylator phenotype. The study also examined the role of disease progression on NAT2 activity, reporting a higher incidence of AIDS among slow acetylators than among fast acetylators. The study also reported that among patients phenotyped more than once, changes in acetylator phenotype were associated with disease progression. O'Neil *et al.* [93] suggested that disease progression in HIV infection and AIDS may alter the expression of the NAT2 gene.

In this study, of the 24 patients in the *EM*→PM group, 6 (25%) patients (#6, 13, 21, 34, 35, 40) had active AIDS-defining illnesses at the time of phenotyping, 2 (8%) patients (#7, 27) had had AIDS-defining illnesses in the past, and 4 (17%) patients (#2, 25, 28, 38) had a history of hepatitis. The *EM*/EM group displayed similar characteristics. Of the 16 patients in the *EM*/EM group, 4 (25%) patients (#10, 19, 20, 24) had active AIDS-defining illnesses at the time of phenotyping, 2 (13%) patients (#3, 36) had had AIDS-defining illnesses in the past, and 2 (12%) patients (#8, 24) had a history of hepatitis. Using the Fisher's Exact Test, an odds

ratio analysis was carried out to determine whether there was a difference in disease status between the EM and PM phenotype. The analysis showed no difference between EM and PM phenotype with regards to AIDS (odds ratio 0.8333; 95% confidence interval: 0.223 to 3.123; $p = 1.000$). Using the same test, no difference was observed between the EM and PM phenotype, with regards to hepatitis (odds ratio 1.4; 95% confidence interval: 0.2246 to 8.728; $p = 1.000$).

These results indicate that AIDS-defining illnesses (acute and historic) do not appear to cause the metabolic "flip" from an extensive CYP2D6 metabolizer, as predicted by genotype, to a poor CYP2D6 metabolizer phenotype. However, there is an observable effect, which may be related to the underlying disease itself (*i.e.* HIV infection). The HIV virus or HIV related fatty infiltration into the liver [124] may have a degenerative effect on this organ's ability to metabolize drugs. *In vivo* studies [125, 126] have shown the presence of HIV-1 in Kupffer cells and hepatocytes, strongly suggesting that HIV-1 could replicate in the liver in the majority of patients with AIDS [126]. The degenerative effect caused by HIV-1 would not be detectable by standard liver function tests and would only manifest itself when the liver is challenged by a drug.

Such an HIV-mediated effect may in fact be secondary to changes in cytokine levels. TNF-α levels are increased in HIV-1 infected patients including children [127], drug users [128], and Africans [129]. Elevated TNF-α levels are detected in symptomatic AIDS patients during acute opportunistic infections [68]. Elevated IL-1

[130] and IL-6 [131] serum levels have been reported in HIV infections as well as the production of these cytokines by monocytic cells infected with HIV [65]. While IL-1 levels can be higher in early disease and decline thereafter, IL-6 levels can be elevated in late disease and associated with elevated TNF-α, IgG and IgA levels [132]. It has been proposed that increased IL-6 production is associated with HIV disease progression [131].

IL-1 has been shown to reduce CYP activities in mice and rats [51-53]. Administration of recombinant IL-1 or LPS-induced inflammation, resulted in a reduction in vitro in the metabolic activities of CYP1A1, -2B1, -2C11, -2D1, -2E1 and -3A. The decrease in CYP2D1 activity in rat liver microsomes prepared from IL-1α treated animals was associated with a decrease in the level of CYP2D mRNA, suggesting that IL-1α down-regulated CYP2D activity at the mRNA level [53]. Studies with IL-2 in rats have indicated that this cytokine increased the enzymatic activities of CYP1A1, -2B1, -2C11, -2D1 and -2E1 [59]. The results of this study also indicated that IL-2 induced an increase in the amounts of immunoreactive CYP2D protein and its mRNA, suggesting that the enzymatic activities were up-regulated at the mRNA level.

In order to assess the effect of cytokines in humans, endotoxin (LPS) was administered to twelve male volunteers for one or two consecutive days [133]. The subjects also received a cocktail of antipyrine, hexobarbital and theophylline before and after endotoxin challenge. Following the second endotoxin challenge, the

systemic clearances of antipyrine, hexobarbital and theophylline were decreased by 35% , 27% and 22% respectively. The decrease in antipyrine correlated with initial peak levels of TNF-α and IL-6. The data suggests that in humans, the LPS-induced inflammatory response decreased hepatic CYP-mediated drug metabolism and that TNF-α and IL-6 may play a role in this response.

Thus, one may speculate that the variation in CYP2D6 activity among HIV/AIDS patients could in part be due to the actions of cytokines such as IL-1, IL-6 or TNF-α, the serum levels of which are all elevated at various times during HIV infection.

CONCLUSION

The treatment of HIV infection is rapidly moving towards polypharmacy, *i.e.* combination antiretroviral therapy, multiple prophylactic therapy, anxiolytics, *etc.* Each new combination carries the risk of metabolic drug interactions with increased toxicity and reduced efficacy. In this environment, knowledge of a patient's metabolic status will be important in determining individual drug response, thus optimizing treatment. The importance of this approach has been illustrated in this work by the demonstration that changes in the expressed activity of CYP2D6 occur within an HIV+ population with the movement from EM to PM phenotypes irrespective of disease state. While the mechanisms underlying the observed phenotypic changes have not been identified, they most likely lie in a combination of drug interactions, disease state, genotype and/or factors associated with the HIV infection itself such as cytokine action, that may play a role in regulating the expression of the CYP2D6 gene. The results from this study suggest that further research is required to identify the possible mechanisms underlying the phenotypic changes observed in this patient population. These results also suggest that probe drug phenotyping with DM (an easy, safe, non-invasive probe of CYP2D6 enzyme activity) may also be useful as a probe of general CYP metabolic activity (CYP2D6 and CYP3A) in this patient population. Selection of tailored dosage regimens for individual patients would benefit from determination of the metabolic phenotype.

REFERENCES

1. Gonzalez FJ, Idle JR. Pharmacogenetic phenotyping and genotyping: present status and future potential. *Clin Pharmacokin* 1994; 26: 59-70.

2. Daly AK, Cholerton S, Gregory W, Idle JR. Metabolic polymorphisms. *Pharmacol Ther* 1993; 57: 129-60.

3. Krishna DR, Klotz U. Extrahepatic Metabolism of Drugs in Humans. *Clin Pharmacokinet* 1994; 26: 144-60.

4. Gibson GG, Skett P. Enzymology and Molecular Mechanisms In *Introduction to Drug Metabolism*. Glasgow: Chapman & Hall; 1994. p. 35-76.

5. Guengerich FP, editor. Mammalian Cytochrome P450, Vols 1 and 2. Boca Raton (Florida): CRC Press; 1987.

6. Guengerich FP. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res* 1988; 48: 2946-54.

7. Kivisto KT, Kroemer HK. Use of Probe Drugs as Predictors of Drug Metabolism in Humans. *J Clin Pharmacol* 1997; 37: 40S-48S.

8. Smith RL. Introduction: human genetic variations in oxidative metabolism. *Xenobiotica* 1986; 16: 361-65.

9. Eichelbaum M, Spanbrucker N, Steincke B, Dengler HJ. Defective N-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur J Clin Pharmacol* 1979; 16: 183-7.

10. Bertilsson L, Lou YQ, Du YL, Liu Y, Kuang TY, Liao XM, Wang KY, Reviriego J, Iselius L, Sjoqvist F. Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. *Clin Pharmacol Ther* 1992; 51: 388-97.

11. Schmid B, Bircher J, Preisig R, Kupfer A. Polymorphic dextromethorphan metabolism: co-segregation of oxidative O-demethylation with debrisoquin hydroxylation. *Clin Pharmacol Ther* 1985; 38: 618-24.

12. Brøsen K, Gram LF. Clinical significance of the sparteine/debrisoquine oxidation polymorphism. *Eur J Clin Pharmacol* 1989; 36: 537-47.

13. Evans DAP, Mahgoub A, Sloan JP, Idle JR, Smith RL. A family population

study of the genetic polymorphism of debrisoquine oxidation in white British population. *J Med Genet* 1980; 17:102-5.

14. Steiner E, Iselieus L, Alvan G, Lindsten J, Sjoqvist F. A family study of genetic and environmental factors determining polymorphic hydroxylation of debrisoquin. *Clin Pharmacol Ther* 1985; 38: 394-401.

15. Kupfer A, Preisig R. Pharmacogenetics of mephenytoin: a new drug hydroxylation polymorphism in man. *Eur J Clin Pharmacol* 1984; 26: 753-59.

16. Drøhse A, Bathum L, Brøsen K, Gram LF. Mephenytoin and sparteine oxidation: genetic polymorphisms in Denmark. *Br J Clin Pharmacol* 1989; 27: 620-25.

17. Evans WE, Relling MV, Rahman A, McLeod HL, Scott EP, Lin JS. Genetic basis for lower prevalence of deficient CYP2D6 oxidative drug metabolism phenotypes in black Americans. *J Clin Invest* 1993, 91: 2150-4.

18. Sachse, C, Brockmöller, J, Bauer, S and Roots, I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am J Hum Genet* 1997; 60: 284-95.

19. Kagimoto M, Heim M, Kagimoto K, Zeugin T, Meyer UA. Multiple mutations of the human cytochrome P450IID6 gene (CYP2D6) in poor metabolisers of debrisoquine. *J Biol Chem* 1990; 265 :17209-14.

