Chemistry for Sustainable Development in Africa

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The Metabolism of Antiparasitic Drugs and Pharmacogenetics in African Populations: From Molecular Mechanisms to Clinical Applications

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Abstract We characterised over 20 antiparasitic drugs with respect to the enzymes responsible for their metabolism. We showed that CYP2C8 is responsible for the metabolism of amodiaquine (ADQ) to desethylamodiaquine and identified a novel reactive metabolite catalysed by extrahepatic CYP1A1 and CYPIB1 which is giving us insights into possible ways of synthesising safer analogues of ADQ. Praziquantel (PZQ) was shown to be metabolised by CYP1A2 and 3A4, knowledge which is being used to explore the possibility of coadministering PZQ with known inhibitors of these enzymes in order to increase its bioavailability. From evaluating over 30 antiparasitic drugs for inhibition of major drug metabolising enzymes, 10 were shown to be potent inhibitors with a potential risk to cause metabolism based drug–drug interactions. The inhibitory effects of artemisinin and thiabendazole on CYP1A2 where further investigated in vivo and the effect of thiabedazole resulted in clinically relevant drug–drug interactions. We studied the genetic polymorphism of drug metabolising enzymes in African populations. We screened genes of 8 drug metabolising enzymes (CYP2B6, 2C9, 2C19, 2D6, FMO, NAT-2, GSTT and GSTM) for over 15 single nucleotide polymorphisms (SNPs) in 9 ethnic groups from across Africa (Ibo, Hausa and Yoruba of Nigeria, Luo, Kikuyu and Masai of Kenya, mixed Bantu volunteers from Tanzania, the Venda of South Africa, the Shona and San of Zimbabwe). Multivariate cluster analysis showed that Caucausian, Oriental and African populations show differential cluster groups, an indication that these major population groups are likely to metabolically handle medicines differently. Further studies led to the discovery of new genetic variants unique to populations of African origin such as CYP2D6*17. Clinical studies on the metabolism and elimination of efavirenz by the polymorphic CYP2B6 showed that

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African populations had a reduced capacity to dispose efavirenz and that patients homozygous for the CYP2B6*6 variant would require as low as half the dose given to Europeans to achieve the same safe and efficacious concentrations.

1 Introduction

In the discovery, development and clinical use of medicines, pharmacokinetics determines how much and how often drugs should be administered to patients for safe and efficacious outcomes. Pharmacokinetics is essentially the time course of a drug and its metabolites in the body and is characterised by the processes of absorption, distribution, metabolism and excretion (ADME). Of these processes, metabolism is the determinant of the clearance of over 75 % of drugs on the market. This makes understanding the biotransformation of drugs and mechanisms of regulation of drug metabolising enzymes important in chemotherapy. Major regulatory mechanisms of enzyme expression and activity have been shown to be induction, inhibition and genetic variability of genes coding for some of the enzymes. Genetic variability that results in variable response to medicines, pharmacogenetics, is pushing the practise of medicine from one-treatment-fits-all to individualised treatment where drugs will be given to people they are predicted to work and at doses they will be safe and efficacious based on their genetic status. The knowledge base and clinical applications of drug metabolism and pharmacogenetics is very advanced in developed countries and significantly lags behind in Africa.

In the 1980s, the laboratory of Professor Julia Hasler at the University of Zimbabwe identified this as a niche for research which could make important contributions towards the safe use of medicines in African populations. With funding from the International Science Programme (ISP), Sweden, www.isp.uu.se, her drug metabolism group started characterising the metabolism of antiparasitic drugs [16–18, 21, 22, 24] and the genetic polymorphism of drug metabolising enzymes in Zimbabweans [17, 18, 20, 21]. The theme of metabolic sciences has been sustained by IPICS funded activities by graduates from the drug metabolism group. Professor Yogi Naik went on to specialise in ecotoxicology at the National University of Science & Technology (NUST) in Zimbabwe. Dr. Stanley Mukanganyama focused on cancer chemotherapy and remained at the University of Zimbabwe. After many years in the pharmaceutical industry, Professor Collen Masimirembwa went on to establish the African Institute of Biomedical Science & Technology, AiBST, www.aibst.com which has drug metabolism and pharmacokinetics (DMPK) as one of its focus areas of research. The seed funding provided by IPICS catalysed a chain reaction of developments in Zimbabwe that has resulted in the training of many postgraduates, establishment of cutting edge research platforms and the conduct of biomedical research, which is beginning to have a clinical impact on Zimbabweans in particular and Africans in general.

The sciences of drug metabolism and pharmacogenetics represent a unique interplay of chemistry, enzymology and molecular biology. The results from our work presented in this chapter, therefore cover aspects of enzyme kinetics, molecular biology, molecular modelling through to clinical evaluations of some of our findings.

2 Metabolism of Antiparasitic Drugs

Most drugs used for the treatment of parasitic diseases were discovered more than 50 years ago, way before pharmacokinetics (PK) was an integral part of the drug discovery and development process. Most of them, therefore carry a number of PK related inadequacies as evidenced by complex dosing regimens and a host of adverse drug reactions associated with their use. Since few new antiparasitic drugs are coming on the market due to poor funding, as a stopgap measure, we decided to invest the modest resources we have into PK characterisation of the available antiparasitic drugs with a view to improving their clinical use. Over 20 antiparasitic drugs (Fig. 1) were evaluated with respect to major enzymes involved in their metabolism and their ability to inhibit the activity of drug metabolising enzymes. The in silico, in vitro and in vivo studies were conducted according to current practices in major pharmaceutical industry [26].

