

**PHARMACOKINETICS AND METABOLISM STUDIES
OF ANTIFILARIAL DRUGS
DERIVATIVES OF BENZIMIDAZOLE CARBAMATE**

by

SURASH RAMANATHAN

**Thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy.**

November 1996

TABLE OF CONTENTS

	Page Numbers
Acknowledgement.....	ii
Publications	xii
List of Conferences.....	xiii
List of Abbreviation	xiv
Abstrak	xv
Abstract.....	xvii
Chapter 1: INTRODUCTION	1
1.1 General Introduction.....	1
1.2 The Pathology and Clinical Manifestation of Lymphatic Filarasis	3
1.2.1 The filarial life cycle	3
1.2.2 Clinical manifestation of lymphatic filariasis infection.....	7
1.3 Chemotherapy of Antifilarial Drugs.....	8
1.3.1 Introduction.....	8
1.3.2 DEC and ivermectin.....	9
1.3.3 Suramin.....	11
1.3.4 Benzimidazole carbamate.....	11
1.4 Mode of Action for Antifilarial Drugs.....	13
1.4.1 Introduction.....	13

1.4.2	Benzimidazole carbamate.....	14
1.4.3	Diethylcarbamazine	15
1.4.4	Suramin	16
1.4.5	Ivermectin.....	17
1.5	The Pharmacokinetic and Metabolism of Antifilarial Drugs	19
1.5.1	Introduction.....	19
1.5.2	Mebendazole.....	20
1.5.3	Albendazole.....	23
1.5.4	Flubendazole	27
1.5.5	Ivermectine	27
1.5.6	Diethylcarbamazine	28
1.5.7	Suramin	28
1.6	Drug Toxicity and Metabolism.....	29
1.7	Racemate and Drug Disposition.....	31
1.8	General Principles of Pharmacokinetics and Drug Metabolism.....	32
1.8.1	Introduction	32
1.8.2	Drug absorption	33
1.8.3	Drug distribution	34
1.8.4	Drug elimination.....	34
1.8.5	Drug excretion.....	36
1.9	Pharmacokinetics Parameters.....	37
1.9.1	Elimination half-life.....	37
1.9.2	Clearance.....	38
1.9.3	Volume of distribution.....	40

1.10 The Scope of This Study.....	41
Chapter 2: CHEMICALS, INSTRUMENT AND STANDARD.....	42
2.1 Chemicals.....	42
2.2 Equipment.....	43
2.3 Preparation of Standard Solutions and buffer.....	44
2.3.1 Standards	44
2.3.2 Extraction Buffer	45
2.4 Chemical Purity of Compounds	45
2.5 Radiolabelled Flubendazole	45
2.6 Solubility of UMF-078 and UMF-289 in various solvents	46
Chapter 3: DETERMINATION OF THE ANTIFILARIAL DRUG UMF-058 AND ITS METABOLITE, MEBENDAZOLE IN WHOLE BLOOD BY HIGH-PERFORMANCE LIQUID CEROMATOGRAPHY.....	48
3.1 Introduction	48
3.2 Determination of Approximate pKa value of UMF-058.....	49
3.3 UV Absorption of UMF-058 and MBZ	50
3.4 HPLC Method Development	50
3.4.1 Chromatography	50
3.4.2 Extraction procedure	51
3.4.3 Detector linearity.....	51
3.4.4 Standard curve.....	51
3.4.5 Analytical recovery, within-day and day-to-day precision	52

3.4.6	Quality control of UMF-058 and MBZ analysis.....	52
3.5	Results and Discussion.....	53
3.5.1	Determination of approximate pKa value of UMF-058.....	53
3.5.2	UV absorption of UMF-058 and MBZ	53
3.5.3	HPLC method development.....	54
3.6	Conclusion.....	55

Chapter 4: DETERMINATION OF THE ANTIFILARIAL DRUG UMF-078

**AND ITS METABOLITES UMF-060 AND FLUBENDAZOLE IN
WHOLE BLOOD USING HIGH PERFORMANCE**

	LIQUID CHROMATOGRAPHY.....	64
4.1	Introduction.....	64
4.2	Determination of approximate pKa value of UMF-078.....	65
4.3	UV Absorption of UMF-078, UMF-060 and FBZ	65
4.4	HPLC method development.....	66
4.4.1	Chromatography	66
4.4.2	Extraction procedure.....	66
4.4.3	Detector linearity.....	67
4.4.4	Standard curve.....	67
4.4.5	Analytical recovery, within-day and day-to-day precision.....	67
4.4.6	Quality control of UMF-078, FBZ and UMF-060 analysis.....	68
4.5	Results and Discussion	68
4.5.1	Determination of approximate pKa value of UMF-078.....	68

4.5.2	UV absorption of UMF-078, UMF-060 and FBZ.....	68
4.5.3	HPLC method development.....	69
4.6	Conclusion.....	70

**Chapter5: THE DETERMINATION OF DECARBOMETHOXY METABOLITES
OF UMF-078, UMF-060 AND FLUBENDAZOLE IN WHOLE
BLOOD USING HIGH PERFORMANCE LIQUID**

CHROMATOGRAPHY	79
5.1 Introduction.....	79
5.2 UV Absorption of The Various Decarbomethoxy Metabolites.....	80
5.3 HPLC Method Development.....	80
5.3.1 Chromatography.....	80
5.3.2 Extraction procedure.....	81
5.3.3 Detector linearity	81
5.3.4 Standard curve.....	81
5.3.5 Analytical recovery, within-day and day-to-day precision.....	82
5.3.6 Quality control of D-UMF-078, D-FBZ and D-UMF-060 analysis.....	82
5.4 Results and Discussion.....	83
5.4.1 UV absorption of D-UMF-078, D-FBZ and D-UMF-060.....	83
5.4.2 HPLC method development.....	83
5.5 Conclusion.....	84

Chapter 6: PHARMACOKINETIC AND EFFICACY STUDIES OF UMF-058 IN

BRUGIA MALAYI INFECTED MONKEYS (*PRESBYTIS*

<i>CRISTATA</i>)	93
6.1 Introduction.....	93
6.2 Material and Methods.....	94
6.2.1 Study design in infected monkeys	94
6.2.2 Blood sampling.....	94
6.2.3 Analysis of Blood	95
6.2.4 Pharmacokinetic calculation	95
6.2.5 Data analysis	96
6.3 Results.....	96
6.3.1 Pharmacokinetics of UMF-058 in infected monkeys following a single and multiple oral doses of UMF-058.....	96
6.3.2 The efficacy of single and multiple oral doses of UMF-058 against <i>B.malayi</i> in infected monkeys.....	97
6.4 Discussion.....	98
6.5 Conclusion.....	100

Chapter7: PHARMACOKINETIC STUDY OF UMF-078 IN HEALTHY

MONKEYS (*MACACA FASCICULARIS*): EFFECT OF

FORMULATION, ROUTES AND DOSE SIZE	107
7.1 Introduction	107
7.2 Materials and Methods.....	107
7.2.1 Study design in healthy monkeys.....	107
7.2.2 Drug administration	108