20. Hanioka N, Kimura S, Meyer UA, Gonzalez FJ. The human CYP2D6 locus associated with a common genetic defect in drug oxidation: a G1934 to A base change in intron 3 of a mutant CYP2D6 allele results in an aberrant 3' splice recognition site. *Am J Hum Genet* 1990; 47: 994-1001.

21. Gaedigk A, Blum M, Gaedigk R, Eichelbaum M, Meyer UA. Deletion of the entire cytochrome P450 gene as a cause of impaired drug metabolism in poor metabolisers of the debrisoquine/sparteine polymorphism. *Am J Hum Genet* 1991; 48:943-50.

22. Steen VM, Andreassen OA, Daly AK, Tefre, Borresen AL, Idle JR, Gulbrandsen AK. Detection of the poor metabolizer-associated CYP2D6(D) gene deletion allele by long-PCR technology. *Pharmacogenetics* 1995; 5: 215-23.

23. Broly F, Meyer UA. Debrisoquine oxidation polymorphism: phenotypic

consequences of a 3-base pair deletion in exon 5 of the CYP2D6 gene. *Pharmacogenetics* 1993; 3: 123-30.

24. Heim MH, Meyer UA. Evolution of a highly polymorphic human cytochrome P450 gene cluster: CYP2D6. *Genomics* 1992; 14: 49-58.

25. Johansson I, Yue QY, Dahl ML, Heim M, Sawe J, Bertilsson L, Meyer UA, Sjöqvist F, Ingelman-Sundberg M. Genetic analysis of the interethnic difference between Chines and Caucasians in the polymorphic metabolism of debrisoquine and codeine. *Eur J Clin Pharmac* 1991; 40: 553-56.

26. Yue QY, Svenssson J-O, Alm C, Sjöqvist F, Sawe J. Codeine O-demethylation co-segregates with polymorphic debrisoquine hydroxylation. *Br J Clin Pharmacol* 1989; 28: 693-45.

27. Sindrup SH, Brøsen K, Bjerring P, Arendt-Nielsen L, Larsen U, Angelo HR, Gram LF. Codeine increases pain threshholds to copper vaper laser stimuli in extensive but not in poor metabolizers of sparteine. *Clin Pharm Ther* 1990; 48: 686-93.

28. Ayesh R, Idle JR, Ritchie JC, Crothers MJ, Hetzel MT. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. *Nature* 1984; 312: 169-70.

29. Caporaso NE, Tucker MA, Hoover, RN, Hayes RB, Pickle LW, Issaq HJ, Muschik GM, Green-Gallo L, Buivys D, Aisner S, Resau JH, Trump BF, Tollerud D, Weston A, Harris CC. Lung cancer and the debrisoquine metabolic phenotype. *J Natl Cancer Inst* 1990; 82: 264-72.

30. Relling MV, Cherrie J, Schell MJ, Petros WP, Meyer WH, Evans WE. Lower prevalence of the debrisoquine oxidative poor metabolizer phenotype in American black versus white subjects. *Clin Pharmacol Ther* 1991; 50: 308-13.

31. Raunio H, Pelkonen O: Cancer genetics: genetic factors in the activation and inactivation of chemical carcinogens. In *Drugs, Diet and Disease: Mechanistic Approaches to Cancer, Volume 1*. Edited by Ioannides C. New York: Ellis Horwood Ltd.; 1995. p.229-58.

32. Law MR, Hetzel MR, Idle JR. Debrisoquine metabolism and genetic predisposition to lung cancer. *Br J Cancer* 1989; 59: 686-87.

33. Roots I, Drakoulis N, Ploch M, Heinemeyer G, Loddenkemper R, Minks T,

Nitz M, Otte F, Kloch M. Debrisoquine hydroxylation phenotype, acetylation phenotype and ABO blood groups as genetic host factors of lung cancer risk. *Klin Wochenschr* 188; 66 (Suppl. IX):87-97.

34. Speirs CJ, Murray S, Davies DS, Biola Mabadeje AF, Boobis AR. Debrisoquine oxidation phenotype and susceptibility to lung cancer. *Br J Clin Pharmacol* 1990; 29:101-9.

35. Duche J-C, Joanne C, Barre J, De Cremoux H, Dalphin JC, DePierre A, Brochard P, Tillement JP, Bechtel P. Lack of a relationship between the polymorphism of debrisoquine oxidation and lung cancer. *Br J Clin Pharmacol* 1991; 31: 533-36.

36. Christensen PM, Gotzsche PC, Brøsen K. The sparteine/debrisoquine (CYP2D6) oxidation polymorphism and the risk of lung cancer: a meta-analysis. *Eur J Clin Pharmacol* 1997; 51: 389-93.

37. Kaisary A, Smith P, Jacqz E, McAllister CB, Wilkinson GR, Ray WA, Branch RA. Genetic predisposition to bladder cancer: ability to hydroxylate debrisoquine and mephenytoin as risk factors. *Cancer Res* 1987; 47: 5488-93.

38. Fleming CM, Kaisary A, Wilkinson GR, Smith P, Branch RA. The ability to 4-hydroxylate debrisoquine is related to recurrence of bladder cancer. *Pharmacogenetics* 1992; 2: 128-34.

39. Barbeau A, Cloutier T, Roy M, Plasse L, Paris S, Poirier J. Ecogenetics of Parkinson's disease: 4-hydroxylation of debrisoquine. *Lancet* 1985; II: 1213-16.

40. Poirier J, Roy M, Campanella G, Cloutier T, Paris S. Debrisoquine metabolism in Parkinsonian patients treated with antihistamine drugs. *Lancet* 1987; II:386.

41. Benitez J, Ladero JM, Jimenez-Jimenez FJ, Martinez C, Puerto AM, Valdivielso MJ, Llerena A, Cobaleda J, Munoz JJ. Oxidation polymorphism of debrisoquine in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1990; 53: 289-92.

42. Fonne-Pfister R, Bargetzi MJ, Meyer UA. MPTP, the neurotoxin inducing Parkinson's disease, is a potent competitive inhibitor of human and rat cytochrome P450 isoenzymes (P450bufl, P450dbl) catalysing debrisoquine 4-hydroxylation. *Biochem Biophys Res Commun* 1987; 148: 1144-50.

43. Suzuki T, Fujita S, Narimatsu S, Masubuchi Y, Tachibana M, Ohta S, Hirobe M. Cytochrome P450 isoenzymes catalyzing 4-hydroxylation of Parkinsonism-related compound 1,2,3,4-tetrahydroisoquinoline in rat liver microsomes. *FASEB J* 1992; 6: 771-76.

44. Armtsrong M, Daly AK, Cholerton S, Bateman DN, Idle JR. Mutant debrisoquine hydroxylation genes in Parkinson's disease. *Lancet* 1992; 339: 1017-18.

45. Siddons MA, Pickering-Brown SM, Mann DM, Owen F, Cooper PN. Debrisoquine hydroxylase gene polymorphism frequencies in patients with amyotropic lateral sclerosis. *Neurosci Lett* 1996; 208: 65-8.

46. Correina MA. Drug Biotransformation In: Katzung BG, Ed. *Basic & Clinical Pharmacology*. 6th ed. Norwalk: Appleton and Lange; 1995. p. 48-59.

47. Hardman JG and Limbird LE, editors.*Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th ed. New York: McGraw-Hill; 1996. p. 3-27.

48. Farrell, GC, Cooksley WG, Hart P, Powell LW. Drug metabolism in liver disease. Identification of patients with impaired hepatic drug metabolism. *Gastroenterology* 1978; 75: 580-8.

49. Narang AP, Datta DV, Nath N, Mathur VS. Impairment of hepatic drug metabolism in patients with acute viral hepatitis. *Eur J Drug Metab Pharmacokin* 1982; 7: 255-8.

50. Levy M, Leibowich I, Zylber-Katz E, Ilan Y, Granit L, Sviri S, Caraco Y. Impairment of the metabolism of dipyrone in asymptomatic carriers of the hepatitis B virus. *Clin Pharmacol Ther* 1997; 62: 6-14.

51. Ghezzi P, Saccardo B, Villa P, Rossi V, Bianchi M, Dinarello CA. Role of interleukin-1 in the depression of liver drug metabolism by endotoxin. *Inf and Immun* 1986; 54: 837-40.

52. Shedlofsky SI, Swim AT, Robinson JM, Gallichio VS, Cohen DA, McClain CJ. Interleukin-1 (IL-1) depresscytochrome P450 levels and activities in mice. *Life Sci* 1987; 40: 2331-6.

53. Kurokohchi K, Yoneyama H, Matsuo Y, Nishioka M, Ichikawa Y. Effects of interleukin 1 α on the activities and gene expression of the cytochrome P450IID6 subfamily. *Biochem Pharmacol* 1992; 44: 1669-74.

54. Bertini R, Bianchi M, Villa P, Ghezzi P. Depression of liver drug metabolism and increase in plasma fibrinogen by interleukin 1 and tumor necrosis factor: a comparison with lymphotoxin and interferon. *Int J Immunopharmacol* 1988; 10: 525-30.

55. Nadin L, Butler AM, Farrell GC, Murray M. Pretranslational down-regulation of cytochromes P450 2C11 and 3A2 in male rat liver by tumor necrosis factor alpha. *Gastroenterol* 1995; 109: 198-205.