2.1 Identification of Enzymes Responsible for the Metabolism of Antiparasitic Drugs

This was done by a combination of three methods: (i) incubating each compound against a panel of seven major drug metabolising recombinant cytochrome P450 (rCYPs) (1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 3A4), (ii) incubating the compounds in human liver microsomes (HLM) and using selective and potent diagnostic inhibitors of each of the various major CYPs, and (iii) using a relative activity factor approach that combines the use of HLMs and rCYPs. These reaction phenotyping studies [14] indicated that 1–3 major CYPs can be involved in the elimination of each drug (Table 1). Knowing which enzyme(s) are involved in the elimination of a drug helps us understand how individuals vary in their ability to eliminate the drug based on our knowledge of the variability of expression and activity of the involved enzyme.

We have shown that amodiaquine is metabolised to desethylamodiaquine by the enzyme, CYP2C8, with high affinity, turnover, and selectivity [13, 14]. This work has resulted in FDA recommending amodiaquine N-deethylation as an acceptable marker reaction for in vitro studies of CYP2C8 in industry (http://www.fda.gov/cder/guidance/index.htm). Using a combination of in vitro and electrochemical oxidation
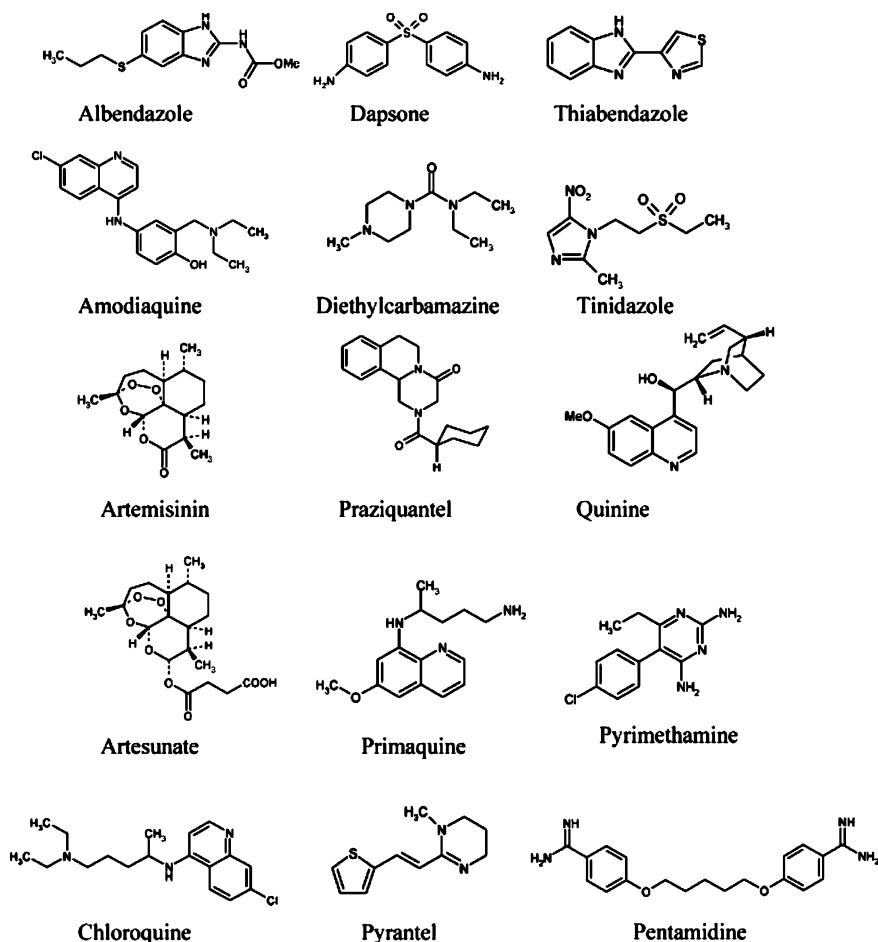
Fig. 1 Structures of some antiparasitic drugs whose metabolism was investigated in this study

methods followed by structural elucidation by MSMS and NMR, we also showed that amodiaquine is metabolised by extrahepatic CYP1A1 and 1B1 to a reactive aldehyde metabolite, Fig. 2, [9, 10, 13]. The reactivity of the metabolites was determined by trapping experiments with nucleophiles such as glutathione, N-acetyl cycsteine (NAC), and methoxyl amine (MOA). This led us to postulate that the two major adverse effects of amodiaquine, liver toxicity and agranulocytosis are caused by separate tissue specific metabolic bioactivation processes, liver toxicity by the bio-activation to the quinonimine metabolite and agranulocytosis by both the quinoni-mine and the aldehyde metabolite. We are, therefore, synthesising potentially safer ADQ analogues in which we aim to block these 2 bioactivation pathways.