7.2.3	Blood sampling.....	108
7.2.4	Analysis of plasma	109
7.2.5	Pharmacokinetic calculations	109
7.2.6	Data Analysis	109
7.3	Results.....	109
7.3.1	Effect of formulation on UMF- 078 drug absorption	110
7.3.2	Effect of dose size on UMF-078 drug absorption	110
7.3.3	Effect of routes on UMF-078 drug absorption.....	112
7.4	Discussion.....	113
7.5	Conclusion.....	116

Chapter 8: PHARMACOKINETIC STUDY OF UMF-078 IN HEALTHY

	DOGS (BEAGLES): EFFECT OF FORMULATION AND	
	DOSE SIZE	128
8.1	Introduction	128
8.2	Materials and Methods.....	129
8.2.1	Study design in dogs.....	129
8.2.2	Blood sampling	130
8.2.3	Analysis of plasma.....	131
8.2.4	Pharmacokinetic calculations	131
8.2.5	Statistical analysis	131
8.3	Results	132
8.3.1	The pharmacokinetic properties of UMF-078 following intravenous administration of 20 mg/kg UMF-078 salt in healthy beagles.....	132

8.3.2	Effect of formulation on UMF-078 drug absorption in healthy beagles.....	132
8.3.3	Effect of various dose regimens of UMF-078 base on UMF-078 drug absorption in healthy beagles.....	133
8.3.4	Effect of oral dose increment of UMF-078 base from 150 mg/kg to 300 mg/kg on UMF-078 drug absorption in healthy beagles.....	134
8.4	Discussion.....	135
8.5	Summary.....	139

Chapter 9: THE DETERMINATION OF DECARBOMETHOXY METABOLITES

OF UMF-078, FBZ AND UMF-060 IN BLOOD AFTER ORAL

ADMINISTRATION OF UMF-078 IN RATS155

9.1	Introduction	155
9.2	Materials and Methods.....	156
9.2.1	Animals.....	156
9.2.2	Blood sampling.....	156
9.2.3	Blood assay procedure	157
9.2.4	Data analysis	157
9.3	Results.....	157
9.4	Discussion.....	158
9.5	Conclusion.....	160

Chapter 10: A PRELIMINARY INVESTIGATION OF UMF-078 AND FLUBENDAZOLE METABOLISM IN RATS.....	165
10.1 Introduction.....	165
10.2 Material and Method.....	166
10.2.1 Study Design.....	166
10.2.2 Surgical procedures for intravenous study.....	167
10.2.3 Drug administration and blood sampling	167
10.2.4 Drug analysis.....	168
10.2.5 Data Analysis.....	169
10.3 Results.....	169
10.4 Discussion.....	171
10.5 Conclusion.....	174
Chapter 11: CONCLUDING DISCUSSION.....	183
References.....	191

PUBLICATIONS

1. Ramanathan, S., Nair, N.K., Mansor, S.M. & Navaratnam, V. (1993). Determination of a new antifilarial drug, UMF-058, and mebendazole in whole blood by high performance liquid chromatography. *Journal of Chromatography* 615, 303-307.
2. Ramanathan, S., Nair, N.K., Mansor, S.M. & Navaratnam, V. (1994). Determination of the antifilarial drug UMF-078 and its metabolites UMF-060 and flubendazole in whole blood using high performance liquid chromatography. *Journal of Chromatography* 655, 269-273.
3. The pharmacokinetic of UMF-078 in healthy dogs: Effect of dose size and formulation. (In preparation).
4. The pharmacokinetic of UMF-078 in healthy monkeys: Effect of dose size, formulation and route. (In preparation).
5. The metabolic products of a new candidate antifilarial drug UMF-078 in rats. (In preparation).

LIST OF CONFERENCES

1. Ramanathan, S., Mansor, S.M., Mak, J.W., Mohamad, M., Nair, N.K. and Navaratnam, V. (1992). Determination of UMF-058 and mebendazole in whole blood using high performance liquid chromatography for application in pharmacokinetic study. 28th. Annual Scientific Seminar of the Malaysian Society of Parasitology and Tropical Medicine, Kuala Lumpur, Malaysia.
2. Ramanathan, S., Mak, J.W., Mansor, S.M., Nair, N.K., Navartnam, V. and Wernsdorfer, W.H. (1992). Pharmacokinetics of candidate antifilarial drug, UMF-058, in leaf monkey infected with *Brugia malayi*, 5th World Conference in Clinical Pharmacology and Therapeutics, Yokohama, Japan.
3. Ramanathan, S., Mansor, S.M., Nair, N.K. and Navaratnam, V. (1994). Pharmacokinetics of UMF-078 and its metabolites in dogs: effects of dose size and formulation. First International Congress of Parasitology and Tropical Medicine, Kuala Lumpur.
4. Ramanathan, S., Mansor, S.M., Mak, J.W., Nair, N.K. and Navaratnam, V. (1994). Pharmacokinetics of UMF-078 and its metabolites in monkeys: effects of formulation dose and route. First International Congress of Parasitology and Tropical Medicine, Kuala Lumpur, Malaysia.
5. Mansor, S.M., Ramanathan, S., Navaratnam, V. (1996). The metabolic products of a new candidate antifilarial drug UMF-078 in rats. XIVth International Congress for Tropical Medicine and Malaria, Nagasaki, Japan.

LIST OF ABBREVIATIONS

%	-	Percentage
±	-	Plus and minus
ABZ	-	Albendazole
AUC	-	Area under the curve
° C	-	Celcius
Cl	-	Clearance
C _{max}	-	Maximum plasma drug concentration
CV	-	Coefficient of variation
DEC	-	Diethylcarbamizine
D-FBZ	-	Decarbomethoxy metabolite of FBZ
D-UMF-060	-	Decarbomethoxy metabolite of UMF-060
D-UMF-078	-	Decarbomethoxy metabolite of UMF-078
F	-	Female
FBZ	-	Flubendazole
g	-	Acceleration due to gravity. Its value is 9.81 meter/second ²
HPLC	-	High performance liquid chromatography
i.m.	-	Intramuscular
i.v.	-	Intravenous
Kg	-	Kilogram
L	-	Litre
M	-	Male
MBZ	-	Mebendazole
mg	-	Milligrammes
mf	-	Microfilarial
min	-	Minutes
ml	-	Millitre
n	-	Number of observation
ng	-	Nanogrammes
p	-	Level of significance
QC	-	Quality Control
r	-	Correlation coefficient
S.D.	-	Standard Deviation
S.E.M.	-	Standard Error of the Mean
T _{1/2}	-	Half-life
T _{max}	-	Time to reach maximum drug concentration
μl	-	Microlitre
μg	-	Microgramme
uv	-	Ultraviolet
V _d	-	Volume of distribution

