Research Article

Absorption, Distribution, Excretion, and Pharmacokinetics of ^{14}C-Pyronaridine Tetraphosphate in Male and Female Sprague-Dawley Rats

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The main objective of this investigation was to determine the absorption, distribution, excretion, and pharmacokinetics of the antimalarial drug pyronaridine tetraphosphate (PNDP) in Sprague-Dawley rats. Following oral administration of a single dose (10 mg/Kg) of ^{14}C-PNDP, it was observed that the drug was readily absorbed from the small intestine within 1 hour following oral administration and was widely distributed in most of the tissues investigated as determined from the observed radioactivity in the tissues. The peak value of the drug in the blood was reached at around 8 hours postadministration, and radioactivity was detected in most of the tissues from 4 hours onwards. ^{14}C-PNDP showed a poor permeability across the blood-brain barrier, and the absorption, distribution, and excretion of ^{14}C-PNDP were found to be gender-independent as both male and female rats showed a similar pattern of radioactivity. Excretion of the drug was predominantly through the urine with a peak excretion post 24 hours of administration. A small amount of the drug was also excreted in the feces and also in the breath. It was found that the C_{max}, AUC (0-inf), and T_{max} values were similar to those observed in the Phase II clinical trials of pyronaridine/artesunate (Pyramax) conducted in Uganda.

1. Introduction

Malaria, a vector borne infectious disease caused by the protozoan parasites (*Plasmodium*) and transmitted to humans by the female anopheles mosquito, has remained a real and longstanding cause for concern, mainly in the tropical world. Malaria has now extended to areas that cover more than 40% of the world's population and according to the World health Organisation's (WHO) latest report there were an estimated 247 million malaria cases worldwide in 2006, of which 91% were due to *P. falciparum*. The vast majority of cases (86%) were found in the African region, followed by the South-East Asia (9%) and Eastern Mediterranean regions (3%). Mortality due to malarial infection was estimated to be about 881,000 deaths worldwide in 2006, of which 90% were in the African region, and 4% in each of the South-East Asia and Eastern Mediterranean regions [1]. Several

antimalarial drugs have been developed over the years and are being used along with antipyretics with varying degree of success. Chloroquine has long been the drug of choice against malaria for several years and has been widely employed in the treatment of *falciparum* malaria. But the extensive deployment of the antimalarial drugs, in the past fifty years, has provided a tremendous impetus for human malaria parasites to evolve mechanisms of resistance. The discovery and spread of chloroquine resistant *P. falciparum* have highlighted the urgent need for developing new antimalarial drugs. Presently, resistance has already been reported to all the antimalarial drug classes except in the case of artemisinin and these drugs have already become an essential component of treatments for multidrug-resistant *falciparum* malaria [2]. The emergence of resistance, particularly in *P. falciparum*, has been a major contributor to the global resurgence of malaria in the last three decades. These factors underscore the urgent

need to identify new antimalarial drugs and to understand their responses so that appropriate measures can be taken for their use to delay possible eventual ineffectiveness.

According to WHO, artemisinin-based combination therapy (ACT) is being recommended as the first-line treatment for *P. falciparum* in 66 countries by the end of 2006 and in almost all countries in the African, South-East Asia, and Western Pacific regions [3]. ACT has become the mainstay of antimalarial treatment in most regions where the disease is endemic [1]. Although, currently available artemisinin combinations show good efficacy and a sustained high cure, rates the tolerability, cost, and impaired patient compliance because of complicated dosing schedules and inappropriate drug formulations are major drawbacks. Specific resistance against drugs currently partnered with artemisinin is rising and novel partner drugs are therefore currently being developed to overcome these limitations. A novel ACT Pyramax (which is a combination of artesunate and pyronaridine tetraphosphate) is currently under development for the treatment of uncomplicated falciparum and vivax malaria under a joint drug development program by the not-for-profit organization Medicines for Malaria Venture (MMV, Geneva, Switzerland) and the pharmaceutical company Shin Poong Pharmaceuticals Co., Ltd. (Seoul, Republic of Korea). The objective is to provide a fixed dose ACT that has a high efficacy, good tolerability and safety profile, low cost (less than $1 per adult-treatment course), long shelf life and an easy dosing regimen of 1 daily dose over 3 days.