The knowledge of enzymes involved in a drug's metabolism also helps us to understand and anticipate drug–drug interactions when a drug is given together

Table 1 Predicted hepatic clearance and relative contribution of major drug metabolising enzymes to the hepatic metabolic clearance of some antiparasitic drugs from in vitro experiments

Drug	Major disease treated	Predicted hepatic clearance CL_H = ml/min/kg	Percent (%) contribution of major drug metabolising cytochrome P450s in the metabolism of the antiparasitic drugs							
			1A2	2A6	2B6	2C8	2C9	2C19	2D6	3A4
Albendazole	Helminths	18.2	53	<10	<10	<10	<10	<10	<10	<10
Amodiaquine	Malaria	19.1	<10	<10	<10	67-100	<10	<10	<10	<10
Artemisinin	Malaria	9.8	<10	<10	10	<10	<10	<10	<10	25
Artesunate	Malaria	12.3	<10	100	<10	<10	<10	<10	<10	<10
Chloroquine	Malaria	1.4	<10	<10	<10	54	<10	<10	53	13
Dapsone	Malaria	5.0	<10	<10	<10	10	48	12	<10	31
Praziquantel	Schistosomiasis	16	39	<10	<10	<10	<10	14	<10	30
Primaquine	Malaria	5.3	60	<10	<10	<10	<10	<10	23	<10
Pyrental	Helminths	3.4	<10	<10	<10	<10	<10	<10	90	<10
Quinine	Malaria	6.1	<10	<10	<10	<10	<10	<10	<10	70
Thiabendazole	Helminths	17.9	100	<10	<10	<10	<10	<10	<10	<10
Tinidazole	Protozoa	1.1	<10	<10	12	<10	<10	<10	<10	77
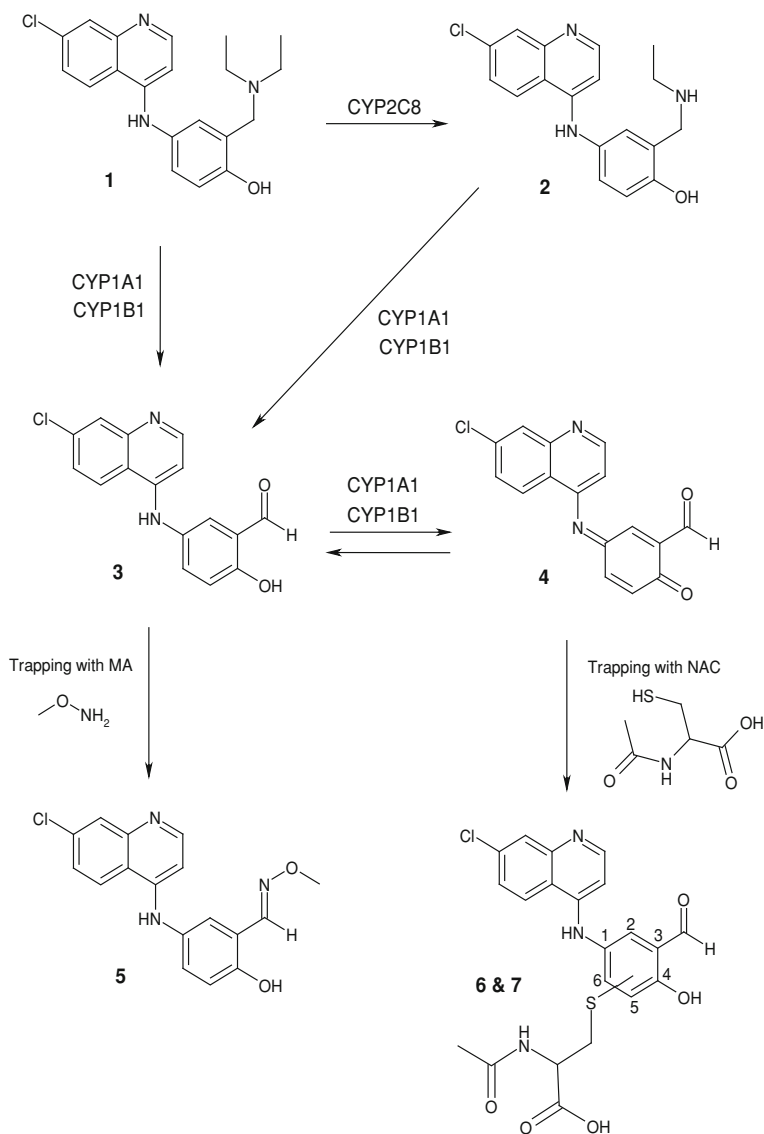
Fig. 2 Metabolic routes of amodiaquine and desethylamodiaquine observed in RLMs and HLMs and recombinant CYP2C8, CYP1A1 and CYP1B1. Trapping reactions were performed on metabolites formed in the rCYP incubations. All the metabolites and trapped adducts were also obtained in the electrochemical system

with another drug which inhibits or induces the major enzyme(s) that eliminate it. Our results on the role of CYP1A2 and CYP3A4 in the metabolism and elimination of praziquantel (PZQ), can explain why earlier combinations of PZQ with dexamethasone, a now-known CYP3A4 inducer, resulted in reduced PZQ levels in

patients being treated for neurocystecosis [32]. Our findings are also providing the rationale for current efforts to increase the oral bioavailability of PZQ by giving it together with grapefruit juice [5], a known inhibitor of CYP3A4. The use of metabolic drug–drug interactions to improve oral bioavailability has precedence in the coadministration of the CYP3A4 substrate drug cyclosporine with ketoconazole, a CYP3A4 potent inhibitor [11]. The clinical benefits of which have been the administration of a lower dose of the expensive immunosuppressant but achieving clinically effective concentrations. Another example is the use of protease inhibitor boosted regimens in which two protease inhibitors (both substrates and inhibitors of CYP3A4) are given together, one as an inhibitor of CYP3A4, retinovir, and the other as the therapeutic agent, e.g. amprenavir, resulting in prolonged exposure of the latter at therapeutically effective concentrations [33]. This strategy facilitated the development of once a day dosing regimens of the otherwise very rapidly cleared protease inhibitors.