KAJIAN FARMAKOKINETIK DAN METABOLISME UBAT-UBATAN ANTIFILARIA TERBITAN BENZIMIDAZOLA KARBAMAT

ABSTRAK

Farmakokinetik dan metabolisme dua jenis drug anifilaria baru UMF-078 dan UMF-058 telah dikaji dalam model haiwan yang sesuai dengan perekaan bagi aspek penyerapan drug dan metabolisme. Kaedah-kaedah kromatografi cecair keupayaan tinggi yang spesifik dan sensitif bagi UMF-078, UMF-058 dan metabolitnya telah diperkembangkan. Kaedah-kaedah ini digunakan untuk mengkaji kesan formulasi, regimen dos dan cara pemberian drug ke atas penyerapan dalam model haiwan monyet dan anjing. Dalam monyet sihat, formulasi garam UMF-078 menunjukkan penyerapan yang lebih tinggi daripada bagi UMF-078 bes. Dalam kajian peningkatan dos, penyerapan drug sebanyak 3 1/2 kali ganda diperhatikan apabila dos ditingkatkan daripada 100 mg/kg ke 200 mg/kg. Penyerapan drug (AUC) melalui laluan otot didapati lebih kecil dan eratik dari laluan oral. Kesan formulasi dan regimen dos ke atas penyerapan drug UMF-078 juga dikaji bagi model anjing. Dalam anjing sihat, penyerapan drug UMF-078 bagi formulasi garam dan bes tidak berbeza. Penyerapan drug UMF-078 didapati bertambah apabila drug UMF-078 diberi sebagai pecahan dos berbanding (150 mg/kg x 2; 50 mg/kg x b.i.d. x 3) dengan dos tunggal (300 mg/kg). Kajian keseluruhan dengan monyet dan anjing juga menunjukkan bahawa UMF-060 dan flubendazola adalah metabolit minor. Farmakokinetik dan efikasi drug UMF-078 dalam monyet yang dijangkiti dengan cacing *Brugia malayi* juga dikaji. kedua-dua regimen dose tunggal dan berganda (50 mg/kg x 5) menunjukkan penyerapan drug

(AUC₀₋₁) yang hampir sama. kedua-dua regimen dos tunggal dan berganda didapati tidak menunjukkan aktiviti antifilaria ke atas cacing dewasa. Walau bagaimanapun, regimen dose berganda menunjukkan aktiviti antifilaria ke atas microfilaria.

Metabolisme drug UMF-078 juga dikaji dalam model haiwan tikus. UMF-078 dan metabolitnya UMF-060, FBZ mengalami hidrolisis karbamat. Kepentingan metabolit adalah dalam susunan berikut D-UMF-078 < D-UMF-060 < FBZ < UMF-060 < D-FBZ. Metabolisme drug UMF-078 dan FBZ juga dibandingkan dengan menggunakan model haiwan tikus. Dalam metabolisme drug UMF-078, metabolit seperti D-UMF-060, UMF-060 dan D-FBZ, di kesan dalam darah tikus yang diberi drug UMF-078. Sebaliknya produk-produk ini tidak dijumpai dalam tikus yang diberi drug FBZ. Perhatian ini mencadangkan bahawa, ada kemungkinan metabolit ini terbit daripada tapak jalan metabolisme UMF-078 yang lain dan bukannya daripada flubendazola. Kajian pra-klinikal ini keseluruhannya menambahkan lagi pengetahuan asas tentang drug UMF-078 yang boleh membantu dalam penyelidikan seterusnya.

ABSTRACT

The pharmacokinetics and metabolism of two new antifilarial drugs, UMF-078 and UMF-058 have been investigated in suitable animal models with special emphasis on drug absorption and drug metabolism, the latter was to determine the possible metabolic products following metabolism in rats. Specific and sensitive high performance liquid chromatography techniques (HPLC) for UMF-078, UMF-058 and their respective putative metabolites has been developed. The availability of these HPLC techniques further facilitated investigation of the effects of dose regimens, formulation and routes on drug absorption in monkeys and dogs. In healthy monkeys, UMF-078 absorption was relatively better for UMF-078 salt than of UMF-078 base. With respect to dose escalation study of UMF-078 base a 3 1/2 fold increment in UMF-078 absorption (AUC) was encountered when the dose was doubled from 100 to 200 mg/kg. The effect of routes (i.m. vs. oral) on drug absorption in monkeys was also investigated. Following i.m. administration UMF-078 absorption was observed to be poor and erratic when compared to oral administration of the same dosing schedule animals.

Studies were also initiated in dogs to investigate the effect of formulation and dose size on drug absorption. The overall absorption of UMF-078 is similar in group of animals receiving either UMF-078 salt or UMF-078 base. UMF-078 absorption was found to increase when the drug was given as divided dose (150 mg/kg x 2; 50 mg/kg x b.i.d. x 3) as compared to those administered with a single bolus dose of 300 mg/kg of UMF-078. In addition studies in dogs and monkeys also tend to suggest that FBZ and UMF-060 were not the major metabolites of UMF-078.

The pharmacokinetics and efficacy of UMF-058 in monkeys infected with *Brugia malayi* were also investigated. The overall UMF-058 oral absorption (AUC_{0-t}) was similar for both single (250 mg/kg) and multiple dose regimen (50 mg/kg x 5). Neither single nor multiple oral dose regimens demonstrated adulticidal activity against *B. malayi* in infected monkeys. However, multiple dose regimen of UMF-058 was microfilaricidal against *B. malayi*. The metabolism of UMF-078 was investigated in rats. UMF-078 and its metabolites FBZ and UMF-060 all undergo carbamate hydrolysis. The importance of these metabolites were found in the order of D-UMF-078 < D-UMF-060 < FBZ < UMF-060 < D-FBZ. A comparative metabolism study of UMF-078 and FBZ in rats were also initiated. D-UMF-060, UMF-060 and D-FBZ were encountered in blood of rats having received UMF-078 but not in animals treated with FBZ. This tend to suggest that these metabolites probably derived from other alternative pathway of UMF-078 rather than via FBZ. The pre-clinical information described has improved our understanding of UMF-078 and thus provides the basis for further evaluation of this new candidate antifilarial compound.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Filariasis was probably known as early as 600 - 250 BC as people with elephantiasis were excluded from the Buddhist priesthood (Laurence, 1967). Microfilariae probably that of *Wuchereria bancrofti* origin was first noted in the hydrocoele fluid of a man who originated from Havana (Demarquay, 1863) subsequently in the urine of haematuric patients in Brazil (Wucherer, 1868) and in human blood in India (Lewis, 1872). Manson, (1899) also first observed the characteristic of nocturnal periodicity in microfilaria of *W. bancrofti* and subsequently describe the localization of microfilaria in lungs and cardiac muscles during the day. Manson, (1878) demonstrated the occurrence of microfilariae in the blood of patients with elephantiasis and latter associated this and other clinical signs of lymphatic obstruction with the infection. Low (1900) on the basis of histological studies on infected mosquitos was the first to propose correctly that human are infected following a mosquito bite. Filariasis is a disease caused by nematodes belonging to the superfamily filarioidea, family onchocercidae. Parasite known to cause human infections belong mainly to genera *Wuchereria*, *Brugia*, *Onchocerca*, *Dipetalonema*, *Mansonella*, *Loa* and *Dirofilaria*. (Manson *et al.*, 1982; Ottesen, 1987). Only *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* are known to cause human lymphatic filariasis. The adults of these three species normally live in the lymphatic system and thus are commonly said to produce lymphatic filariasis. *W. bancrofti* and