One group of alternative antimalarial drugs that are being currently considered is the Mannich bases, especially pyronaridine tetraphosphate (PNDP; Figure 1(a)). It was first synthesized in China and introduced for the treatment of malaria in certain malaria infested regions of China [4]. Chemically, it is 7-chloro-2-methoxy-10-[3, 5-bis (pyrolidinyl-1-methyl)-4-hydroxyanilino] benzo [*b*]-1, 5-naphthyridine tetraphosphate. It is a derivative of benzonaphthyridine, with a side chain similar to amodiaquine and has both acidic and basic functional groups [5]. PNDP is a blood schizonticidal drug active against the erythrocytic stages of the malarial parasite and has already undergone limited trials in humans against both *Plasmodium falciparum* and *Plasmodium vivax* [6]. Preliminary clinical studies conducted in China have demonstrated its safety and efficacy against the malarial parasites [7]. Pyronaridine tetraphosphate is also reported to be effective against chloroquine and multi-drug-resistant *Plasmodium falciparum* malaria [8]. It is reported to be less toxic than chloroquine and highly effective against chloroquine-sensitive and resistant parasites both in rodent malarias in vivo and *Plasmodium falciparum in vitro* [9]. Clinical studies carried out in Thailand [10] and in Cameroon [11] have reported that oral administration of PNDP is well tolerated and effective against *falciparum* malaria. In spite of its clinical efficacy being documented by several investigators, its pharmacological action still remains unclear. Studies have shown that PNDP affects haemoglobin degradation, possibly through an effect on the parasite topoisomerase II [12] and also by inhibiting P-glycoprotein mediated multidrug resistance [13]. Auparakkitanon et al. [14] have shown that pyronaridine targets hematin and

inhibits in vitro beta-hematin formation (at a concentration equal to that of chloroquine), by forming a complex with hematin with a stoichiometry of 1 : 2, to enhance hematin-induced red blood cell lysis (but at 1/100 of the chloroquine concentration) and also inhibits glutathione-dependent degradation of hematin.

The present investigation was designed to study and characterize the absorption, distribution, excretion, and pharmacokinetics of ^{14}C-PNDP in male and female Sprague-Dawley rats for a period of 10 days, following administration of a single target dose of 10 mg/kg.

2. Materials and Methods

2.1. Chemicals. Pyronaridine base was supplied by Shin Poong Pharmaceuticals Co., Ltd. Seoul, Republic of Korea. Pyronaridine tetraphosphate (PNDP) and ^{14}C-pyronaridine tetraphosphate (^{14}C-PNDP) were synthesized at KAERI laboratory. ^{14}C-PNDP (Figure 1(b)) had a radiochemical purity >98% and specific activity >10 μCi/mg which was checked according to previously reported method [15]. All other chemicals were purchased from Sigma Chemical Co., St. Louis, USA.

2.2. Animals. Male and female Sprague-Dawley rats were obtained from Central Lab Animal Inc., Seoul, Republic of Korea and weighed 150–200 g on the day of dosing. Within a study, the individual body weights were within ±10 standard deviations from the mean body weight of each sex and group mean body weights for each group of each sex were not statistically different at the 5% probability level. Rats were divided in 10 groups with 6 animals in each group corresponding to the time intervals at which the samples were analyzed. The animals were housed in polypropylene cages before and after dosing and were provided with a certified rodent pellet diet and pure drinking water. Animals were maintained under controlled conditions of temperature (22±2°C), relative humidity (55±5%) and approximately 12 hours light and dark cycle. For excretion studies, male and female Sprague-Dawley rats were housed in glass metabolism cages (Tecniplast, Italy). All the animal experiments were performed in compliance with the guidelines prescribed by the institutional animal ethical committee.

2.3. Dose Preparation. ^{14}C-PNDP (radiochemical purity >98%) was orally administered to male and female Sprague-Dawley rats at a dose of 10 mg/kg b·w (2.07 MBq/animal) in 0.4% methylcellulose prepared in saline. Rats were fasted over night before the administration of dose and also for approximately 4 hours postdosing. The target dose of 10 mg/kg b·w was administered by a single oral gavage using a ball tipped needle. All doses were freshly prepared on the day of dosing and administered within 4 hours of preparation.

