# THE PHARMACOKINETICS OF IVERMECTIN IN RABBIT

**By**
**SHU EN AND OKONKWO PO**
***Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu Campus***

## SUMMARY

**Objective:** To establish the pharmacokinetics of ivermectin in the rabbit model as a basis for future screening of newly developed micro and macrofilaricides.

**Method:** Pharmacokinetic parameters of ivermectin were investigated in 5 rabbits after a single subcutaneous dose (150ug/kg), as a basis for screening micro- and macro-filaricides. Plasma ivermectin levels were measured by a sensitive High Performance Liquid Chromatography (HPLC) with fluorometric detection method.

**Results:** The mean ±SEM pharmacokinetic parameters were as follows: time taken from dosing to maximum concentration ($T_{max}$), 1.4 ±0.4 hrs; maximum concentration ($C_{max}$), 34.0 ±1.6ng/ml; volume of distribution (V), 4.8 ±1.3L/kg; area under the plasma concentration-time curve (AUC), 475.6 ±5.4ng.hr/ml; plasma clearance (CI), 9.2 ±1.8ml/min and elimination half life (t), 10.4 ±2.3hrs.

**Conclusion:** The elimination phase of ivermectin pharmacokinetics reveals a secondary peak suggestive of an enterohepatic recycling.

***Key Words:*** *HPLC, Pharmacokinetics, Ivermectin*

## INTRODUCTION

Ivermectin, a macrocyclic lactone produced by the actinomycete, *streptomyces avermitilis* is a potent new antihelminthic agent. Its has proven to be effective, well tolerated and has revolutionized the treatment of onchocerciasis and other nematode diseases of man[1] and ectoparasitic infections[2,3]. The drug is effective in a single oral dose and has less severe side effects[4,5]. Community-based trials indicate that the single dose will markedly reduce skin microfilarial level for up to 12 months, with transient fall in transmission levels[5,6].

The pharmacokinetic parameters of ivermectin are a function of the species in which the compound is studied[7]. The microfilaria worm, M*onanema martini* with skin-dwelling microfilariae in its natural muride host represents the model for the study of pathogenesis and chemotherapy of onchocerciasis[8].

Since the pharmacokinetic properties of ivermectin have been reported in a wide variety of hosts such as cattle, sheep, dog, swine and horses[7], but not in rabbit, there is need to establish pharmacokinetic parameters of the drug in rodent models before and after infection with *Monanema martini.*

In this study, ivermectin levels have been measured in non-infected rabbits as a basis for screening newly developed micro- and macro-filaricides.

## MATERIALS AND METHODS

### Administration of ivermectin and sample collection

After an overnight fast, the standard single dose (150ug/kg) of ivermectin[9,10] was administered subcutaneously to each of five non-infected rabbits weighing between 1.70 and 1.90kg. Venous blood was collected from the ear lobe of each rabbit before drug administration and 1,2,3,4,5,6,7,8,9,10,12,48 and 52 hours later, into heparinized tubes. The blood was centrifuged at 3000g for 10 minutes and the plasma separated. All plasma samples were stored frozen at -20°C pending analysis.

**Corresponding Author:**
*E. N. Shu, Department of Pharmacology and Therapeutics College of Medicine, University of Nigeria, Enugu Campus*
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**Instrumentation**

Ivermectin concentrations were measured by a sensitive high performance liquid chromatography (HPLC) analytical method of Krishna and Klotz[11] with fluorescent detection. The HPLC-system consisted of a Rhodyne injector ($100_{ud}$ sample loop), a pump (LC-6A), fluorescence detector (RF 535) and a computing integrator (C-R6A), all obtained from Shimadzu, Kyoto, Japan. The detector was set at an excitation wavelength of 364nm and an emission wavelength of 440nm. The integrator and detector were run at an attenuation of 4 and 16 respectively. The analytical column (12.5 x 0.4cm) was packed with reversed phase Hypersil ODS H (5um; Machery & Nagel, Duren FRG); and the mobile phase with a flow rate of 1.2ml/min consisted of acetonitrile (HPLC-grade) and distilled water (5%).