2.2 Inhibition of Drug Metabolising Enzymes

In Africa, most patients are on more than one drug at any one time. This polypharmacy is usually due to the need to treat coinfections, the need to use drug combinations for improved efficacy and to avoid the emergence of drug resistance in the treatment of diseases such as malaria, TB and HIV/AIDS. Simultaneous exposure to many drugs predisposes a patient to drug–drug interactions, most of which are based on the inhibition or induction of drug metabolising enzymes. In the case of inhibition, one drug (the perpetrator) will inhibit the enzyme responsible for the elimination of another (the victim) resulting in the latter increasing in plasma concentration which can lead to increased adverse drug reactions. In the case of induction, the perpetrator increases the expression and activity of the enzyme(s) responsible for the elimination of the victim drug which can lead to the latter failing to reach therapeutic plasma levels. Subtherapeutic levels not only affect efficacy, but promote the emergence of drug resistance in the treatment of HIV, TB and malaria.

Our studies [2] indicate that of the over 30 drugs screened for inhibitory effect against 5 major CYPs, 1A2, 2C9, 2C19, 2D6, and 3A4, 10 showed in vitro potential for inhibition of either CYP1A2 or CYP2D6 (Table 2). Thiabendazole's inhibitory effects on CYP1A2 were studied in detail and were shown to cause mixed mode inhibition (competitive, non-competitive and time dependent (TDI) mechanism based inhibition (MBI)) [31]. We used molecular modelling studies to rationalise thiabendazole's route of metabolism and possible binding modes in the CYP1A2 active site associated with the various mechanisms of inhibition (Fig. 3). Two of the drugs, artemisinin and thiabendazole where further evaluated for inhibitory effects on CYP1A2 in vivo in humans. This was done using caffeine as an in vivo probe for CYP1A2 activity and volunteers took caffeine alone or in combination with thiabendazole or artemisinin [4]. The in vitro data was in good

Table 2 Inhibitory effects of antiparasitic drugs on major drug metabolising enzymes

| CYP and inhibitory compounds	Ki (μM)	Type of inhibition	Plasma concentrations (Cmax)	Predicted % inhibitory effects from in vitro data	Observed % inhibitory effects in vivo
CYP1A2					
Artemisinin	0.43	Competitive	1.38	76	66
Thiabendazole	1.54	Mixed	89	98	92
Primaquine	0.22	Competitive	0.44	67	ND
Dihydroartemisinin	3.67	Competitive	2.50	41	ND
CYP2D6					
Quinine	15.51	Competitive	15.41	50	
Chloroquine	12.68	Competitive	0.39	<10	ND
Amodiaquine	2.1	Competitive	0.074	<10	ND
Desethylamodaiquine	4.13	Mixed	0.444	<10	ND
Proguanil	6.76	Mixed	0.76	<10	ND
Cycloguanil	5.97	Competitive	0.21	<10	ND

ND - not determined
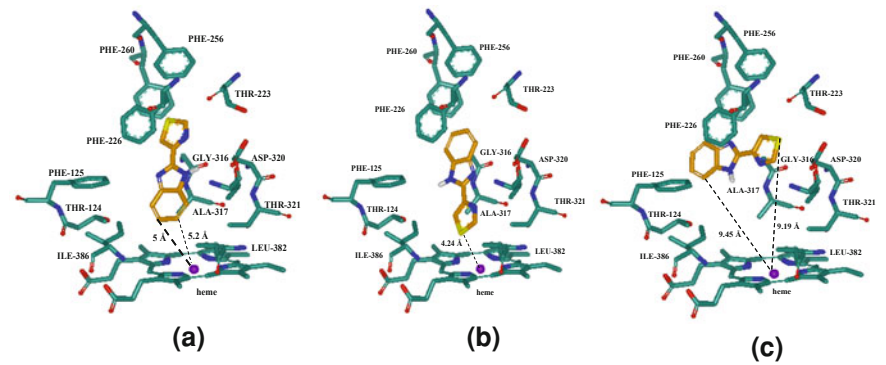
Fig. 3 Examples of different orientations in which thiabendazole docks into the active site of CYP1A2. The docking experiment was performed in GLUE. In 5 of the top 10 ranked solutions, the thiazole group was the group closest to the haem (**b**); in 3 solutions, the benzene ring in which hydroxylation occurs was closest (**a**); and in 2 solutions, both groups were further away (**c**). Interactions of the benzene or thiazole moiety of thiabendazole with phenylalanine 226 of CYP1A2 seems to be important in determining the orientation of thiabendazole in the enzyme active site

agreement with in vivo observations which gives credibility to the predictive power of the in vitro systems we use. Our data on the inhibitory effects of thiabendazole explains earlier clinical observations of an interaction between thiabendazole and theophylline and proposition of a 50 % theophylline dose reduction in asthma patients also taking thiabendazole [12]. Knowing the mechanistic basis for this interaction will now enable us to predict potential drug–drug interactions involving thiabendazole and other CYP1A2 substrate drugs.