56. Fantuzzi G, Cantoni M, Sironi M, Ghezzi P. Inhibitors of cytochrome P450 suppress tumor necrosis factor production. *Cellular Immunol* 1993; 150:417-24.

57. Thal C, Kahwaji JE, Loeper J, Tinel M, Doostzadeh J, Labbe G, Leclaire J, Beaunne P, Pessayre D. Administration of high doses of human recombinant interleukin-2 decreases the expression of several cytochromes P-450 in the rat. *J Pharmacol Exp Ther* 1994; 268: 515-21.

58. Aussel C, Breittmayer JP, Ticchioni M, Pelassy C, Bernard A. Regulation of T cell activation by cytochrome P450 inhibitors. *Cellular Immunol* 1994; 155: 436-45.

59. Kurokohchi K, Matsuo Y, Yoneyama H, Nishioka M, Ichikawa Y. Interleukin 2 induction of cytochrome P450-linked monooxtgenase systems of rat liver microsomes. *Biochem Pharmacol* 1993; 45: 585-92.

60. Meyer UA, Skoda RC, Zanger UM, Heim M, Broly F. The genetic polymorphism of debrisoquine/sparteine metabolism - molecular mecanisms. In Kalow W, Ed. *Pharmacogenetics of drug metabolism.* Pergamon, New York, 1992. p. 609-23.

61. Bock KW, Schrenk D, Forster A, Griese EU, Morike K, Brockmeier D, Eichelbaum M. The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronsyltransferases in man using sparteine, caffeine and paracetamol as probes. *Pharmacogenetics* 1994; 4: 209-18.

62. Ansher SS, Thompson W. Modulation of hepatic mRNA levels after administration of lipopolysaccharide and diphteria and tetanus toxoids and pertussis vaccine adsorbed (DTP vaccine) to mice. *Hepatology* 1994; 20: 984-91.

63. Abdel-Razzak Z, Loyer P, Fautrel A, Gautier JC, Corcos L, Turlin B, Beaune P

Guillouzo A. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol* 1993; 44: 707-15.

64. Li X,Youngblood GL, Payne AH, Hales DB. Tumor necrosis factor-alpha inhibition of 17 alpha-hydroxylase/c17-20 lyase gene (*Cyp* 17) expression. *Endocrinology* 1995; 136: 3519-26.

65. Molina JM, Schindler R, Ferriani R, Sakaguchi M, Vannier E, Dinarello CA, Groopman JE. Production of cytokines by peripheral blood monocytes/ macrophages infected with human immunodeficiency virus type 1 (HIV). *J Inf Dis* 1990; 161: 888-93.

66. Revilllard JP. Immunologie de l'infection par le VIH et du syndrome d'immunodeficience acquise (SIDA) In *Immunologie*. Bruxelles: DeBoeck-Wesmael S.A.; 1994. p. 261-268.

67. Bender BS, Davidson BL, Kline R, Brown C, Quinn TC. Role of the mononuclear phagocyte system in immunopathogenesis of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *Rev Infect Dis* 1988; 10: 1142-54.

68. Lahdevirta J, Maury CPJ, Teppo AM, Repo H. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired human immunodeficiency syndrome. *Am J Med* 1988; 85: 289-91.

69. Wright SC, Jewett A, Mitsuyasu R, Bonavida B. Spontaneous cytotoxicity and tumor necrosis factor production by peripheral blood monocytes from AIDS patients. *J Immunol* 1988; 141: 99-104.

70. deWitt R, Raasveld MHM, Ten Berge RJM, van der Wouw PA, Bakker PJ, Veenhof CH. Interleukin-6 concentrations in the serum of patients with AIDS-associated Kaposi's sarcoma during treatment with interferon-alpha. *J Intern Med* 1991; 229: 539-42.

71. Kovacs JA, Baseler M, Dewar R et al. Increases in CD4+ lymphocytes with intermittent courses of continuous interleukin-2 infusions in patients with human immunodeficiency virus infection: a preliminary study. *New Engl J Med* 1995; 332: 567-575.

72. Rizzardi GP, Barcellini W, Tambussi G, Lillo F, malnati M, Perrin L, Lazzarin A. Plasma levels of soluble CD30, tumor necrosis factor (TNF)-alpha and TNF receptors during primary HIV-1 infection: correlation with HIV-1 RNA and the clinical outcome. *AIDS* 1996; 10: F45-F50.

73. Kalinkovich AA, Engelmann H, Harpaz N, Burstein R, Barak V, Kalickman I, Wallach D, Bentwich Z. Elevated serum levels of soluble tumor necrosis factor in patients with HIV infection. *Clin Exp Immunol* 1992; 89: 351-55.

74. Godfried MH, van der Poll T, Weverling GJ, Mulder JW, Jansen J, van Deventer SJH. Soluble receptors for tumor necrosis factor as predictors of progression to AIDS in asymptomatic human immunodeficiency virus type1 infection. *J Inf Dis* 1994; 169: 739-45.

75. Montagnier L. *AIDS: Facts and hopes*. 8th ed. Paris: Med-Edition; 1995.

76. Broder S, Merigan TC, Bolognesi D, editors. *Textbook of AIDS Medicine*. Baltimore (MD): Williams & Wilkins; 1994.

77. Devita VT, Hellman S, Rosenberg SA, editors. *AIDS: Etiology, Diagnosis, Treatment and Prevention*, 4th ed. Philadelphia: Lippincott-Raven Publishers; 1997.

78. Gries JM, Troconiz IF, verotta D, Jacobson M, Sheiner LB. A pooled analysis of CD4 response to zidovudine and zalcitabine treatment in patients with AIDS and AIDS-related complex. *Clin Pharmacol Ther* 1997; 61: 70-82.

79. Malone JL, Simms TE, Gray GC, Wagner KF, Burge JR, Burke DS. Sources of variability in repeated T-helper lymphocyte counts from human immunodeficiency virus type 1- infected patients: total lymphocyte count fluctuations and diurnal cycle are important. *J Acquir Immune Defic Syndr* 1990; 3: 144-51.

80. Giorgi JV, Cheng HL, Margolick JB et al. Quality control in the flow cytometric measurement of T-lymphocyte subsets: the Multicenter AIDS Cohort Study experience. *Clin Immunol Immunopathol* 1990; 55: 173-86.

81. Hughes MD, Stein DS, Gundacker HM, Valentine FT, Phair JP, Volberding PA. Within-subject variation in CD4 lymphocyte count in asymptomatic human immunodeficiency virus infection: implications for patient monitoring. *J Inf Dis* 1994; 169: 28-36.

82. Garcia F, Vidal C, Gatell JM, Miro JM, Soriano A, Pumarola T. Viral load in asymptomatic patients with CD4+ lymphocyte counts above 500 x 10^6/L. *AIDS* 1997; 11: 53-7.

83. Smith KJ, Skelton HG, Yaeger J, Baxter D, Angritt P, Johnson S, Oster CN, Wagner KF. Clinical features of inflammatory dermatoses in human immunodeficiency virus type 1 disease and their correlation with Walter Reed stage. *J Am Acad Dermatol* 1993; 28: 167-73.

84. Soeprono FF, Schinella RA, Cockerell CJ, Comite SL. Seborrheic-like dermatitis of acquired immunodeficiency syndrome. *J Am Acad Dermatol* 1986; 14: 242-8.

85. Valle S-L. dermatologic findings related to human immunodeficiency virus infection in high-risk individuals. *J Am Acad Dermatol* 1987; 17: 951-61.

86. Merenich JA, McDermott MT, Asp AA, Harrison SM, Kidd GS. Evidence of endocrine involvement early in thecourse of human immunodeficiency virus infection. *J Clin Endocrinol Metab* 1990; 70: 566-570.

87. Sellemeyer DE, Grunfeld C. Endocrine and metabolic disturbances in human immunodeficiency virus infection and the acquired immune deficiency syndrome. *Endocrine Reviews* 1996; 17: 518-32.

88. Moss AR, Bacchetti P, Osmond D, Krampf W, Chaisson RE, Stites D, Wilber J, Allain JP, Carlson J. Seropositivity for HIV and the development of AIDS or AIDS-related condition: three year follow up of the San Francisco General Hospital Cohort. *Br Med J* 1988; 296: 745-50.

89. Centers for Disease Control. 1993 Revised classification system for HIV infection and expanded surveillance of definition of AIDS among adolescents and adults. *MMWR* 1992; 41 (RR-17): 1-19.

90. Moss AR, Bacchetti P. Natural history of HIV infection. *AIDS* 1989; 3: 55-61.

91. Lee BL, Wong D, Benowitz NL, Sullam PM. Altered patterns of drug metabolism in patients with acquired immunodeficiency syndrome. *Clin Pharmacol Ther* 1993; 53: 529-35.

92. Delomenie C, Grant DM, Mathelier- Fuscade P, Jacomet C, Leynadier F, Jacqz-Aigrain E. N-acetylation genotype and risk of severe reactions to sulphonamides in AIDS patients. *Br J Clin Pharmacol* 1994; 38: 581-2.

93. O'Neil WM, Gilfix BM, Di Girolamo A, Tsoukas CM, Wainer IW. N-acetylation among HIV-positive patients and patients with AIDS: When is fast, fast and slow, slow? *Clin Pharmacol Ther* 1997; 62: 261-71.

94. Barry M, Gibbons S, Back D, Mulcahy F. Protease inhibitors in patients

with HIV disease: Clinically important pharmacokinetic considerations. *Clin Pharmacokin* 1997; 32: 194-207.