2.4. Blood Collection and Plasma Separation. Post administration of ^{14}C-PNDP, blood (approximately 1 mL) was collected from each rat via a cannula placed in the jugular

PNDP

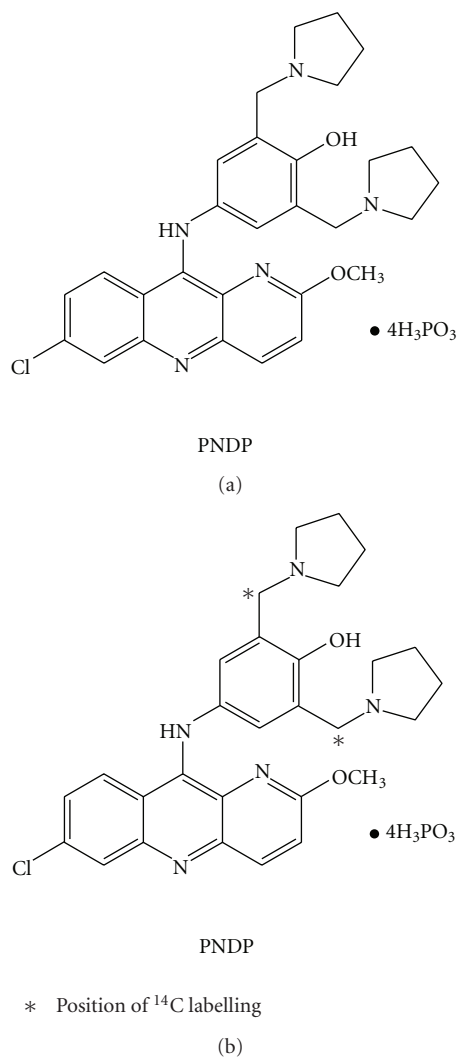(a)

PNDP

∗   Position of ^{14}C labelling

(b)

Figure 1: (a) Structure of pyronaridine tetraphosphate (PNDP). (b) Structure of ^{14}C-pyronaridine tetraphosphate (^{14}C-PNDP).

vein at time intervals of 1, 4, 8, 24, 48, 96, 144, 192, and 240 hours after administration of the drug. Whole blood from each animal was collected in 2 separate vials with and without heparin. Plasma was collected from the blood collected in heparinized tubes by centrifugation at 3000 rpm for 15 minutes and stored in separate vials at 4°C until analysis. The whole blood (collected without heparin) and plasma samples obtained from each rat from both genders were mixed with appropriate volumes of Hionic-Fluor scintillation cocktail and tested for radioactivity using Liquid Scintillation Counter (LSC) (TRI-CARBTM 1600TR, Packard, USA). Although, PNDP has a good stability for up to 4 weeks in refrigerated and frozen whole blood samples [16], in this study all the samples were analyzed for radioactivity within 48 hours of sample collection, until which they were stored at 4°C.

2.5. Biodistribution Studies. Following collection of blood samples, the animals were killed by halothane anesthesia at time intervals of 1, 4, 8, 24, 48, 96, 144, 192, and 240 hours postdosing and selected tissues, namely, liver, heart, lung, brain, spleen, kidney, stomach, small and large intestines were removed, rinsed in ice-cold saline and blotted to dryness. The tissues were then weighed and stored at −20°C until further analysis. The tissues were digested using solvable (Packard Bioscience, CT, USA) and mixed with 0.5 mL of methanol to prevent precipitation. Appropriate volumes of Hionic-Fluor scintillation cocktail were added and then analyzed by LSC for radioactivity. After the removal of selected tissues, the carcass was digested by submerging the entire rat in 500 mL of 6N potassium hydroxide solution. Aliquots (0.5 mL) of each rat carcass homogenate were mixed with 0.5 mL of methanol to prevent precipitation. Appropriate volumes of Hionic-Fluor scintillation cocktail were added to the samples and they were counted for radioactivity LSC. All samples were analyzed for radioactivity within 48 hours of collection and processing.

2.6. Mass Balance/Excretion Studies. For mass balance /excretion studies, animals were placed in glass metabolic cages after the administration of a target dose of ^{14}C-PNDP. Urine was collected in glass containers containing 10% H_3PO_4 (about 1 mL) at time intervals of 0−4, 4−24, 24−48, 48−72, 72−96, 96−120, 120−144, 144−168, 168−192, 192−216, and 216−240 hours postdosing. The urine samples were stored at 4°C until further analysis. They were mixed with Hionic-Fluor liquid scintillation cocktail and analyzed by LSC for the detection of radioactivity. The feces were also collected in the same manner at same time intervals as mentioned above. The feces was then homogenized with water (1 : 3) using a homogenizer and oxidized using Packard TRICARB Oxidizer. The residues were mixed with appropriate volumes of Hionic-Fluor scintillation cocktail and analyzed by LSC. After the last excreta collection, the cages were washed with about 400 mL of 1% trisodium phosphate and wiped with gauze pads. The cage wash was also collected into plastic containers, containing 12 mL of 10% H_3PO_4. The cage wash was mixed with appropriate volumes of Hionic-Fluor scintillation cocktail and analyzed by LSC.