3 Pharmacogenetics of Drug Metabolism

Based on our understanding that DNA is the blue print of life, the international community embarked on a project to sequence the whole human genome, a feat which was completed in 2003. The human genome is composed of 3 billion base pairs, about 25,000 genes, and approximately 10–30 million single nucleotide polymorphism (SNPs) (www.hugo.org). Genetic variability is thought to be the mechanism that facilitates the evolutionary process, where organisms with certain genetic status survive or succumb to some environmental selection pressures. For humans, this is supported by a number of known genetic variants that have been associated with disease susceptibility/resistance, with longevity, and with variable ability to tolerate potentially poisonous chemical exposures. Our work focuses on how genetic variability can affect our responses to medicines, a field of study referred to as pharmacogenetics. As indicated before, levels of drug exposure (PK) will determine the drug's pharmacological effect (PD). Since metabolism is the key determinant of the PK of most drugs, genetic variability of genes coding for drug metabolising enzymes could result in altered PK of a drug and subsequently affect the pharmacological effects of the affected drugs.
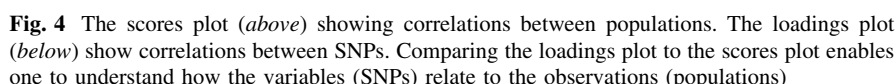
Genetic variation in the major drug metabolising enzymes such as CYP2D6, 2C9, 2C19, 2B6, FMO, and NAT-2 has been shown to influence the plasma concentrations of drug substrates and has been associated with increased incidences of adverse drug reactions in carriers of defect alleles. Most such studies have been done in Caucasian populations of Europe and North America and an increasing body of knowledge is being published for Asian populations. Our work, therefore sought to establish the status of some of these polymorphisms in African populations.

Following a workshop organised by AiBST on pharmacogenetics of drug metabolism in 2003 (the year the completion of the human genome was announced) in Nairobi, African scientists from six different countries (Nigeria, Kenya, Tanzania, Zimbabwe, Uganda, and South Africa) formed a consortium for biobanking and pharmacogenetic databasing in African populations. Samples from volunteers were subsequently collected from nine ethnic groups (Yoruba, Ibo, Hausa, Luo, Masai, Kikuyu, Shona, San, Venda and other mixed Bantu populations) and screened for genetic variants of key drug metabolising enzymes (Table 3). The data was subjected to multivariate analysis (Fig. 4) and clearly showed that the frequency of many genetic variants clusters Caucasian, Oriental and African populations into distinct groups [7, 8, 15–18, 21, 24, 28]. The general implication of this observation is that for drugs metabolised by these enzymes, the capacity to metabolise and eliminate them will differ significantly among these three major population clusters. This has implications for the use of medicines discovered and optimised for clinical use in Europe and then used in African populations [23].

The molecular epidemiological studies were followed by mechanistic investigations which lead to the discovery of a number of novel variants of CYPs and NAT, some of them unique to the African populations. Based on the phenotypic

Table 3 Genetic polymorphisms (% allele frequencies) of major drug metabolising enzymes in major African populations compared to Caucasian and Oriental populations

	CYP2C19		CYP2D6								NAT2				GST		CYP2B6	FMO		
	*2	*3	*2/2	*3	*4	*5	*10	*9	*17	*29	*5	*6	*7	*14	M1 del/del	T1 del/del	*6	K158	M257	G308
Orientals	30	10	2	0	1	6	51	0	0	0	5	25	13	0	55	65	18	–	–	–
Chinese	37	8	1	0	1	6	51	0	0	0	6	31	16	0	58	53	21	22.9	20.3	14.8
Japanese	35	11	1	0	1	3	43	0	0	0	2	19	10	0	44	44	16	22.7	14.5	21.0
Koreans	21	12	0	0	2	6	51	0	0	0	3	19	11	0	53	60	15	18.9	–	18.3
Caucasian	15	0	5	2	25	5	2	2	0	0	49	27	2	0	50	15	21	–	–	–
Swedes	17	0	1	3	23	5	1	0	0	0	51	28	2	0	51	20	–	44.3	7.1	22.4
Germans	18	0	2	2	20	2	2	0	0	0	46	27	4	0	51	21	–	42.6	6.9	22.5
American	14	0	2	–	–	–	–	–	0	–	45	28	2	0	54	15	–	39-53	6.7-22	15-22
Mix African	16	1	2	0	2	4	6	0	30	15	34	20	5	13	30	–	40	–	–	–
African American	25	0	1	0	7	6	4	1	15	5	30	22	2	9	28	24	47	41-52	5-7	0-5.2
Tanzanian	18	1	3	0	2	4	4	0	18	20	34	21	3	13	33	25	39	–	–	–
Shona	13	0	2	0	2	4	6	0	34	17	31	21	6	14	24	26	38	50	2	1.5
Venda	21	0	–	0	3	5	12	0	24	6	39	22	5	11	23	20	36	48.4	2.4	1.6
Ghanaian	–	–	2	0	7	6	3	0	28	–	–	–	–	–	39	–	49	–	–	–
Ethiopians	14	2	15	0	4	3	9	0	9	–	–	–	–	–	–	–	–	–	–	–
Kikuyu	16	0	–	0	1	–	–	0	33	14	58	24	–	–	28	25	34	49	4.6	1.0
Luo	18	0	–	0	4	–	6	0	23	16	34	22	3	14	29	22	37	50	4.5	0
Maasai	11	0	–	0	8	–	5	0	18	8	42	27	4	9	16	40	35	42	4.2	0
Igbo	29	0	–	0	8	–	10	0	14	20	28	29	4	11	23	36	38	43.9	0.5	0.5
Yoruba	10	0	–	0	3	–	7	0	22	10	33	27	3	8	31	35	42	52	1.0	0.5
Hausa	12	0	–	0	2	–	13	0	18	10	27	33	3	3	37	42	42	44	4.0	0.5
San	12	–	–	–	9	–	–	0	22	2	20	8	–	–	45	–	40	33.3	0.0	0.8