95. Fitzsimmons ME, Collins JM. Selective biotransformation of the human immunodeficiency virus protease inhibitor saquinavir by human small-intestinal cytochrome P4503A4, potential contribution to high first-pass metabolism. *Drug Metab Dispos* 1997; 25: 256-66.

96. Chiba M, Hensleigh M, Nischime JA, Balani SK, Lin JH. Role of cytochrome P4503A4 in human metabolism of MK639, a potent human immunodeficiency virus protease inhibitor. *Drug Metab Dispos* 1996; 24: 307-14.

97. Eichelbaum M, Gross AS. The genetic polymorphism of debrisoquine/ sparteine metabolism in clinical aspects. *Pharmacol Ther* 1990; 46: 377-94.

98. Chen S, Chou Wh, Blouin RA, Mao Z, Humphries LL, Meek QC, Neill JR, Martin WL, Hays LR, Wedlund PJ. The cytochrome P450 2D6 (CYP2D6) enzyme polymorphism: Screening costs and influence on clinical outcomes in psychiatry. *Clin Pharmacol Ther 1996*; 60: 522-34.

99. Jones DR, Gorski JC, Hamman MA, Hall SD. Quantification of dextromethrorphan and metabolites: a dual phenotypic marker for CYP450 3A4/5 and 2D6 activity. *J Chromatogr B* 1996; 678: 105-11.

100. Ducharme J, Abdullah S, Wainer IW. Dextromethorphan as an in-vivo probe for the simultaneous determination of CYP2D6 and CYP3A activity. *J Chromatogr B* 1996; 678: 113-28.

101. Jacqz E, Dulac H, Mathieu H. Phenotyping polymorphic drug metabolism in the French caucasian population. *Eur J Clin Pharmacol* 1988; 35: 167-71.

102. Dahl ML, Iselius L, Alm C, Svensson JO, Lee D, Johansson I, Ingelman-Sundberg M, Sjoqvist F. Polymorphic 2-hydroxylation of desipramine: A population and family study. *Eur J Clin Pharmacol* 1993; 44: 445-50.

103. Brϕsen K, Zeugin T, Meyer UA. Role of P450 IID6, the target of sparteine-debrisoquin oxidation polymorphism in the metabolism of imipramine. *Clin Pharmacol Ther* 1991; 49: 609-17.

104. Llerena A, Alm C, Dahl ML, Ekqvist B, Bertilsson L. Haloperidol

disposition is dependent on debrisoquin hydroxylation. *Ther Drug Monit* 1992; 14: 92-7.

105. Stevens JC, Wrighton SA. Interaction of enantiomers of fluoxetine and norfluoxetine with human liver cytochromes P450. *J Pharmacol Exp Ther* 1993; 266: 964-71.

106. Kumar GN, Rodrigues AD, Buko AM, Denisson JF. Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. *J Pharmacol Exp Ther* 1996; 277: 423-31.

107. Hamelin BA, Turgeon J, Valee F, Belanger PM, Paquet F, Lebel M. The disposition of fluoxetine but not srtraline in altered poor metabolizers of debrisoquin. *Clin Pharmacol Ther 1996*; 60: 512-21.

108. Hou ZY, Chen CP, Yang WC, Lai MD, Buchert ET, Chung HM, Pickle LW, Woosley RL. Determination of dextromethorphan metabolic phenotype by salivary analysis with a reference to genotype in Chinese patients receiving renal hemodialysis. *Clin Pharmacol Ther* 1996; 59: 411-17.

109. Douglas AM, Atchison BA, Somogyi AA, Drummer OH. Interpretation of a simple PCR analysis of the CYP2D6(A) and CYP2D6(B) null alleles associated with the debrisoquine/sparteine genetic polymorphism. *Pharmacogenetics* 1994; 4: 154-8.

110. Daly AK, Steward A. Use of the Expand™ long template PCR system in genotyping for polymorphisms in the human cytochrome P450 CYP2D6 gene. Boehringer Mannheim *Biochemica* 1995; 4: 31-7.

111. Daly AK, Brockmoller J, Broly F, Eichelbaum M, Evans WE, Gonzalez FJ, Huang JD, Idle JR, Ingelman-Sundberg M, Ishizaki T, Jacqz-Agrain E, Meyer UA, Nebert DW, Steen VM, Wolf CR, Zanger UM. Nomenclature for human CYP2D6 alleles. *Pharmacogenetics* 1996; 6: 193-201.

112. Linnet K, Wiborg O. Steady-state serum concentrations of the neuroleptic perphenazine in relation to CYP2D6 genetic polymorphism. *Clin Pharmacol Ther* 1996; 60: 41-7.

113. Gonzalez FJ, Meyer UA. Molecular genetics of the debrisoquin-sparteine polymorphism. *Clin Pharmacol Ther* 1991; 50: 233-8.

114. Evans WE, Relling MV. Concordance of P450 2D6 (debrisoquine hydroxylase) phenotype and genotype: inability of dextromethorphan metabolic ratio to discriminate reliably heterozygous and homozygous extensive metabolizers. *Pharmacogenetics* 1991; 1: 143-8.

115. Kaufmann GR, Wenk M, Taeschner W, Peterli B, Klaus G, Meyer UA, Haefeli WE. N-acetyltransferase 2 polymorphism in patients infected with human immunodeficiency virus. *Clin Pharmacol Ther* 1996; 60: 62-7.

116. Paintaud G, Bechtel Y, Brientini MP, Miguet JP, Bechtel PR. Effects of liver disease on drug metabolism. *Therapie* 1996; 51: 384-9.

117. Feingold KR, Krauss RM, Pang M, Doerrler W, Jensen P, Grunfeld C. The hypertriglyceridemia of Acquired Immunedeficiency Syndrome is associated with increased prevalence of low density lipoprotein subcalss pattern B. *J Clin Endocrinol Metab* 1992; 76: 1423-7.

118. Zangerle R, Sarchetti M, Gallati Reibnegger G, Wachter H, Fuchs D. Decreased plasma concentrations of HDL cholesterol in HIV-infected individuals are associated with immune activation. *J Acquir Immune Defic Syndr* 1994; 7: 1149-56.

119. Oster MH, Enders SR, Samuels SJ, Cone LA, Hooton TM, Browder HP, Flynn NM. Megastrol acetate in patients with AIDS and cachexia. *Ann Intern Med* 1994; 121: 400-8.

120. Von Roenn JH, Armstrong D, Kotler DP, Cohn DL, Klimas NG, Tchekmedyian NS, Cone L, Brennan PJ, Weitzman SA. Megastrol acetate in patients with AIDS-related cachexia. *Ann Intern Med* 1994; 121: 393-9.

121. Struwe M, Kaempfer SH, Geiger CJ, Pavia AT, Plasse TF, Shepard KV, Ries K, Evans TG. Effect of dronabinol on nutritional status in HIV infection. *Ann Pharmacother* 1993; 27: 827-30.

122. Merry C, Barry MG, Mulcahy F, Ryan M, Heavey J, Tjia JF, Gibbons SE, Breckenbridge AM, Back DJ. Saquinavir pharmacokinetics alone and in combination with ritonavir in HIV-infected patients. *AIDS* 1997; 11: F29-F33.

123. Kempf DJ, Marsh KC, Kumar G, Rodrigues AD, Denissen JF, McDonald E, Kukulka MJ, Hsu A, Granneman GR, Baroldi PA, Sun E, Pizzuti D, Plattner JJ, Norbeck DW, Leonard JM. Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by

coadministration with ritonavir. *Antimicrob Agents Chemother* 1997; 41: 654-60.

124. Grunfeld C, Pang M, Doerler W, Shigenaga JK, Jensen P, Feingold KR. Lipids, lipoproteins, triglyceride clearance and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J Clin Endo Metab* 1992; 74: 1045-52.

125. Hufert FT, Schmitz J, Schreiber M, Schmitz H, Racz P, von Laer DD. Human Kupffer cells infected with HIV-1 in vivo. *J Acquir Immune Defic Syndr* 1993; 6: 772-7.

126. Cao YZ, Dieterich D, Thomas PA, Huang YX, Mirabile M, Ho DD. Identification and quantification of HIV-1 in the liver of patients with AIDS. *AIDS* 1992; 6: 65-70.

127. Rautonen J, Rautonen N, Martin NL, Philip R, Wara DW. Serum interleukin-6 concentrations are elevated and associated with elevated tumor necrosis factor-alpha and immunoglobulin G and A concentrations in children with HIV infection. *AIDS* 1991; 5: 1319-25.

128. Ayehunie S, Sonnenborg A, Yemane-Berhan T, Zewdie DW, Britton S, Strannegard O. Raised levels of tumor necrosis factor and HIV p24 antigen levels in serum of HIV-infected populations. *Clin Exp Immunol* 1993; 91: 37-42.

129. Martin-Chollet S, Simon F, Matheron S, Joseph CA, Elbim C, Gougerot-Pocidalo MA. Comparison of plasma cytokine levels in African patients with HIV-1 and HIV-2 infection. *AIDS* 1994; 8: 879-84.

130. Cox RA, Anders GT, Cappelli PJ, Johnson JE, Blanton HM, Seaworth BJ, Treasure RL. Production of tumor necrosis factor-alpha and interleukin-1 by alveilar macrophages from HIV-1 infected persons. *AIDS Res Human Retoviruses* 1990; 6: 431-41.