2.7. $^{14}CO_2$ Breath Test. To determine the amount of drug excreted in breath the animals were placed in specialized cages that were attached to a carbon dioxide analyzer (CAPSTAR-100) fitted with a pump (LeticaR Oxilet 4 LE400-4) which regulated the flow of air in and out of the cages. Animals were maintained under controlled conditions of temperature (22±2°C), relative humidity (55±5%), and approximately 12 hours light and dark cycle. Male and female Sprague-Dawley rats were administered the target dose of 10 mg/kg b·w of ^{14}C-PNDP and were placed in specialized polypropylene cages where the air flow was tightly regulated as explained above. The expired air from the cage was bubbled through a solution of sodium hydroxide (1 M) to trap the $^{14}CO_2$. One mL of the trapping solution (NaOH) was collected at time intervals of 1, 4, 8, 24, 48, 96, 144, 192, and 240 hours postdosing and stored in vials until further
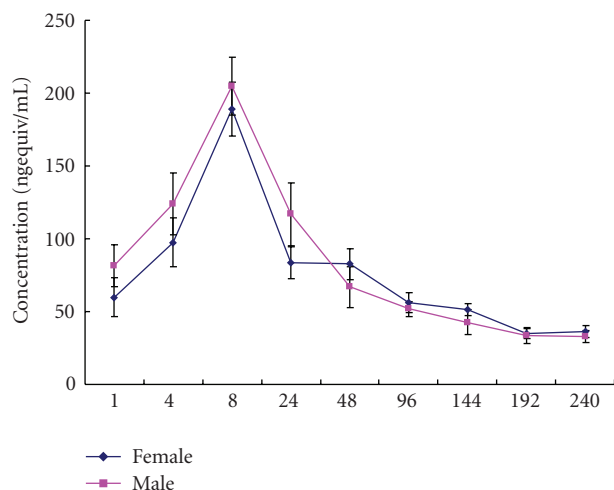
FIGURE 2: Mean plasma concentration-time profiles in male and female Sprague-Dawley rats after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean±S.D.).
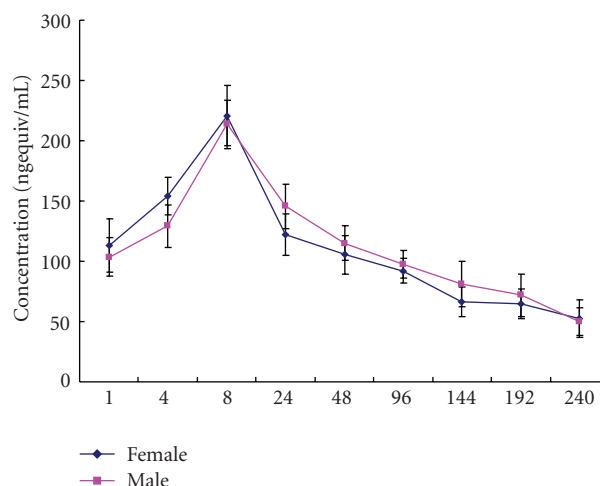

FIGURE 3: Mean blood concentration-time profiles in male and female Sprague-Dawley rats after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean±S.D.).

analysis. The aliquots were then mixed with appropriate volumes of Hionic-Fluor scintillation cocktail and analyzed by LSC.

3. Results

The results of absorption, distribution, excretion, and pharmacokinetic of ^{14}C-PNDP in male and female Sprague-Dawley rats following a single oral administration are presented hereunder.

3.1. Biodistribution of ^{14}C-PNDP in Blood and Plasma. ^{14}C-PNDP was quickly absorbed and delivered and was present in measurable levels throughout the entire body following oral administration. It was observed that ^{14}C-PNDP can easily pass through a number of compartments to reach targets around the whole body. This was evident by the observed radioactivity in the blood and plasma within 1 hour of dosing. The distribution of ^{14}C-PNDP was found to be almost similar across both genders of Sprague-Dawley rats. The mean plasma C_{max} of ^{14}C-PNDP was 207.2 μg/mL in male rats and 189.2 μg/mL in female rats. Corresponding blood C_{max} values for ^{14}C-PNDP were 222.8 μg/mL in male rats and 220.6 μg/mL in female rats. The area under the curve (AUC) in plasma was 14.1 and 14.7 μg·h/mL in male and female rats, whereas in blood it was 25.0 and 23.1 μg·h/mL in male and female rats, respectively (Table 1; Figures 2 and 3).