B. malayi are mainly responsible for human filariasis in Malaysia and the surrounding countries.

Filariasis is a morbid condition disease which leads to considerable socio economic effects. Clearly, people with gross elephantiasis are severely impaired both physically and socially and the prevalence of elephantiasis alone makes lymphatic filariasis an important public health problem. Lymphatic filariasis infection is most prominent in subtropical and tropical regions of the world. To date an estimated 78.9 million are infected, as evidenced by microfilariae in blood samples and clinical symptoms. Of this figure, 72.8 million are infected by *W. bancrofti* and 5.8 million with *B. malayi* or *B. timori* (Ramachandran, 1994). Ideally for any control programme to be effective, the community must be acquainted with not only the modern tools and techniques used but also an acceptable way of thinking about the causation of the disease and its treatment. A successful programme for the control of lymphatic filariasis is usually based on a thorough understanding of its distribution and the dynamics of the disease in the aimed populations. However, the diverse demographic environmental and socioeconomic characteristics of population in endemic areas of lymphatic filariasis as well as differences in vector, parasite and disease parameters, do not allow a simple, uniform approach for control programmes to be implemented. Hence, each of the control strategies need to be tailored to the unique circumstances of a particular affected population and also its environment and ecology. Unfortunately, in most of the endemic areas of the world, there is at present no effective filariasis control. The main reasons are filariasis is not accepted as a public health priority by health officials and most control programmes are too complicated and costly to be sustained. Methods used to control this vector borne disease such as the use of insecticides have

demonstrated vague results (Chiang *et al.*, 1989). This being mainly due to the resistance developed by these mosquitos towards insecticides. Biological control which is an innovative method of controlling this vector borne disease is still in the early stages. For instance *Bacillus sphericus* a biological insecticide is currently effectively used agains *Culex* species for large scale control (Arunachalam *et al.*, 1991). Still more work need to be done to assess the possibility of using this method to disrupt the filarial vector in the remaining endemic areas. However, it might be more appropriate to emphasize on the development and implementation of simple, cost-effective and sustainable programmes for morbidity control. In view of this, chemotherapy plays a key role in controlling lymphatic filariasis. The situation has stimulated research into better and more effective usage of the currently available drugs and development of new antifilarial agents.

1.2 The pathology and clinical manifestation of lymphatic filariasis.

1.2.1 The filarial life cycle

In order to develop control strategies for this disease it is essential to delineate the host, parasite and vector relationship by knowing the lifecycle of filariasis (Fig.1.1). The life cycle of *B. malayi* and *W. bancrofti* are essentially similar. The adult worms normally resides in the lymphatic systems of infected humans. Following mating, female worms release blood circulating microfilariae into surrounding tissue. The microfilaremic host then serves as the source of infection to vector mosquitoes which depending on the locality belong to either or a combination of the genera mansonina, anopheles, culex and aedes. Following mosquito bite, microfilariae is taken up to the thoracic muscles of the mosquito, where the parasite that undegoes development becomes thicker and shorter than its initial size. The first stage of larva (L1) at 2.5 days has an average length of

143.7 ± 3.8 µm and a maximum width of 16.5 ± 0.7µm. The development of L1 to second stage larva (L2) occurs at about day 5. The length and maximum width of this L2 at day 5.5 are 441 ± 27 µm and 29.4 ± 1.8 µm respectively. The L2 moults again to become L3 or infective stage by the 9th to 10th day of entry following mosquito bite. L3 has a mean length of 1503 ± 40.6µm and a maximum width of 30.5 ± 0.7 µm at day 10.5. L3 at maturity, migrates mainly to the proboscis but can be seen also in another parts of the insect body. It is responsible for the propogation of disease and may take several months to a year to become an adult worm. When an infective mosquito bites a susceptible host, L3 actively migrate into the wound and travel to the different lymphatics and subcapsular sinus. In the final host, L3 moults again to the fourth stage (L4) by 9 to 10 days post-entry . In experimentally infected leaf monkey *Presbytis melalophos* at two weeks post infections, *B. malayi* female larvae L4 have a mean length of 4710 ± 214.3 µm and a maximum width of 38.6 ± 2.7 µm . The male L4 larvae measures 4236 ± 120.8 µm and 37.4 ± 1.9 µm respectively (Mak, 1983). The final development of L4 to adult worms occurs approximately 35 - 40 days after initial infection (Edeson & Buckley, 1959). *B. malayi* juvenile adult worms in *P. melalophos* have an average lengths of 13.7 ± 0.3 mm and 12.6 ± 0.5 mm in females and males respectively (Mak, 1983). Sexual maturity occurs two to three months after infection in *B. malayi*. Variability in mean prepatent period are noted in both *B. malayi* and *W. bancrofti* species of filarial parasite in different hosts. For instance, the prepatent peiod for *B. malayi* in *P. melalophos* is about 67 days, whereas in cats and jirds it is 80 days (Edeson & Wharton, 1957; Ash & Riley, 1970). The prepatent period for *W. bancrofti* in monkeys is about 8 - 18 months (Cross *et al.*, 1979). *W. bancrofti* demonstrated a longer prepatent period in man (11 months) (Colwell *et al.*, 1970) than *B. malayi* which

is about 3.5 months (Edeson & Wharton, 1957). However, variation in mean prepatent period were observed in both *B. malayi* and *W. bancrofti*.