131. Breen EC, Rezai AR, Nakajima K, Beall GN, Mitsuyasu RT, Hirano T, Kishimoto T, Martinez-Maza O. Infection with HIV is associated with elevated IL-6 levels and production. *J Immunol* 1990; 144: 480-4.

132. Mildvan D, Machado SG, Wilets II, Grossberg SE. Endogenous interferon and triglyceride concentrations to assess response to zidovudine in AIDS and advanced AIDS-related complex. *Lancet* 1992; 339: 453-6.

133. Shedlofsky SI, Israel BC,McClain CJ, Hill DB, Blouin RA: Endotoxin administration to humans inhibits hepatic cytochromeP450-mediated drug metabolism. *J Clin Inv* 1994; 94: 2209-14.

APPENDIX I

CDC CLASSIFICATION FOR AIDS

Centers for Disease Control 1993 Classification System

	Symptoms		
CD4 Count	A	B	C
> 500 cells/mm^3	A1	B1	C1
200-520 cells/mm^3	A2	B2	C2
< 200 cells/mm^3	A3	B3	C3

Symptom Category

A: Acute retroviral syndrome
Generalized lymphadenopathy
Asymptomatic disease

B: Symptoms of AIDS-related complex
Candidiasis, mucosal
Cervical dysplasia
Constitutional symptoms
Herpes zoster
Idiopathic thrombocytopenic purpura
Listeriosis
Oral hairy leukoplakia
Pelvic inflammatory disease
Peripheral neuropathy

C: AIDS-defining conditions
CD4 count < 200 cells/mm^3
Candidiasis, pulmonary or esophageal
Cervical cancer
Coccidioidomycosis
Cryptosporidiosis
Cytomegalovirus infection
Herpes esophagitis
HIV encephalopathy
Histoplasmosis
Isosporiasis
Kaposi's sarcoma
Lymphoma
Mycobacterial disease
Pneumocystis carinii infection
Pneumonia, bacterial
Progressive multifocal leukoencephalopenia
Salmonellosis

APPENDIX II

PHENOTYPING PROTOCOL

THE MONTREAL GENERAL HOSPITAL
CLINICAL RESEARCH PROTOCOL

DETERMINATION OF METABOLIC INDICES IN AIDS PATIENTS

AIMS AND OBJECTIVES

This study will explore the role of metabolic profile/phenotype in the response/toxicity ratio of medications commonly used in the management of AIDS. The aim of the research is to discover the key factors which follow or predict the metabolic changes in the AIDS wasting syndrome in order to guide physicians in the prescription of drugs to those patients and in the timing of nutritional intervention.

The study described herein aims to:

1) establish the association between individual patients' ability to metabolize drugs and the safety and effectiveness of those drugs;
2) monitor the metabolic capacity of AIDS patients;
3) determine changes in their ability to metabolize drugs as their disease progresses and their nutritional status wanes.

RATIONALE

Pronounced interindividual variations in response to usual doses of drugs have been recognized in clinical practice and many of those agents are activated or eliminated by metabolic routes that are under genetic control. The genetic control results in at least two distinct subgroups in the population which differ in their ability to perform certain biotransformations. Individuals that are deficient in their ability to metabolize certain drugs, relative to the mean rate and extent of metabolism, are called poor metabolizers or PM-phenotypes; extensive metabolizers, EM-phenotypes, have normal to fast metabolism. Which phenotype is affected by toxicity or lack of efficacy depends on the drug's mode of action. If, as in the case of encainide, the metabolite is the major active component, then a lack of efficacy is apparent in PM's. Alternatively, as with tricyclic antidepressants, the parent drug is the active component. Therefore, EM's may not respond due to the rapid rates of metabolism making it difficult to reach steady-state therapeutic ranges with normal doses.

The classification of patients as to metabolic polymorphism/capacity is possible through the use of innocuous probe drugs. These drugs are metabolized by well characterized hepatic microsomal enzymes and the level of those

enzymes is reflected in the metabolite to parent ratio of the appropriate drug. The antitussive dextromethorphan (DM), for example, is metabolized in two steps by two isozymes of the cytochrome P450 (CYP) family of drug-metabolizing enzymes. Thus the ratio of the O-desmethyl metabolite to its parent (DM) is an index of CYP2D6 activity while the N-desmethylDM/DM ratio is indicative of CYP3A4 activity. In equivalent manner, caffeine and acetaminophen, serve as probes for N-acetyltransferase and glucuronidation, respectively.

The frequency distribution of the levels of urinary parent/metabolite ratios is analyzed in the studied population. Probe drugs are chosen for the pathway (hepatic microsomal isozyme) of interest. EM's and PM's fall into two distinct groups, distributed below or above a certain level (determined by probit analysis of the data). Two of the most widely studied hepatic routes showing genetic polymorphism of drug metabolism are the debrisoquin/sparteine oxidation pathway (CYP2D6 probed with DM) and the N-acetylation pathway (probed with caffeine). Some of the drugs which co-segregate with the debrisoquin/sparteine metabolism are various ß-blockers (metoprolol, propranolol), antiarrhythmics (encainide, perhexiline), antidepressants (desmethylimipramine, nortryptyline) and codeine. The adverse side-effects linked to this pathway are CNS toxicity, peripheral neuropathy, and excessive ß-blockade. Codeine is metabolized to morphine via that pathway which may represent an important activation pathway. Drugs that are subject to N-acetylation polymorphism include sulfonamides (sulfamethazine), antidepressants (phenelzine), antiarrhythmics (procainamide), antihypertensives (hydralazine) and dapsone, which is also metabolized by CYP3A4. Some adverse therapeutic consequences of the acetylator phenotype are peripheral neuropathy and hepatitis. Recent evidence suggests that CYP3A4 may also be polymorphic.

Variations in drug metabolism occur within individuals depending on smoking, the intake of numerous compounds (e.g. drugs) known to inhibit or induce hepatic drug metabolizing enzymes or other factors. Nutritional status has also been shown to have an effect on drug metabolism. In malnourished subjects, drug metabolism can be reduced or increased, depending on the pathways involved.

Malnutrition is an almost inescapable aspect of AIDS which is complicated by metabolic abnormalities caused by the host response to HIV-infection. These abnormalities have been studied extensively in terms of protein and energy metabolism but the effect of malnutrition on drug metabolism in AIDS has not been studied nor has the molecular basis of the observed changes been investigated.

We are proposing to monitor in AIDS patients the functional levels of four metabolic pathways involved in the hepatic metabolism of drugs: 4-hydroxylation (CYP2D6), N-demethylation (CYP3A4), N-acetylation and glucuronidation. In our study, we plan to use probe drugs (DM, caffeine and acetaminophen) and to also monitor the metabolism of codeine (CYP2D6), dapsone (CYP3A4 and N-acetyltransferase) and AZT (glucuronidation) in those patients so prescribed. Metabolic index values will be based on their metabolic ratios. The probes (dextromethorphan, caffeine and acetaminophen) are fairly innocuous and the sample collection is non-invasive (urine). The method of analysis will be by HPLC or capillary electrophoresis. It is expected that differences in the metabolism of the probes (e.g. DM for CYP2D6) will be parallelled by differences in substrate metabolism (e.g. codeine), that as caffeine metabolism increases (or decreases) so will that of dapsone. Indices of nutritional status, including body weight, serum albumin level and bioelectric impedance analysis, and liver function will also be assembled for each patient and multivariate analysis used to test correlations between drug-metabolic capacity and nutritional status.

The knowledge obtained as to the metabolic capacity of individuals and changes that may be effected during the course of their illness may be of great help in tailoring drug regimens which balance efficacy and toxicity and may provide an early indicator of the hepatic abnormalities which lead to cachexia.

STUDY DESIGN

Study Population

Sixty (60) patients will be asked to participate from the Immune Deficiency Treatment Centre of the Montreal General Hospital. All individuals will be asked to sign an informed consent prior to participation.

1. Inclusion Criteria

1.1 Asymptomatic Western Blot (or RIPA) confirmed HIV positive patients;

1.2 Age between 12 and 60 years old;

1.3 Mean CD4 count of 200 cells/mm^3 or less, based on two (2) separate determinations, performed at intervals over no more than eight (8) weeks prior to the first experiment;

1.4 Patients must sign a written informed consent; patients under

the age of 18 require written informed parental consent;

1.5 Negative pregnancy test for women of childbearing potential;

1.6 Body weight range of 80-120% the Life Insurance Underwriters Standard for age and height.

2. Exclusion Criteria

2.1 Active clinically significant medical problems. Patients with haemophilia are permitted if considered clinically stable at the time of entry;

2.2 Liver abnormalities defined as SGOT/SGPT greater than 200 IU/L, or albumin less than 3.0 g/dL or prothrombin time > 15 seconds (except patients with haemophilia);

2.3 Renal dysfunction defined as: BUN > 10 mmol/L or creatinine > 100 μmol/L;

2.4 Haematologic abnormalities as defined by: haemoglobin < 120 g/L or WBC < 3,000/mm^3 or platelet count < 70,000/mm^3;

2.5 Chronic alcohol or drug abuse within 12 months prior to this study;

2.6 Women of child bearing potential, defined as a pre-menopausal female who is biologically capable of becoming pregnant; unless a negative pregnancy test is obtained and the patient agrees to use two (2) means of artificial birth control (condom plus an additional hormonal or mechanical method).