Fig. 4 The scores plot (*above*) showing correlations between populations. The loadings plot (*below*) show correlations between SNPs. Comparing the loadings plot to the scores plot enables one to understand how the variables (SNPs) relate to the observations (populations)

observations that African populations had reduced capacity to metabolise CYP2D6 substrate, sequence analysis led to the discovery of a novel variant, CYP2D6*17 (originally called CYP2D6*Z in recognition of its discovery in our work on Zimbabweans) [17, 18, 21]. The enzyme kinetic impact of key amino acid changes associated with this variant, T107I, R296C, and S486T was investigated using both in silico modelling and in vitro metabolism [3, 30] and shown to result in reduced affinity for CYP2D6 in a substrate dependent manner. Many publications have now demonstrated that CYP2D6*17 is the molecular basis of reduced CYP2D6 function in all populations of African origin [1]. Our group has also discovered variants of NAT-2 [6] and of CYP2C19, 2C5 and NAT-2 ([27], www.cypalleles.ki.se) whose functional significance is yet to be established.

Efavirenz, is a non-nucleoside analogue HIV reverse transcriptase inhibitor (NNRTI) which is part of the highly active antiretroviral therapy (HAART) being used to treat HIV/AIDS. It is normally used in patients who will have shown adverse drug reactions to nevirapine, a cheaper NNRTI and in patients who will be under treatment of both HIV/AIDS and TB. The latter use is done to avoid the drug–drug interactions between nevirapine, a mainly CYP3A4 substrate and rifampicin, a component of the TB treatment regimen which is a potent inducer of CYP3A4. Efavirenz on the other hand is mainly metabolised by CYP2B6 which is associated with reduced metabolic interaction with rifampicin. Genetic polymorphism of CYP2B6 has been associated with high plasma levels of efavirenz and increased incidence of CNS adverse drug reactions in patients homozygous for the
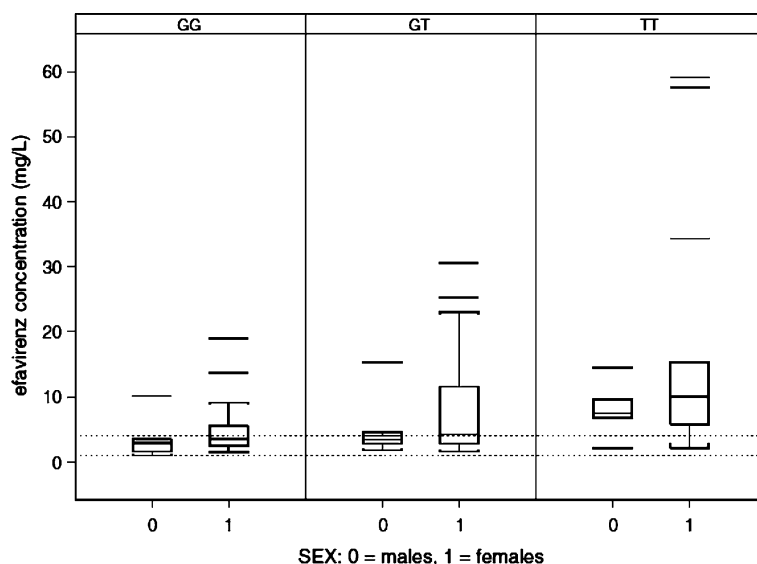
Fig. 5 Box and whiskers plot showing the observed efavirenz plasma concentrations at steady state in 71 HIV/AIDS patients of Zimbabwean origin. GG extensive metabolizer, GT intermediate metabolizer, TT poor metabolizer. The dotted horizontal lines show the optimum concentration interval (1–4 mg/L)

CYP2B6*6 variant. Following our observation that up to 20 % of African people (in contrast to <5 % in Caucasian and Oriental populations) were homozygous for CYP2B6*6 (Table 3), we conducted studies to evaluate the clinical impact of this polymorphism in HIV/AIDS patients.