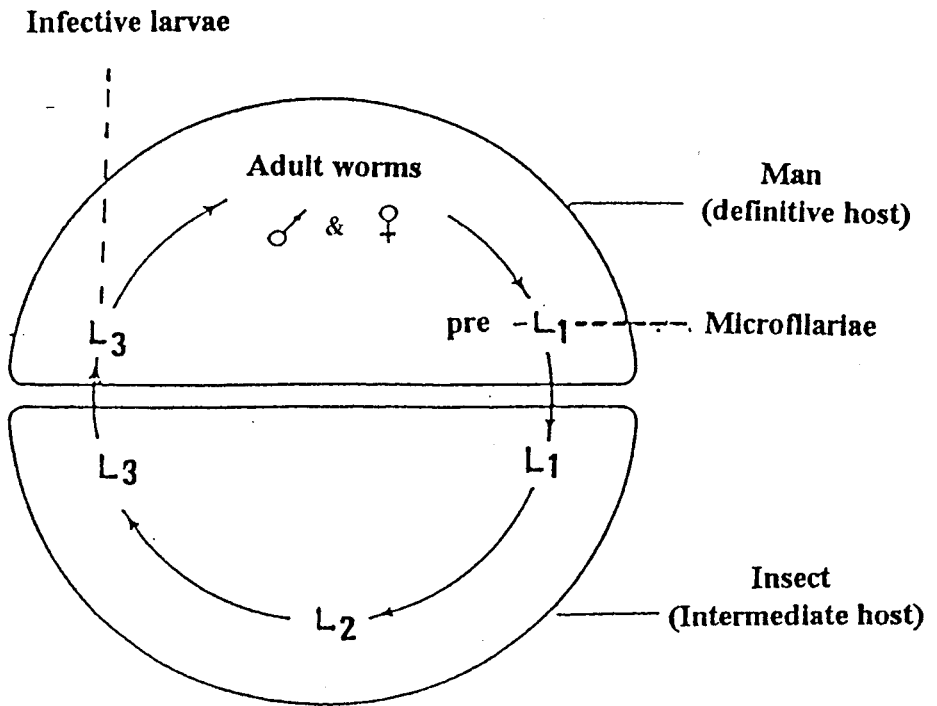


Fig. 1.1 Life cycle of the filarial parasite

1.2.2. Clinical manifestation of lymphatic filariasis infection.

The general sequence of events following filarial infection are: as prepatent period, asymptomatic microfilaremia, acute and finally chronic clinical filariasis. Lymphatic filariasis causes various acute, occult and chronic manifestations. The acute manifestation involves episodic attacks of adenolymphangitis associated with fever and malaise. The attack may last a week or more and incapacitate the patient for several days. It would appear that acute attacks are a significant cause of morbidity and loss of workdays. The most prevalent chronic manifestation of lymphatic filariasis are hydroceles and lymphoedema of the extremities, including its most advanced and feared state, elephantiasis. Milky urine, which is painless but could result in weight loss is also noted. Chyluria occurs when lymph flow is obstructed in the thoracic duct, above the lymphatic branches of the kidney. The sediment may contain microfilariae and red blood cells. Lymphoedema and elephantiasis more commonly observed in brugian filariasis than in cases of bancroftian filariasis. In brugian filariasis leg, arm, scrotum, vulva and breast are effected. In the contrast to the brugian type, bancroftian elephantiasis occurs beyond the knee or elbow, affecting the whole leg or arm. Occult filariasis is another clinical manifestation of lymphatic filariasis infection which occurs when microfilaria are produced and destroyed by the host immune response (Lie, 1962). The destruction of the microfilaria is thought to be responsible for the clinical syndrome. The clinical features include lymph node enlargement, usually affecting a single group of glands or generalised lymphadenopathy. These glands are painless, firm, movable and may reach a diameter of 5 cm. Tropical pulmonary eosinophilic (TPE) is also a form of occult filariasis and is believed to be due to a hyperimmune response to filarial parasite mainly microfilariae.

1.3 Chemotherapy of antifilarial drugs.

1.3.1 Introduction

In situations where vector control is relatively complex and unaffordable the first approach to control lymphatic filariasis is by use of antifilarial drugs that kill the infecting parasites such as *W.bancrofti* and *B. malayi* in human host. The currently available drugs are primarily microfilaricidal, with still poorly defined degrees of macrofilaricidal activity. However, the pursuit of filariasis control using only microfilaricidal drugs is still appropriate for at least two reasons: first, because the clearance of microfilariae from the blood can be expected to have a positive effect toward reducing transmission of the infection; and the other reason is that decreasing microfilaremia in a community yields a positive "clinical effect" on infected subjects (i.e.; decreasing incidence of clinical lymphoedema and adenolymphangitis attacks). The choice of various drugs and regimens for filariasis control must certainly depend on the relative effectiveness of the regimens, adverse reaction induced, as well as the relative cost for controlling programmes and also widely used upon. Drugs currently available and widely used for the treatment of lymphatic filariasis are ivermectin and diethylcarbamazine (DEC). The structure of these compounds are shown in Fig. 1.2. These two drugs are described mainly in this chapter since they are widely used in the treatment of lymphatic filariasis in human. The usage of other antifilarial drugs such as mebendazole (MBZ), flubendazole (FBZ) and suramin for the treatment of lymphatic filariasis will be described briefly since their efficacy studies in human and animal models are limited (Fig. 1.2).

1.3.2. DEC and Ivermectin

Currently five drug regimens are available for the control of lymphatic filariasis. The administration of "WHO-recommended standard course" of DEC 6mg/kg per day x 12 days for *W.bancrofti* and x 6 days for *B. malayi* were found to be effective in treating lymphatic filarial infections (Ottesen, 1985). However, such regimes are expensive, require patients compliance and causes possible adverse reactions (usually, fever, local inflammatory reactions, gastrointestinal symptoms) (Ottesen, 1987) making them impractical for most control programmes.

The administration of single dose (spaced dose) of DEC given at weekly, monthly, 6 monthly, or yearly intervals has been advanced for many years by a number of public health workers in *W. bancrofti* endemic areas. (Laigret *et al.*, 1980). A more frequent yearly single dose of DEC (about 6 mg/kg) regimens are found to be effective in decreasing microfilarial prevalence and density in both *B. malayi* and *W. bancrofti* infected communities; Tahiti (Laigret *et al.*, 1980); India (Panicker *et al.*, 1991); Samoa (Kimura *et al.*, 1985) and French polynesia (Cartel *et al.*, 1992).

The chemical stability of DEC permits its use as a DEC fortified salt in both cooking and other flavouring. The effectiveness of DEC-fortified salt in decreasing the microfilarial prevalence of both bancroftian and brugian filariasis in large population in China, Taiwan and India are well reviewed by Gelband (1994). Similar observation was made in an individual patient in whom prevalence of *W. bancrofti* microfilaraemia decreased by 97.8% after 4 months of using DEC- salt and whose corresponding microfilarial (mf) density fell even more dramatically greater than 99% (Jingyuan, 1992). However, the effectiveness of this strategy primarily determined by the

exclusive availability of salt fortified with DEC as well as its consumption is determined by choice within the households.

Ivermectin, a new antifilarial drug has been long employed effectively for control of onchocerciasis (Aziz *et al.*, 1982; Upp *et al.*, 1986; Albiez *et al.*, 1988). Recently its effectiveness against microfilariae of both *W. bancrofti* and *B. malayi* has been evaluated in individual patients for a period of 12 - 24 months following drug administration (Richards *et al.*, 1991; Addis *et al.*, 1993; Kazura *et al.*, 1993; Eberhard *et al.*, 1992). It is well recognised that a single dose of ivermectin 400 ug/kg yields superior microfilaricidal activity (Richards *et al.*, 1974). At this dose level there were 36 - 70% and 86 - 99% decrease of mf prevalence and mf densities respectively after 12 months post-treatment.

The combination of single dose of DEC together with ivermectin seems to offer a more effective drug regimen than either single dose DEC or single dose ivermectin. The results of using this regimen mainly comes from individual patients with *W. bancrofti* infections receiving this ivermectin/DEC combination (Richards *et al.*, 1991 ; Addison *et al.*, 1993). Studies comprising a total of 33 patients demonstrated a decrease in mf prevalence of 45 - 70% and mf density of 96 - 99% after 12 and 24 months of post-treatment. In this study the dose of ivermectin used in the ivermectin/DEC combination was only 20 µg/kg (along with 6 mg/kg DEC). In addition safety trials have already been successfully carried out to demonstrate the feasibility of using ivermectin/DEC combination (Navaratnam *et al.*, 1992; Glaziou *et al.*, 1994)

1.3.3 Suramin

Suramin is a potent filaricide used for the treatment of onchocerciasis (Hawking, 1978). However, its toxicity and the need for intravenous dosing under medical supervision for up to six weeks greatly limit its use, (Katzung, 1985). The antifilarial properties of suramin against human lymphatic parasite have not been extensively studied, since, DEC and ivermectin were the better choice of drugs in the treatment of brugian and bancroftian filariasis. However, investigators have reported the filaricidal activity of suramin against lymphatic parasite in experimentally infected animal models. Denham and co-workers in their study demonstrated the adulticidal activity of suramin against *B. pahangi* in cats at five weeks post-treatment (Denham & Mc Greevy, 1977). On the other hand tertiary screening of potential filaricides, in *Brugia malayi* - *Presbytis cristata* animal model has identified suramin to possess adulticidal activity (Mak *et al.*, 1990). Intravenous suramin at 10 mg/kg daily x 5 days or 17mg/kg weekly x 5 weeks substantially reduced the recovery of live adult worms to 50.6% and 13.6% of control respectively. But no microfilaricidal activity was accounted. However, the suramin as antifilarial agent for lymphatic filariasis obviously depends on envisaged preclinical and clinical evaluation, while DEC and ivermectin still remain as the drugs of choice.