**Standard Solutions**

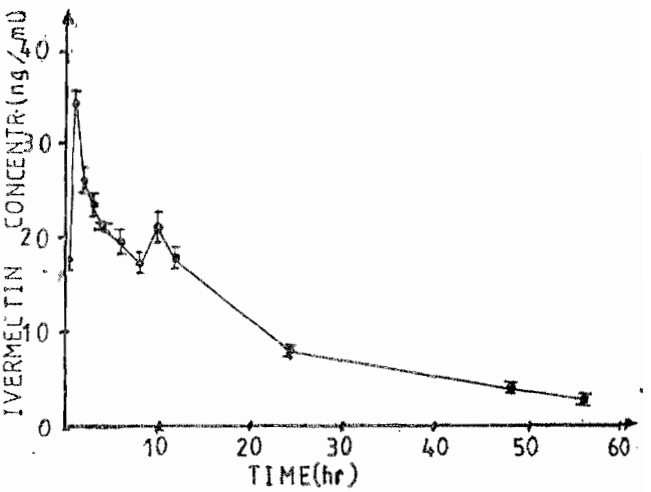
Ivermectin reference standard and the monosaccharide of 22, 23-dihydroavermectin $B_{1a}$ (internal standard) were kind gifts of Merck Sharp & Dohme Research Laboratories Rayway, New Jersey, USA and Dr. Edward Liverpool University, England respectively. A stock ivermectin solution (5ug/ml) was obtained in methanol (HPLC-grade). Plasma standards (10, 30, 40, 60, 80 ng/ml) and quality control samples (20ug/ml) were prepared by dilution with drug-free blank plasma. The concentration of the internal standard stock solution was 0.8ug/ml methanol. The retention times ($R_t$) for internal standard and ivermectin were about 6 and 11 minutes, respectively.

**RESULTS**

The recovery of ivermectin from plasma averaged 95% while the intra- and inter- assay variability averaged 3.6% and 5.8%, respectively.

The mean ± SEM plasma concentration-time profile for ivermectin is shown in figure 1. Ivermectin was rapidly absorbed, achieving a peak concentration within 1-2 hours. Thereafter, plasma concentrations fell throughout the study except in the tenth hour when it peaked again.

**Figure 1: Mean ± SEM Plasma Concentration-time Profile for Ivermectin in Rabbits**




The pharmacokinetic parameters are summarized in table 1. There were wide inter-subject variations in the pharmacokinetic parameters. Out of the five rabbits, three were characterized by high $C_{max}$ (38.9 ±0.7ng/ml) and an early $T_{max}$ (1hr) while the rest of the rabbits had a low $C_{max}$ (26.6 ± 0.2ng/ml) and a late $T_{max}$ (2hrs).

**Table 1 Pharmacokinetic in Rabbits**

| Parameter | Mean ±SEM | Range |
|---|---|---|
| $T_{max}$ (hr) | 1.4 ± 0.4 | 1.0 – 2.0 |
| $C_{max}$ (ng/ml) | 34.0 ± 1.6 | 32.5 – 36.1 |
| V (L/kg) | 4.8 ± 1.3 | 3.0 – 6.9 |
| AUC (ng.hr/ml) | 475.6 ± 59.4 | 414.1 – 501.4 |
| CL (ml/min) | 9.2 ± 1.8 | 7.0 – 11.3 |
| $t_{½}$ (hr) | 10.4 ± 2.3 | 8.2 – 12.8 |

**DISCUSSION**

Ivermectin is an extremely effective single dose *Onchocerca* microfilaricide which greatly reduces parasite levels in the dermis and suppresses the release of new microfilariae from female worms for over 12 months[5,6]. It lacks any obvious macrofilaricides effects and it has fewer adverse effects in man[5].

The elimination phase of ivermectin in this study does not consist of a simple exponential curve and examination of individual plasma concentration profile, reveals a secondary peak. This is suggestive of enterohepatic recycling. A similar observation has been reported in human subjects after the administration of a 14-mg dose of $^{3}$H-labelled ivermectin[7]. Other research workers[12] have reported that a peak concentration was followed by a mono-exponential decline in the plasma levels.

In the present study, plasma elimination half-life (0.4days) of ivermectin is comparable with that of swine (0.5days) but it shows wide variability with those of other species: dogs (1.8days), sheep (2.7days), cattle (2.8days) and man (2.3days)[7]. This is suggestive of other routes of elimination in the rabbit and swine, apart from the faeces. Low levels of ivermectin have been found in breast milk and saliva samples of man[13]. It is not clear why ivermectin does not appear in urine samples of man[11,12]. Such investigations (of ivermectin in urine) have not been carried out in laboratory animals. The urinary tract could be an additional route of ivermectin elimination in the rabbit.

In a study carried out in sheep[14], a volume of distribution of 4.6L/kg was obtained, similar to the value (4.8L/kg) for this study. Other studies show variability in the volume of distribution in various animals: cattle (1.9L/kg), dog (2.4L/kg)[7] and in humans (9.9L/kg)[12]. The rapid absorption; the comparatively large volume of distribution and area under the plasma concentration-time curve (AUC); and a slow elimination phase of ivermectin achieve a rapid elimination and long-lasting suppression of microfilariae from the skin.

Our findings therefore contribute to the knowledge on the fate of ivermectin in laboratory models. Since there is variability in various tissues and species[13], there is still need to find out how much of the drug gets to the target sites (skin and eyes) and its pharmacokinetic parameters after infection of rodents with *Monanema martini*.

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