Over 50 % of the 71 patients had concentrations above the minimum toxic concentration (MTC) of 4 μg/ml and none were below the minimum effective concentration (MEC) of 1 μg/ml. There was a clear gene dose concentration relationship with CYP2D6*1/*1, *1/*6, and *6/*6 patient groups showing increasing plasma concentrations. We also observed that in each genotype category, women had much higher concentrations than the men (Fig. 5). We conducted pharmacokinetic simulations to estimate the likely impact of CYP2B6 genotype on efavirenz dose requirements. The data indicated that patients homozygous for the CYP2B6*6 genotype might require 300 mg/day instead of the standard dose of 600 mg/day [29]. Based on this prediction, we are now conducting a large clinical study to evaluate the effect of demographical, physiological, and genetic factors on efavirenz dosing. If we observe similar results, it could be the basis to introduce a genetic pharmacodiagnsotic test and dosing algorithm to guide the use of efavirenz in Zimbabwe and other African countries.

4 Tools for Drug Discovery and Development Research

In the process of characterising the DMPK properties of antiparasitic drugs and demonstrating the utility of such information in the clinical use of these drugs, AiBST has also setup a platform for the integration of DMPK in the discovery and development of new chemical entities against infectious diseases. We now have in silico and in vitro methods for the prediction and measurement of physicochemical parameters such as solubility, lipophilicity (logP), ionisability (pKa), protein binding, blood/plasma partitioning and ADME parameters such as permeability, volume of distribution, metabolic stability, enzyme and metabolite identification, and enzyme inhibition. We have applied this platform in the characterisation of over 100 compounds from various drug discovery projects in Africa and for WHO–TDR funded discovery projects. The data is used to either guide the design of new chemical entities predicted to have better PK or towards the design of the first-time in men dose finding and drug–drug interactions studies (Masimirembwa et al. 2002) [19, 25, 26]. WHO–TDR has recognised our expertise and the utility of this platform and has nominated AiBST as DMPK Centre of Excellence.

The work on pharmacogenetics has evolved towards the development of a Biobank of African populations [28]. The Biobank will be used as a biomedical research tool towards healthcare solutions tailored for African populations. It will be useful in early stages of drug discovery in target identification and validation where the pharmaceutical industry needs to ensure that molecular drug targets against which hits, leads and candidate drugs are discovered do not have genetic variability that might result in the drug not working in some people. The tool will also be used to design clinical studies that evaluate exposure levels and possible adverse drug reactions profiles in subjects who might be carriers of genetic defects in ADME genes for a new chemical entity under investigation. Our work has also been a wake-up call to Research & Ethics committees in Africa to upgrade their basic and clinical research guidelines to address the potentially complex ethical and intellectual property issues that come with genomic research.

5 Conclusion

Work from our laboratory demonstrates that with modest but long term funding that focuses on human resource development (through postgraduate training) and research capacity strengthening (through purchase of equipment and support of effective South–North collaborations), Africa can achieve international standards in biomedical research. As this is being achieved, there is now a need to increase research collaborations among African institutions towards sharing expertise acquired over many years of North–South collaborations.

References

1. Akililu E, Dandara C, Bertilsson L et al (2007) Pharmacogenetics of cytochrome P450s in African populations: clinical and molecular evolutionary implications. In: Suarez-Kurtz G (ed) Pharmacogenomics in admixed populations, Ed. Landes Bioscience, Germany (ISBN 978-1-58706-130-9)
2. Bapiro TE, Egnell AC, Hasler JA et al (2001) Application of higher throughput screening (HTS) inhibition assays to evaluate the interaction of antiparasitic drugs with cytochrome P450s. Drug Metab Dispos 29:30–35
3. Bapiro T, Hasler JA, Ridderström M et al (2002) The molecular and enzyme kinetic basis for the diminished activity of the cytochrome P450 2D6*17 variant. Implications for CYP2D6 phenotyping studies and the clinical use of substrate drugs in some African populations. Biochem Pharmacol 7422:1–12
4. Bapiro TE, Sayi J, Hasler JA et al (2005) Artemisinin and thiabendazole are potent inhibitors of cytochrome P450 1A2 (CYP1A2) activity in humans. Eur J Clin Pharmacol 61:755–761
5. Castro N, Jung H, Medina R et al (2002) Interaction between Grapefruit Juice and Praziquantel in Humans. Antimicrob Agents Chemother 46:1614–1616
6. Dandara C, Masimirembwa C, Magimba A et al (2002) Arylamine N-acetyltransferase (NAT2) genotypes in Africans: the identification of a new allele with nucleotide changes 481C > T and 590 > A. Pharmacogenetics 12:1–4
7. Dandara C, Masimirembwa CM, Magimba A et al (2001) Genetic polymorphism of CYP2D6 and CYP2C19 in east- and southern African populations including psychiatric patients. Eur J Clin Pharmacol 57:11–17
8. Dandara C, Sayi J, Masimirembwa CM et al (2002) Genetic polymorphism of cytochrome P450 1A1 (Cyp1A1) and glutathione transferases (M1, T1 and P1) among Africans. Clin Chem Lab Med 40:952–957
9. Johansson T, Jurva U, Grönberg G et al (2009) Novel metabolites of amodiaquine formed by CYP1A1 and CYP1B1: structure elucidation using electrochemistry, mass spectrometry, and NMR. Drug Metab Dispos 37:571–579
10. Jurva U, Holmén A, Grönberg G et al (2008) Electrochemical generation of electrophilic drug metabolites: characterization of amodiaquine quinoneimine and cysteinyl conjugates by MS, IR, and NMR. Chem Res Toxicol 21:928–935
11. Keogh A, Spratt P, McCosker C et al (1995) Ketoconazole to reduce the need for cyclosporine after cardiac transplantation. N Engl J Med 333:628–663
12. Lew G, Murray WE, Lane JR et al (1989) Theophylline-thiabendazole drug interaction. Clin Pharm 8:225–227
13. Li XQ, Björkman A, Andersson TB et al (2002) Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate. J Pharmacol Exp Ther 300:399–407
14. Li XQ, Björkman A, Andersson TB et al (2003) Identification of human cytochrome P(450)s that metabolise antiparasitic drugs and predictions of in vivo drug hepatic clearance from in vitro data. Eur J Clin Pharmacol 59:429–442
15. Mao M, Matimba A, Scordo MG et al (2009) Flavin-containing monooxygenase 3 polymorphisms in 13 ethnic populations from Europe, East Asia and sub-Saharan Africa: frequency and linkage analysis. Pharmacogenomics 10:1447–1455