1.3.4 Benzimidazole carbamate

MBZ and FBZ derivatives of benzimidazole carbamate are widely used in the treatment of onchocerciasis (Dominguez-vazques *et al.*, 1993; Rivas-Alcala *et al.*, 1981 a, b). In the treatment of *O. volvulus* infections patients receiving 1 gm of MBZ twice daily for 28 days had a 50% decrease in their skin microfilariae counts for over 6 months (Dominguez-Vazques *et al.*, 1983). However, poor oral absorption of MBZ as

well as prolonged dosing regimen do not seem to be a suitable regimen for routine practice. On the other hand, intramuscular (i.m.) injection of FBZ 750 mg/week for 5 weeks demonstrated a profound reduction of skin microfilariae in patients compared to patients receiving DEC 200 mg/day for 2 weeks (Rivas-Alcala *et al.*, 1981). However, ulceration at the site of injections currently limit the usage of FBZ in the treatment of onchocerciasis. Both MBZ and FBZ are known well for their limited solubility and poor absorption from the gastrointestinal (GI) tract (Brugmans *et al.*, 1971; Michiels *et al.*, 1982), thus making them suitable for intestinal helminth treatment (Keystone & Murdoch, 1979; Van den Bossche *et al.*, 1982).

The filaricidal activity of MBZ and FBZ against lymphatic parasite have been demonstrated both in animal and humans. Denham *et al.* (1978) showed the adulticidal activity of MBZ against *B. pahangi* in experimentally infected jirds and cats. A similar observation was also noted following filaricidal screening of FBZ in *B. pahangi* infected jirds and cats (Denham *et al.*, 1979). However, no microfilaricidal activity was observed in FBZ studies above. In clinical trials, the macrofilaricidal action of MBZ was reported in few patients suffering from *B. malayi* and *W. bancrofti* infection. These patients were treated with large doses of mebendazole 500 mg three times daily for 21 days (WHO 1984). However, this high doses are not recommended because its absorptions is erratic, it is teratogenic in some animals and it can be toxic in large doses. A successful therapy for systemic infections disease such as lymphatic filariasis requires a sufficient quantity of drug to be absorbed to achieve a therapeutic plasma concentration. In view of this poorly soluble drugs such as MBZ and FBZ do not seem to offer a viable alternative to current drugs (DEC, ivermectin) in the treatment of this disease. In accordance with this new benzimidazole carbamate derivatives with better solubility need to be synthesized. In this thesis the bioavailability of two new

benzimidazole carbamate derivatives UMF-078 and UMF-058 have been investigated in suitable animal models.

1.4 Mode of Action for antifilarial drugs

1.4.1 Introduction

Generally, extensive studies on the mode of action of parasite drugs were done mainly on anthelmintic agents especially benzimidazole carbamates, which have shown themselves to be highly effective and safe for the treatment of the majority of intestinal helminth infections both in human and veterinary medicine (Van Den Bossche *et al.*, 1982). Knowledge of the mechanism by which antifilarial drugs are absorbed and their mechanism of action on filarial parasites are vital in the discovery of new drugs and development of more effective delivery system. However, present studies are mainly focused on large nematodes such as *Ascaris sp* (Thompson *et al.*, 1993). This probably might be due to its wide distribution, its importance in both human and veterinary medicine as well as the fact that it is large enough to study using a variety of techniques.

Little is known on the mechanism of action of antifilarial drugs on lymphatic parasites such as *B. malayi* and *W. bancrofti*. The studies on the antifilarial mechanism mode of action of drugs such as DEC, ivermectin, suramin and benzimidazole carbamate on filarial parasite is still in the early stages. This probably is due in part to the lack of knowledge regarding these organisms as well as lack of suitable animal model for the study of these parasites in the laboratory. Indeed parasites material are also scarce thus limiting the scope of physiological and biochemical studies on these worms which may reveal promising targets for research. However, the antifilarial mechanism of these drugs will be discussed briefly herein.

1.4.2 Benzimidazole carbamate

The mode of action of benzimidazole carbamates are usually assayed in terms of its antitubulin activity against microtubulin polymerization in parasite (Lacey, 1990). Tubulin is the functional subunit of microtubules which participate in several important cell functions e.g. the transport of materials within cells. Microtubules exist in dynamic equilibrium with tubulin and are being controlled by a range of endogenous regulatory proteins and co-factors. The equilibrium can be interrupted both *in vivo* and *in vitro* by exogenous substances known as microtubule inhibitors. Most of the inhibitors exert their action by binding to tubulin to prevent the self-association of sub-units onto the growing microtubules. This results in "capping" of the microtubule at the associating end with a net loss of microtubule length. The disintegration of the microtubule led to impaired cell functions such as mitosis, secretion and regulation of cell form.

Both MBZ and FBZ induced the disappearance of the cytoplasmic microtubules of the tegumental or intestinal cells of cestodes and nematodes, which results in blocking the transport of secretory vesicles (Van Den Bossche, 1976; Van Den Bossche, 1986). This subsequently lead to impaired coating of the membranes thus decreasing the digestion and absorption of nutrients. This probably might be the basis of MBZ induced impairment of glucose absorption in nematodes and cestodes both *in vivo* and *in vitro* studies (Van Den Bossche, 1976; Van Den Bossche 1986). The antitubulin activity was also demonstrated by other congeners of MBZ such as albendazole (ABZ), parbendazole, oxbendazole and fenbendazole (Van Den Bossche *et al.*, 1982). Further evidence of tubulin dependent benzimidazole action was obtained in charcoal binding stability study. Colchicine, a microtubule inhibitor forms a tight pseudoirreversible complex with tubulin that enables the colchicine - tubulin complex to survive

extraction with charcoal. In addition, studies with benzimidazole resistant isolates parasites demonstrated a reduced charcoal-stable [H^3] mebendazole binding (Lacey, 1988). This observation further supported tubulin as the site of action of benzimidazole carbamate. It is also important to note that other postulated mode of action was also been proposed. McCracken & Stillwell (1991) showed that benzimidazole anthelmintic activity in part may be due to bioenergetic disruptions of natural membrane system resulting from transmembrane proton discharge. However, it is not yet possible to determine whether the action of these group of drugs on microtubules or on energy producing systems represents their primary mode of action. Further study is necessary to determine the exact mode of action of these chemotherapeutic agents.