3. Dose

On the day of the test, the subject will first provide a pre-dose (blank) urine sample before being given one tablet containing 100 mg caffeine (NoDoz, Bristol Myers Squibb Co., Wakeups, Adrem Ltd. or Chase Caffeine Tablets, A.W. Chase) and one tablet containing 500 mg acetaminophen (Atasol Forte, Frank W. Horner Inc. or Tylenol Extra-Strength, McNeil Consumer Products Co.) with approximately 100 mL water and 10 mL of cough syrup containing 30 mg dextromethorphan (Robitussin-DM, A. H. Robins Co. Inc. or Balminil D.M., Rougier Inc.).

Sample Collecting and Handling

A 10 mL aliquot of the pre-dose (blank) urine sample will be retained frozen for analysis. At 4 h post-dose, the patients will be asked to collect a sample of their urine in the container provided. Three (3) 10 mL aliquots will be separated from the 4 hour specimen. The three aliquots will be retained in a freezer at -20°C until analyzed for phenotype/metabolic index.

POTENTIAL HAZARDS

All the administered medications are non-narcotic, non-prescription and available over the counter. A recognized but rare side effect of a single dose of NoDoz is agitation which is transient and the side effects of a single dose of Robitussin DM are rare and include nausea and dizziness.

APPENDIX III

CONSENT FORM

MONTREAL GENERAL HOSPITAL

Principal Investigators: Drs. I.W. Wainer, C. Tsoukas and J. Falutz

DETERMINATION OF METABOLIC INDEX IN AIDS PATIENTS
PATIENT'S INFORMED CONSENT FORM

Dextromethorphan, Acetaminophen and Caffeine

The way in which your body responds to the medication(s) you are taking may be due to a specific and inherited pattern of metabolizing drugs. Further, the way in which you metabolize drugs may change as your disease state and/or nutritional status changes. In order to determine what pattern you have and how it might change, you will take:

- approximately two teaspoons (10 mL) of cough syrup containing the test drug dextromethorphan (DM) (Robitussin DM^R or Balminil DM^R - 30 mg/10 mL),
- a caffeine tablet (Wake-up^R) or a cup of coffee
- and an extra-strength acetaminophen tablet (Tylenol^R or Atasol^R) with a glass of water (200 mL)

You can take the syrup and the tablets together, and this will be done on one occasion only but you may be asked to repeat the process on subsequent occasions.

On the day of the test you will abstain from coffee or any caffeinated beverage (colas, hot chocolate, tea).

You will collect two (2) spot urine samples (of about 20 mL, or four teaspoons, each): the first one before ingesting the syrup and tablets (pre-dose urine sample) and the second one 4 hours after taking the syrup and tablets (4-h urine sample). During those 4 hours, you need not collect your urine nor abstain from urinating. After 4 hours, you will be able to drink coffee or any caffeinated beverage.

Dextromethorphan is a non-narcotic, non-prescription drug commonly used to treat coughs. Robitussin DM^R and Balminil DM^R are cough syrups that are available over the counter. The uncommon side effects of a single dose of Robitussin^R/Balminil DM^R may include nausea and dizziness; but these effects are transient and disappear rapidly.

The Wake-up^R tablet contains 100 mg of caffeine, which is

approximately equivalent to a cup of regular coffee. A single dose of caffeine doesn't usually induce side effects, but rarely, it may cause nausea, nervousness, insomnia, palpitations or headache; the possible side effects are transient and disappear rapidly.

The Tylenol[R] or Atasol[R] tablet contains 500 mg of acetaminophen, equivalent to less than two regular strength tablets. Acetaminophen is a common headache remedy available over the counter. Side effects from a single dose are exceedingly uncommon.

PCP Medications

Patients infected with the Human Immunodeficiency Virus (HIV) usually take dapsone (Avlosulfon) or Septra (sulphamethoxazole/trimethoprim) to treat or prevent *pneumocystis carinii* pneumonia (PCP). These patients may later require other medications such as cough suppressants, analgesics or antibiotics. Since these drugs' use in treating PCP is relatively new, their interactions with other drugs in the human body is not completely known.

In order to understand how such medications are metabolized by your liver, you will take one or both of the following:

Ketoprofen

Two Apo-Keto tablets (100 mg ketoprofen) with a glass of water. Ketoprofen is a non-steroidal anti-inflammatory drug used in the management of pain. The uncommon side effects of a single dose of ketoprofen include stomach upset, but that would be transient and disappear rapidly. As in the test described above (cough syrup/ caffeine), urine samples pre-dose and 4 hours post-dose will be required.

This test may be performed in conjunction with that described above (cough syrup/ caffeine) with the ketoprofen replacing acetaminophen. Additional urine samples, therefore, would not be involved.

For certain subjects, depending on which medications are currently prescribed and other factors, the test will have to be performed in addition to the syrup/caffeine test, on a separate day.

Aminosalicylate Sodium

Septra is a very common medication in the management of HIV-related diseases (*i.e.* PCP) but many patients react badly to it. Adverse reactions to drugs, like rashes which sometimes affect people taking Septra, may in part be due to the way in which the drugs are metabolized. The component of Septra which most likely causes such adverse reactions is metabolized by an enzyme called NAT1. The medicine aminosalicylate sodium (Nemasol Sodium) which is sometimes prescribed for tuberculosis, is also metabolized by NAT1. The way in which you metabolize Nemasol should parallel the way you would metabolize Septra.

In order to determine the status of your NAT1 system, you will:

- provide 20 mL (about 4 teaspoons) of blood to test for NAT1 in your blood cells;
- take two 500 mg Nemasol Sodium tablets, about one tenth of a normal daily dose;
- collect two (2) urine samples, the first, of about 20 mL (4 teaspoons), before taking the pills (pre-dose urine sample) and the second one to consist of all your urine over the 2 hours after taking the pills (0-2 h urine sample). During those 2 hours, you need to collect all your urine.

You would take the Nemasol and provide the blood and urine on a different day from the one when you take the cough syrup.

Aminosalicylate sodium is used in conjunction with other drugs to treat tuberculosis. Side effects such as nausea, vomiting, abdominal pain or diarrhoea have been reported, but only in patients taking 20 - 24 tablets a day, the normal dosage. A single 2-tablet dose of Nemasol is very unlikely to cause any such effects. The tablets should be taken with a meal so as to minimize any gastrointestinal discomfort which might occur. Any side effects from a single dose would be transient and disappear rapidly.

Other Medications

As usual, when you visit the clinic, blood will be drawn. Should you be receiving other medications, some of that blood and some of the pre-dose urine you collect for this study may be measured for drug and metabolite concentrations.

Metabolic Genotype

Your pattern of metabolizing drugs can in part be due to what you inherited. In order that your pattern may be studied at the genetic level (the level of your DNA), you will provide a sample of 10 mL (about 2 teaspoons) of blood.

Data Collection and Confidentiality

You will fill out a short questionnaire designed to collect information which will be used to interpret the results of the study. The investigators might need access to your medical record, in order to gather additional relevant information. This questionnaire and a case report form containing all the collected information will be kept confidentially at the Montreal General Hospital.

No information by which you can be identified will be released or published. The results of this study may be published in medical journals or reported at medical research meetings. Individual patients will never be identified. At the end of the study, if you so wish you can be informed of the results.

Benefits

With this study, we aim to improve the management of AIDS by understanding how the way in which individual patients metabolize drugs might change with nutritional status and/or disease progression. Participating in this study may not give any direct benefit, but it may help physicians and scientists choose the best treatment for other patients suffering from AIDS and related diseases.

Voluntary Participation

Your participation in this study is totally voluntary. You are free not to participate or to withdraw your consent to participate in this study at any time, without any penalty or loss of medical care.

If the results of the present study warrant further investigation, you will be asked to participate by repeating the tests. If this occurs, you will be fully informed and your participation will be totally voluntary.

Written Consent

I have read all the above and the attached patient's information sheets. I asked questions and received answers on all matters I did not understand. I willingly give my consent to participate in this study. Upon signing, I will receive a copy of this consent form and of the information sheets.

I agree to participate,

PATIENT _____ DATE _____

INVESTIGATOR _____ DATE _____

WITNESS _____ DATE _____

HÔPITAL GÉNÉRAL DE MONTRÉAL

Chercheurs principaux: Dr I.W. Wainer, Dr C. Tsoukas et Dr J. Falutz

DÉTERMINATION D'UN INDEX MÉTABOLIQUE CHEZ LES PATIENTS ATTEINTS DU SIDA

Formule de consentement éclairé

Dextrométhorphane, Acétaminophène et Caféine

La réponse de votre organisme aux médicaments que vous prenez peut dépendre de la façon dont vous les métabolisez. Votre capacité à métaboliser les médicaments vous est propre et peut être acquise par voie héréditaire. De plus, cette capacité métabolique peut changer selon votre état nutritionnel ou l'évolution de votre maladie. Afin de déterminer quelle est votre capacité métabolique, vous allez prendre:

- deux cuillerées à thé (10 mL) de sirop contre la toux contenant un médicament-test, le dextrométhorphane (DM) (Robitussin DM^R ou Balminil DM^R - 30 mg/10 mL),
- un comprimé de caféine (Wake-up^R) ou une tasse de café
- et un comprimé de 500 mg d'acétaminophène (Tylenol^R ou Atasol Forte^R) avec un verre d'eau (200 mL).

Vous pouvez prendre le sirop et les comprimés ensemble et vous n'aurez à le faire qu'une seule fois. Cependant, il se peut qu'on vous demande de refaire le test à une autre reprise.