16. Masimirembwa C, Bertilsson L, Johansson I et al (1995) Phenotyping and genotyping of S-mephenytoin hydroxylase (cytochrome P450 2C19) in a Shona population of Zimbabwe. Clin Pharmacol Ther 57:656–661
17. Masimirembwa C, Hasler JA, Bertilsson L et al (1996) Phenotype and genotype analysis of debrisoquine hydroxylase (CYP2D6) in a black Zimbabwean population: reduced enzyme activity and evaluation of metabolic correlation of CYP2D6 probe drugs. Eur J Clin Pharmacol 51:117–122
18. Masimirembwa C, Persson I, Bertilsson L et al (1996) A novel mutant variant of the CYP2D6 gene (CYP2D6*17) common in a black African population: association with diminished debrisoquine hydroxylase activity. Br J Clin Pharmacol 42:713–719
19. Masimirembwa CM, Bredberg U, Andersson TB (2003) Metabolic stability for drug discovery and development: pharmacokinetic and biochemical challenges. Clin Pharmacokinet 42:515–528
20. Masimirembwa CM, Dandara C, Sommers DK et al (1998) Genetic polymorphism of cytochrome P4501A1, microsomal epoxide hydrolase, and glutathione S-transferases M1 and T1 in Zimbabweans and Venda of southern Africa. Pharmacogenetics 18:83–85
21. Masimirembwa CM, Gustafsson LL, Dahl ML et al (1996) Lack of effect of chloroquine on the debrisoquine (CYP2D6 and S-mephenytoin (CYP2C19) hydroxylation phenotypes. Br J Clin Pharmacol 41:344–346
22. Masimirembwa CM, Hasler JA (1994) Characterisation of praziquantel metabolism by rat liver microsomes using cytochrome P450 inhibitors. Biochem Pharmacol 48:1779–1783
23. Masimirembwa CM, Hasler JA (1997) Genetic polymorphism of drug metabolising enzymes in African populations: implications for the use of neuroleptics and antidepressants. Brain Res Bull 44:561–571
24. Masimirembwa CM, Hasler JA, Johansson I (1995) Inhibitory effects of antiparasitic drugs on cytochrome P450 2D6. Eur J Clin Pharmacol 48:35–38
25. Masimirembwa CM, Ridderström M, Zamora I et al (2003) Combining pharmacophore and protein modeling to predict CYP450 inhibitors and substrates. Methods Enzymol 357:133–144
26. Masimirembwa CM, Thompson R, Andersson TB (2001) In vitro high throughput screening of compounds for favourable metabolic properties in drug discovery. Comb Chem High Throughput Screen 4:245–263
27. Matimba A, Del-Favero J, Van Broeckhoven C et al (2009) Novel variants of major drug-metabolising enzyme genes in diverse African populations and their predicted functional effects. Hum Genomics 3:169–190
28. Matimba A, Oluka MN, Ebeshi BU et al (2008) Establishment of a biobank and pharmacogenetics database of African populations. Eur J Hum Genet 16:780–783
29. Nyakutira C, Röshammar D, Chigutsa E et al (2008) High prevalence of the CYP2B6 516G–>T(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. Eur J Clin Pharmacol 64:357–365
30. Oscason M, Hilderstrand M, Johansson I et al (1997) A combination of mutations in the CYP2D6*17 (CYP2D6Z) allele causes alterations in enzyme function. Mol Pharmacol 52:1034–1040
31. Thelingwani RS, Zvada SP, Hugues D et al (2009) In vitro and in silico identification and characterisation of thiabendazole as a mechanism-based inhibitor of CYP1A2 and simulation of possible pharmacokinetic drug–drug interactions. Drug Metab Dispos 37:1286–1294
32. Vazquez ML, Jung H, Sotelo J (1987) Plasma levels of praziquantel decrease when dexamethasone is given simultaneously. Neurology 37:1561–1562
33. Zeldin RK, Petruschke RA (2004) Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients. J Antimicrob Chemother 53:4–9