1.4.3 Diethylcarbamazine (DEC)

DEC is the oldest and the most effective antifilarial drug used in the treatment of lymphatic filariasis. However, the exact mechanism of action is still unknown. Fujimaki *et al.* (1988) proposed that DEC inhibited the development of *Brugia pahangi* larvae cultured *in vitro* in the presence of feeder cells (LLC-M cells). It was discovered recently by Fujimaki and co-workers that DEC also inhibits proliferation of LLC-MK2 cells, disrupts the cytoplasmic microtubules complex, inhibits the assembly of microtubules *in vitro* and induces the disassembly of the performed microtubules *in vitro* (Fujimaki *et al.*, 1990). They suggested that the inhibition of larvae development by DEC probably might be due to the antitubulin effect of DEC on feeder cells and finally these cells lose their supporting function for filarial larvae.

Subsequent study by Fujimaki *et al.* (1990b) also showed that *B. pahangi* larvae exposed to DEC *in vitro* were retarded in their development in jirds. These

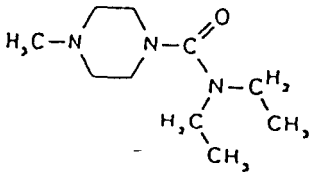
result indicate that DEC has a direct action against the infective larvae of *B. pahangi*. The other postulated mode of action was that DEC might enhance the host parasite defence system by increasing the adherence of leukocytes to microfilariae mediated by antibodies reacting with the surface of the worms (Willy *et al.*, 1979). Other investigators suggested that DEC might affect the neuromuscular system and surface layers of the larvae (Hawking, 1979). Further studies need to be done since knowledge regarding the physiological and biochemical aspects of the neuromuscular system are insufficient.

1.4.4. Suramin

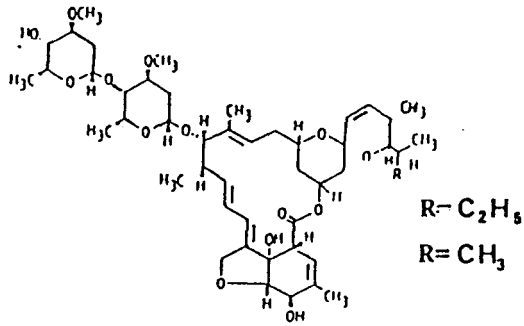
The antifilarial action of suramin upon *B. pahangi* has been investigated *in vivo* and *in vitro* (Howells *et al.*, 1983). With regard to its polyanioninature, suramin was found to be bound to the surface of worms *in vitro*. Subsequent *in vivo* studies with jirds fail to demonstrate the ability of suramin to alter the rates of glucose utilization, uptake rates of glucose, leucine and adenosine. However, ultrastructural changes were noted in the intestinal epithelium of worms from suramin treated jirds study, thus suggesting the intestinal epithelium itself might be the site of action of the drug. Further studies are needed to investigate suramins effect on filarial gut system since the knowledge regarding the pysiological aspects of the gut of filarial worm are still lacking.

1.4.5 Ivermectin

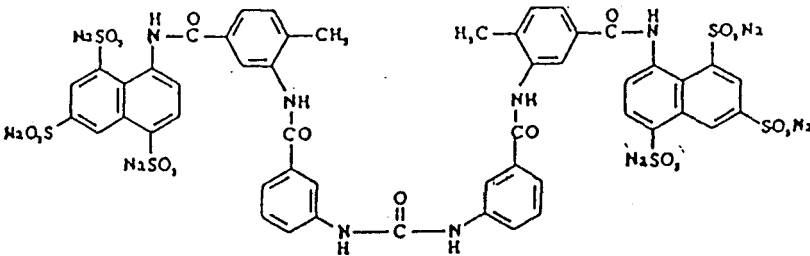
The exact mechanism of action of ivermectin remains unknown but is thought to involve the activation of γ -aminobutyric acid (GABA) pathways in the parasite via an effect on the GABA receptor-chloride ion channel complex (Vande Wan, 1991). Ivermectin potentiates the release and binding of GABA at postsynaptic sites on the neuromuscular junction thus paralysing the nematode worms (Gutaffson *et al.*, 1987). However, ivermectin is not filaricidal at 100-200 ug/kg dose level as recurrence of microfilaraemia was encountered in polynesians infected with *W. bancrofti* following ivermectin efficacy study (Cartel *et al.*, 1993). In addition, recent study in Brazil showed that high dose of ivermectin (400 ug/kg) has no observable macrofilaricidal effect on adult *W. bancrofti*, although it is known to be a potent microfilaricidal agent (Dreyer *et al.*, 1995).



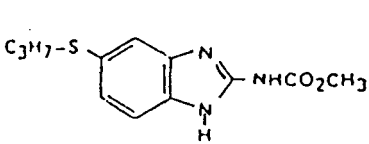
a. diethylcarbamazine



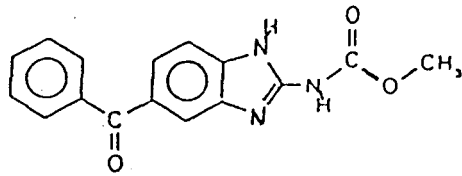
b. ivermectin



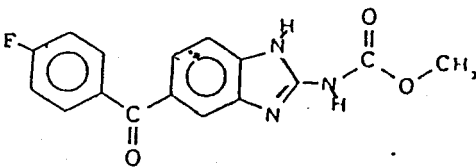
c. suramin



d. albendazole



e. mebendazole



f. flubendazole

Fig 1.2: Structural formula of antifilarial drugs.

1.5 The Pharmacokinetic and Metabolism Of Antifilarial Drugs

1.5.1 Introduction

The primary objective of pharmacokinetic and drug metabolism studies in development of new drugs is to understand how the drug molecule is handled by the living animal, which involves the absorption, distribution, metabolism and excretion of the drug in the living system. Before reaching the stage of clinical evaluation the new compound, potentially a new drug is usually evaluated in numerous animal models in order to determine its pharmacological potency, toxicological potential and metabolic fate. Frequently, several animal species are used in the evaluation. Metabolism studies conducted in living animals provide the ultimate information regarding the pharmacokinetic properties and metabolic pathway of the drug molecule which can be used to correlate or interpret efficacy and toxicity data collected in the same species.

Work in this thesis describe the pharmacokinetic and metabolism of new benzimidazole carbamate derivatives UMF-078 and UMF-058. In view of this it is essential to review the pharmacokinetic and metabolism of benzimidazole carbamate related drugs for our reference to the current status of this compound in this aspect. In addition the metabolic fate of other important antifilarial drugs such as DEC, ivermectin and suramin will also be discussed briefly herein. Clinical results were presented wherever essential. However, when human studies are not available, results of animal experiment from literature were used to described the pharmacokinetic and metabolism of the drugs.

Benzimidazole carbamates are important broad-spectrum drugs primarily employed in the treatment of infections of helminth parasite. In human clinical practice only three

benzimidazole compounds; MBZ, FBZ and ABZ are currently used. However, their poor solubility in gastrointestinal tract limited their usage in the treatment of systemic disease such as lymphatic filariasis. Nevertheless, the antifilarial activity of MBZ (WHO 1984) as well as FBZ (Denham *et al.* 1979) in limited animal and human studies have been demonstrated. This section will discuss some of the pharmacokinetic and metabolism aspect of MBZ, FBZ and ABZ.