Le jour du test avant que celui-ci ne soit fini, vous ne pourrez prendre aucune boisson contenant de la caféine, que ce soit du café, du thé, du chocolat chaud ou un cola.

Vous allez recueillir 2 échantillons de votre urine, environ 20 mL ou quatre cuillerées à thé chacun, le premier avant la prise du DM (échantillon d'urine pré-dose) et le deuxième 4 heures après le DM (échantillon d'urine à 4-h). Pendant ces 4 heures, vous n'aurez besoin ni de vous abstenir d'uriner ni de recueillir votre urine.

Le dextrométhorphane n'est pas un narcotique. Il peut être obtenu dans les pharmacies sans prescription. Les sirops contenant du DM, comme le Robitussin DM^R ou le Balminil DM^R, sont couramment utilisés pour combattre la toux. Après

une dose unique de sirop DM, les effets indésirables sont rares. Ceux-ci peuvent inclure des nausées et des étourdissements transitoires qui disparaissent rapidement.

Le comprimé de Wake-up[R] contient 100 mg de caféine, soit à peu près l'équivalent d'une tasse de café régulier. Une dose unique de caféine ne donne habituellement pas d'effets indésirables. Rarement, des nausées, de la nervosité, des palpitations ou des maux de tête peuvent se produire. Ces éventuels effets indésirables sont transitoires et disparaissent rapidement.

Le comprimé de Tylenol[R] ou d'Atasol Forte[R] contient moins d'acétaminophène que deux comprimés réguliers. L'acétaminophène est un médicament couramment utilisé contre les maux de tête, disponible dans les pharmacies sans prescription. Après une dose unique les effets indésirables sont très rares.

Les médicaments contre la PPC

Les patients infectés par le virus d'immunodéficience humaine (VIH) prennent d'habitude de la dapsone (Avlosulfon) ou du Septra (sulphaméthoxazole/triméthoprime) pour traiter ou prévenir les pneumonies à *pneumocystis carinii* (PPC). Ces patients peuvent par ailleurs recevoir d'autres médicaments (antitussifs, analgésiques, antibiotiques). L'utilisation de ces médicaments dans le traitement de la PPC étant relativement nouvelle, leurs interactions avec autres médicaments dans le corps humain ne sont pas complètements connues.

Afin de comprendre comment de tels médicaments sont métabolisés par votre foie, vous prendrez soit l'un ou soit les deux médicaments suivant:

Kétoprophène

Deux comprimés d'Apo-Keto[R] (100 mg de kétoprophène) avec un verre d'eau. Le kétoprophène est un médicament anti-inflammatoire non-stéroïdien utilisé contre la douleur. La prise d'une dose unique de kétoprophène n'entraîne que rarement des effets indésirables, de type troubles digestifs, toujours transitoire et disparaissant rapidement. Comme dans le test décrit ci-dessus (sirop/caféine), des prélèvements d'urine pré-dose et quatre heures après la prise seraient requis.

Ce test pourrait être fait conjointement avec celui décrit ci-dessus (sirop/caféine) en remplaçant l'acétaminophène par du kétoprophène. Aucun échantillon supplémentaire d'urine ne serait alors nécessaire.

Dans certains cas, en fonction des médicaments qui vous sont

couramment préscrits ainsi que d'autres facteurs, le test devra être réalisé en plus du test sirop/caféine, un jour différent de celui-ci.

Aminosalicylate de sodium

Le Septra est un médicament très fréquemment utilisé contre les infections associées au SIDA (par exemple la PPC) mais, malheureusement, beaucoup de patients développent des effets indésirables, particulièrement des éruptions cutanées. Selon votre capacité métabolique, de telles réactions peuvent ou non se produire. Le Septra est métabolisé par une enzyme appelée la NAT1, enzyme qui pourrait être impliquée dans le développement des réactions cutanées associées au Septra. L'aminosalicylate de sodium (Nemasol Sodium^{R}), médicament parfois prescrit contre la tuberculose, est aussi métabolisé par la NAT1. Les deux médicaments, le Septra et le Nemasol^{R}, sont métabolisés de façon parallèle.

Pour déterminer la capacité métabolique de votre système "NAT1", vous allez:

- donner un échantillon de votre sang d'environ 20 mL, ou 4 cuillerées à thé;
- prendre deux (2) comprimés de 500 mg de Nemasol Sodium^{R}, soit donc à peu près un dixième de la dose usuelle;
- recueillir deux (2) échantillons d'urine: le premier, d'environ 20 mL (4 cuillerées à thé), avant de prendre les comprimés (échantillon d'urine pré-dose); le deuxième pendant les deux heures qui suivent l'administration des comprimés. Vous devrez recueillir toute l'urine éliminée pendant cette période de 2 heures et la collecter dans un même flacon.

Vous ne prendrez le Nemasol Sodium^{R} ni ne recueillerez les échantillons d'urine et de sang le même jour où vous prenez le sirop contre la toux.

Le Nemasol Sodium^{R} (aminosalicylate de sodium) avec d'autres médicaments, est utilisé pour traiter la tuberculose. Certains effets indésirables comme des nausées, des vomissements, des douleurs abdominales ou des diarrhées ont été rapportés, mais uniquement chez des patients consommant 20 à 24 comprimés par jour (la posologie usuelle). Une seule dose de deux comprimés de Nemasol^{R} ne devrait pas causer d'effets indésirables. Les comprimés pourraient être pris avec un repas pour minimiser tout inconfort gastro-intestinal. Tout effet indésirable qui pourrait survenir après la prise de 2 comprimés de Nemasol Sodium^{R} serait transitoire et disparaîtrait rapidement.

Autres médicaments

A chaque visite à la clinique, un échantillon d'urine et de sang sont habituellement prélevés. Si vous prenez d'autres médicaments, une partie de ces échantillons pourraient être utilisée pour mesurer la concentration de ces médicaments et de leurs métabolites.

Génotype métabolique

Votre capacité à métaboliser les médicaments dépend des gènes que vous avez hérité de vos parents. Il est possible d'étudier des gènes spécifiques (au niveau de votre ADN) à partir d'un échantillon de sang. Pour ce faire, nous vous demandons de donner un échantillon de votre sang, d'environ 10 mL ou deux cuillerées à thé.

Collecte des données et confidentialité

Vous allez remplir un court questionnaire destiné à recueillir des informations qui seront utilisées pour interpréter les résultats de l'étude. Les chercheurs auront besoin d'avoir accès à votre dossier médical afin d'obtenir des informations additionnelles nécessaires à l'étude. Le questionnaire et les feuilles de collecte de données seront gardés de façon confidentielle à l'Hôpital Général de Montréal.

Toutes les données seront conservées sous le sceau de l'anonymat et aucune information par laquelle vous pourriez être identifié ne sera divulguée ou publiée. Les résultats de cette étude pourraient être publiés dans des journaux médicaux ou présentés à des congrès scientifiques. Dans un tel cas, les patients ne seraient jamais identifiés ou identifiables. A la fin de l'étude, vous pourrez, si vous le désirez, être informé des résultats obtenus et des conclusions formulées.

Avantages

Le but de cette étude est d'améliorer le traitement du SIDA en cherchant à comprendre pourquoi différents patients métabolisent les médicaments de façon différente. Votre participation à cette étude ne vous apporterait aucun bénéfice direct mais aidera les médecins et les scientifiques à choisir le meilleur traitement possible pour les patients atteints du SIDA.

Participation volontaire

Votre participation à cette étude est totalement volontaire. Vous êtes entièrement libre de ne pas participer à cette étude ou de retirer votre consentement à tout moment, sans pénalité ou perte d'avantages.

Si les résultats de la présente étude justifient une recherche encore pousée, vous serez contacté pour refaire le(s) test(s). Si tel est le cas, vous serez dûment informé de la recherche et votre participation sera, à nouveau, entièrement volontaire.

Consentement écrit

J'ai lu tout ce qui précède ainsi que les feuilles d'information destinées aux patients. J'ai posé des questions et reçu des réponses sur tout ce que je ne comprenais pas. Je consens donc, de mon plein gré, à participer à cette étude. Après avoir signé, je recevrai une copie de la présente formule de consentement et des feuilles d'information destinées aux patients.

J'accepte de participer à cette étude,

PATIENT _____ DATE _____

CHERCHEUR _____ DATE _____

TÉMOIN _____ DATE _____

APPENDIX IV

PATIENT QUESTIONNAIRE

MONTREAL GENERAL HOSPITAL

Patient Questionnaire

Investigators: Drs. I. W. Wainer , C. Tsoukas and J. Falutz

METABOLIC INDEX STUDY

Subject's Initials _____

Treating Physician _____

Date ____/____/____
dd mm yy

- When were you born ____/____/____ Age _____ years
  dd mm yy

- How would you classify your ethnic origin?

☐ White	Black:	☐ Asian
☐ Native American	☐ African-American	☐ Hispanic
☐ Arabic	☐ African	Other _____
	☐ West Indian	

- How tall are you? _____

- When were you first diagnosed with HIV? _____ ☐ N/A

- Are dapsone (Avlosulfon) or Septra (sulfamethoxazole/trimethoprim) currently prescribed for you?

  ☐ No

  ☐ Yes, please specify:

Medication	Dose/frequency	Time of last pill
_____	_____	_____

➢ Have either dapsone or Septra been prescribed for you in the past?