1.5.2 Mebendazole

MBZ is poorly absorbed after oral administration due to its poor aqueous solubility. Following administration of MBZ tablets (1.5 gm) to three fasting volunteers, plasma levels of MBZ remained below 17 nmol/L in two volunteers and a peak concentration of 17 nmol/L was noted in the third subject. When the same dose regimen was employed with a fatty meal, plasma concentration mounted up to 91,112 and 142 nmol/L within 2 to 4 hr in the three treated subjects (Munst *et al.*, 1980). The increase in the absorption was probably due to the ability of the fatty meal to aid dissolution, through solution of the drug in the fatty media, from which it is partitioned into the aqueous GI contents. Dawson *et al.* (1985) in their attempt to determine the absolute bioavailability of MBZ utilised a solution of radiolabelled MBZ in dimethylsulphoxide (DMSO) (0.25%) for oral and intravenous studies in volunteers. Following intravenous administration of tracer dose radiolabelled MBZ (1.18 ug), the average distribution half life, elimination half-life ($T_{1/2}$) and rate of clearance (Cl) were 0.2 hr, 1.12 hr and 1.163 l/min respectively. After oral administration of the similar solution the mean $T_{1/2}$, Cl and time to achieve the maximum concentration (T_{max}) were 0.93 h, 0.846 l/min and 0.42 hr respectively. The bioavailability of MBZ was about 22%. Comparisons of area under the curve (AUC) data for two MBZ metabolites after

administration of the parent drug by each route indicate that absorption of MBZ from the GI tract at this dose is almost complete. The low bioavailability observed following oral administration at this subtherapeutic dose level is due to high first pass elimination. However, at higher dosages the poor bioavailability of MBZ is due to a combination of high first pass metabolism and very low solubility of the drug. (Dawson *et al.*, 1985)

In patients with hydatid disease, oral administration of MBZ 10 mg/kg yield a plasma concentration time curves which differed considerably among subjects. The $T_{1/2}$, C_{max} and T_{max} ranged from 2.8 to 9.0 hr, 17.5 to 500 ug/L and 1.5 to 7.3 hr respectively (Witassek *et al.*, 1981). Greater values of half lives were encountered following oral administration of large doses of MBZ compared to intravenous administration of MBZ. This difference is probably due to the absorption rate limitation of MBZ from GI tract. This is further evident, when the $T_{1/2}$ (0.92 hr) value of MBZ following a subtherapeutic oral administration of MBZ solution compares favourably with the $T_{1/2}$ (1.12 hr) value of MBZ after an intravenous treatment as described previously in this section (Dawson *et al.*, 1985).

The metabolic transformation of MBZ *in vitro* and *in vivo* has been reported in literature. The *in vitro* metabolic pattern of MBZ in rat, dog and pig liver has been described by Meuldermans *et al.* (1976) (Fig. 1.3). In all the three species studied hydroxy metabolite (methyl [5-(α -hydroxy- α phenylmethyl)-H benzimidazole-2-yl] carbamate was the major biotransformation product resulting from reduction of the ketone of the drug molecule. The major hydroxy metabolite of MBZ was also formed by the three different subcellular fractions (10,500 g supernatant, 100,500g supernatant, and microsomal enzyme) of rat, dog and pig liver. The relative activity of both soluble

and cellular microsomal enzyme differed for the three species. A much greater part of MBZ was reduced by the dog enzyme preparations than by the rats or pigs. A species differences was encountered for a minor amine metabolite of MBZ in the 10,000 g supernatant fraction and the microsomal fraction of pig liver. This metabolite (2-amino-1-H benzimidazole-5yl) phenylmethane was produced from carbamate hydrolysis and was not noted in the incubates with the rat or dog liver enzyme preparations. The carbamate hydrolysis of MBZ was inhibited significantly by SKF-525A indicating the involvement of the microsomal mixed function oxidation enzyme system. Conversely the formation of hydroxy metabolite of MBZ was only slightly influenced in the presence of SKF-525A inhibitor. From the overall study they conclude the ketone reduction of MBZ was to be the most important *in vitro* metabolic pathway of MBZ in dog, rat and pig liver preparations (Meuldermans *et al.*, 1976).

Allan *et al.* (1982) successfully identified three biliary metabolites of MBZ in the rats after intravenous administration of a mixture of MBZ and pentadeuteromebendazole. These metabolites are methyl-5(6)-(α-hydroxybenzyl)-2-benzimidazole carbamate, 2-amino-5(6)-α-hydroxybenzyl benzimidazole and 2-amino 5(6)-benzoylbenzimidazole which were obtained after enzymic conjugate hydrolysis. They conclude that the major route of metabolism for MBZ was carbonyl or ketone reduction followed by conjugation to form glucuronides and sulphates. Conjugation appeared to be extensive since only unmetabolized MBZ could be detected in bile extracts taken before enzyme hydrolysis. Subsequent intravenous metabolic study in rats using [³H]-MBZ and [¹⁴C]-MBZ further ascertain the ketone reduction of MBZ to be the more important metabolic route in MBZ biotransformation (Allan *et al.*, 1983). The carbamate hydrolysis remain as a trivial pathway in MBZ metabolism in rats. The

major hydroxy MBZ accounted for about 77% of the the total recovered and 99% of it was the conjugate metabolite (Allan *et al.*, 1983).

1.5.3. Albendazole

There is little data available on the pharmacokinetics of ABZ in man because it is largely undetectable in human plasma due to its low GI absorption and rapid metabolism (Penicaut *et al.*, 1983; Marriner *et al.*, 1986). When ABZ is taken orally either as tablets or a 2% suspension (400 mg) the plasma concentration of active metabolite ABZ sulfoxide peaked in the range of 0.22 to 0.25 mg/L at 2.0 to 3.0 hours post dose. The elimination $T_{1/2}$ of this metabolite was 8.5 hours (Penicaut *et al.*, 1983). Marriner *et al.* (1986) reported an inconsistent increase in ABZ sulphoxide concentration when ABZ was administered with oil and milk. Lange *et al.* (1988) in their study demonstrated a significant increase of ABZ sulphoxide concentration in patients with echinococcosis when ABZ was given with a fatty breakfast (42.5% relative to fasting patients).

ABZ undergoes extensive metabolism. The metabolic pathway noted in man (Penicaut *et al.*, 1983), cattle, sheep, rats and mice (Gyurik *et al.*, 1981) was similar; the hydrolysis of the carbamate moiety and oxidation of the sulphur atom, alkyl side chain and aromatic ring (Fig. 1.4). Sulphoxide and sulphone are the major metabolites of ABZ biotransformation in the urine and plasma of all species and the proportion varies considerably among species (Delatour *et al.*, 1991; Lanusse *et al.*, 1992). However, the parent compound was detected in minor amount in the urine in all species (Gyurik *et al.*, 1981). Microsomal incubation studies of ABZ demonstrated the formation of sulfoxide metabolite. The formation was mediated by cytochrome P-450 and/or FAD

containing monooxygenase depending on the system used; rat (Fargetton *et al.*, 1986), sheep (Galtier *et al.*, 1986), pig (Souhaili-El Amri *et al.*, 1987) or human (Rolin *et al.*, 1989) liver microsomes. Unlike ABZ sulfoxidation, the oxidation to ABZ sulfone has been exclusively related to cytochrome P-450 in perfused rat liver preparation. ABZ is also known to induce its own metabolism (Souhail-El Amri *et al.*, 1988).