☐ No

☐ Yes, please specify:

Medication	Dose/frequency	When you stopped (month/year)

➢ In the last 24 hours have you taken any of the following medications (except those taken for the test)?

	Yes	No	If yes, please specify the brand
Cough syrup	☐	☐	
Pain medication such as aspirin, acetaminophen or ibuprofen	☐	☐	

➢ Are you currently (within the last month) taking any over the counter medications, vitamins, other supplements, herbal extracts, etc.? Please specify:

Product	Amount	Frequency

➢ How many coffees or other caffeine containing beverages (e.g. tea, hot chocolate, cola) do you usually have per day ?

0 ☐
1-4 ☐
more than 5 ☐

- Before performing this test, when was your last cup of coffee (or tea/cola)?

  Day _____ Time _____

- How many alcoholic drinks do you have per week?

  0-7 ☐
  8-14 ☐
  more than 14 ☐

- Do you smoke (tobacco)?

  No ☐
  Yes ☐ How long have you been smoking? _____ years
  How many cigarettes per day? _____

- Did you ever smoke?

  No ☐
  Yes ☐ When did you stop? ___/___ (month / year)
  For how long did you smoke? _____ yrs
  How many cigarettes per day? _____

- Does anyone with whom you live smoke?

  No ☐
  yes ☐ For how long, during your cohabitation, has he/she smoked? _____ years
  How many cigarettes per day? _____

- Do you use any other drugs?

  No ☐
  Yes ☐ Please specify drug(s), amount and frequency:

  _____

  _____

  _____

HÔPITAL GÉNÉRAL DE MONTRÉAL

Questionnaire destiné aux Patients

Chercheurs: Drs I. W. Wainer, C. Tsoukas et J. Falutz

ÉTUDE D'INDEX MÉTABOLIQUE

Initiales du patient _____

Médecin traitant _____

Date _____/_____/_____
jj mm aa

- Quand êtes-vous né _____/_____/_____ Age _____ ans
  jj mm aa

- Comment décririez-vous votre origine ethnique?

☐ Blanc	Noir:	☐ Asiatique
☐ Amérindien	☐ Africain-Américain	☐ Hispanique
☐ Arabique	☐ Africain	Autre _____
	☐ Antillais	

- Votre grandeur _____ cm ou pousses

- Quand votre premier diagnostic de séropositivité VIH a-t-il été posé? _____ ☐ N/A

- Avez-vous déjà pris de la dapsone (Avlosulfon) ou du Septra (sulphaméthoxazole/ triméthoprime) dans le passé?
  - ☐ Non
  - ☐ Oui, précisez:

Médicament	Posologie/fréquence	Date d'arrêté (m/a)
_____	_____	_____

- Prenez-vous actuellement de la dapsone ou du Septra?

  ☐ Non

  ☐ Oui, précisez:

Médicament	Posologie/fréquence	Heure de votre dernier comprimé
_____	_____	_____
_____	_____	_____

- Pendant les dernières 24 heures avez-vous pris l'un des médicaments suivants (hormis la prise des médicaments-test)?

	Oui	Non	Si oui, précisez la marque
Sirop contre la toux	☐	☐	_____
Analgésique comme l'aspirine, l'acétaminophène ou l'ibuprofène	☐	☐	_____

- Prenez-vous régulièrement (au cours du dernier mois) des médicaments hors-prescriptions, les vitamines, d'autres suppléments, des extraits herbacés, etc.? Précisez:

Produit	Quantité	Fréquence
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

- Habituellement, combien de cafés ou d'autres breuvages contenant de la caféine (ex. thé, chocolat chaud, cola) prenez-vous par jour?

  0 ☐
  1-4 ☐
  plus de 5 ☐

- Avant de commencer ce test, quand avez-vous pris votre dernier café (ou thé/cola)?

  _____ _____
  Jour Heure

- Combien de boissons alcoolisées prenez-vous par semaine?

  0-7 ☐
  8-14 ☐
  plus de 14 ☐

- Fumez-vous (tabac)?

  non ☐

  oui ☐ Vous avez fumé pendant combien de temps? _____ ans
  Combien de cigarettes par jour? _____

- Avez-vous fumé dans le passé?

  non ☐

  oui ☐ Quand avez-vous arrêté? _____
  mm/aa
  Vous fumiez pour combien de temps? _____ ans
  Combien de cigarettes par jour? _____

- Demeurez-vous avec quelqu'un qui fume?

  non ☐

  oui ☐ Depuis que vous vivez avec elle, cette
  personne fume depuis combien de temps? _____ ans
  Combien de cigarettes par jour? _____

- Prenez-vous d'autres drogues?

  non ☐

  oui ☐ Veuillez spécifier la (les) drogue(s), la quantité et la
  fréquence:

  _____

  _____

  _____

APPENDIX V

DATA COLLECTION FORM

MONTREAL GENERAL HOSPITAL

DATA COLLECTION FORM

METABOLIC PHENOTYPING STUDY

IDTC Treating physician_____

Patient's initials_____ **Patient's study number**_____

MGH Chart number_____

Date_____(d/m/yr) Date of Birth_____(d/m/yr)

- Weight _____kg _____lb
- Height _____cm _____in
- Body surface area _____m^2 G___ P___ A__

Social History

I Smoking

- ❑ Never used
- ❑ In past, but not presently
- ❑ Current use (specify) _____

II Alcohol use

- ❑ Never used
- ❑ In past, but not presently
- ❑ Light use (< 8 drinks/week)
- ❑ Moderate use (8-14 drinks/week)
- ❑ Heavy use (>14 drinks/week)

III Drug use

- ❑ None
- ❑ In past, but not presently
- ❑ Present use (specify) _____

HIV History

AIDS defining conditions

	No	Yes	Onset (m/yr)	Active
Candidiasis, bronchi, trachea, lungs, esophageal	❑	❑	_____	_____
Coccidioidomycosis	❑	❑	_____	_____
Cryptococcosis, extrapulmonary	❑	❑	_____	_____
Cryptosporidiosis, chronic intestinal (> 1 month's duration)	❑	❑	_____	_____
Cytomegalovirus disease (CMV)	❑	❑	_____	_____
Cytomegalovirus retinitis (loss of vision)	❑	❑	_____	_____
Herpes simplex: chronic ulcers (> 1 month's duration)	❑	❑	_____	_____
Histoplasmosis, disseminated or extrapulmonary	❑	❑	_____	_____
Isosporiasis, chronic intestinal (> 1 month's duration)	❑	❑	_____	_____

	No	Yes	Onset (m/yr)	Active
Lymphoma, specify:_____	❑	❑	_____	_____
Mycobacterium avium or M. kansasii disseminated or extrapulmonary	❑	❑	_____	_____
Mycobacterium tuberculosis, any site	❑	❑	_____	_____
Mycobacterium (other), disseminated or extrapulmonary	❑	❑	_____	_____
Pneumocystis carinii pneumonia (PCP)	❑	❑	_____	_____
Pneumonia, recurrent	❑	❑	_____	_____
Salmonella septicemia, recurrent	❑	❑	_____	_____
Toxoplasmosis of brain	❑	❑	_____	_____
Cervical cancer, invasive	❑	❑	_____	_____
Dementia, HIV-related	❑	❑	_____	_____
Encephalopathy, HIV-related	❑	❑	_____	_____
Kaposi's sarcoma (KS)	❑	❑	_____	_____
Nocardiosis	❑	❑	_____	_____
Progressive multifocal leukoencephalopathy(PML)	❑	❑	_____	_____
Strongyloidosis	❑	❑	_____	_____
Wasting syndrome due to HIV	❑	❑	_____	_____

HIV-associated conditions

	No	Yes	Onset (m/yr)	Active
Candidiasis vaginalis	❑	❑	_____	_____
Cervical dysplasia	❑	❑	_____	_____
Constitutional Sx (fever, diarrhea > 1 month)	❑	❑	_____	_____
Herpes simplex (oro-genital)	❑	❑	_____	_____
Herpes zoster, multidermal	❑	❑	_____	_____
Human papilloma virus (HPV)	❑	❑	_____	_____
Idiopathic thrombocytoponic purpura (ITP)	❑	❑	_____	_____
Oral candidiasis	❑	❑	_____	_____
Oral hairy leukoplakia (OHL)	❑	❑	_____	_____
Pelvic inflammatory disease (PID)	❑	❑	_____	_____
Persistent generalized lymphodenopathy (PGL)	❑	❑	_____	_____
Other _____	❑	❑	_____	_____

Date of HIV diagnosis _____ (d/m/yr)

CDC classification

❑ A1	❑ A2	❑ A3
❑ B1	❑ B2	❑ B3
❑ C1	❑ C2	❑ C3

CD4 _____(%) _____(cells/mm^3) Date: _____
CD8 _____(%) _____(cells/mm^3)

Medication History

Allergies No ❑ Yes ❑

Drug	Brief description of reaction
_____	_____
_____	_____
_____	_____
_____	_____

Prior PCP prophylaxis therapy No ❑
Yes ❑

Drug:_____ Dose/Frequency:_____ Start date:_____ End date:_____

Reason stopped: _____

Drug:_____ Dose/Frequency:_____ Start date:_____ End date:_____

Reason stopped: _____

Medications: (taken in the last month)

Medication	Dose	Indication	Start Date	Stop Date

Investigational Drugs: No ❑ Yes ❑

Specify:	Date started	Date stopped
_____	_____	_____
_____	_____	_____
_____	_____	_____