3.2. Biodistribution of ^{14}C-PNDP in Tissues. ^{14}C-PNDP was rapidly adsorbed mainly from the small intestine than from stomach following oral administration and it was rapidly distributed in most of the tissues studied which is evidenced by the observed radioactivity in all the tissues studied within 1 hour of drug administration. The C_{max} in the stomach (47.9
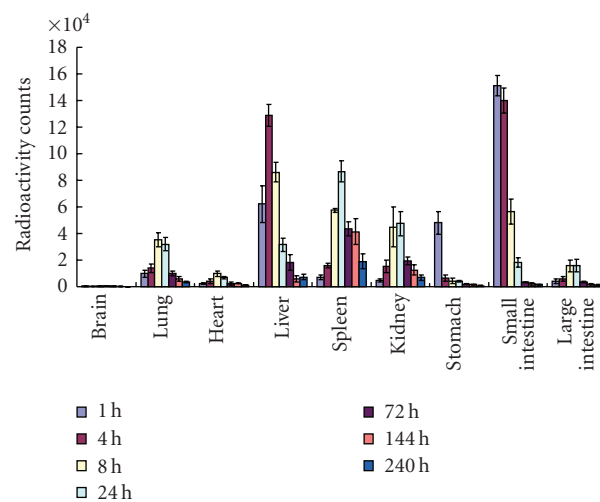

FIGURE 4: Mean concentrations of radioactivity/g tissue in male Sprague-Dawley rats after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean±S.D.).

and 30.7 μg eq/g) was reached in 1 hour in both male and female rats, while in small intestine (166.3 and 186.1 μg eq/g) it was observed after 2 hours in male rats and 4 hours in female rats. The drug was rapidly distributed to various organs with the C_{max} in the liver (186.8 and 189.8 μg eq/g) being observed by 4.7 hours both in male and female rats. The C_{max} in kidney (53.6 and 31.0 μg eq/g), heart (10.1 and 7.9 μg eq/g) and lungs (36.4 and 41.9 μg eq/g) was reached in 7−10 hours in both male and female rats. The C_{max} in spleen (88.0 and 76.6 μg eq/g) was reached in 21 hours and in brain (6.7 and 8.2 μg eq/g) it was in about 36 hours. The low levels of radioactivity observed in the brain indicate that ^{14}C-PNDP diffuses poorly through the blood-brain barrier. The radioactivity in all the tissues gradually decreased to less

Table 1: Plasma and blood pharmacokinetic parameters in male and female Sprague-Dawley rats after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP (mean$\pm$SD, $n = 6$).

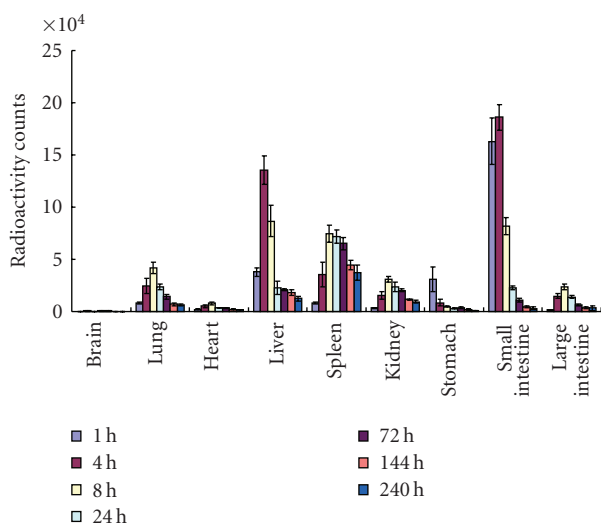|		C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{240\,hr}$ (μg·hr/mL)	AUC_{inf} (μg·hr/mL)
Male SD-rat	Plasma	207.2 ± 31.9	7.3 ± 1.6	148.5 ± 39.1	14.1 ± 1.5	21.4 ± 4.0
	Whole blood	222.8 ± 20.9	10.7 ± 6.5	138.1 ± 52.7	25.0 ± 1.8	35.6 ± 5.4
Female SD-rat	Plasma	189.2 ± 48.4	8.0 ± 0.0	157.5 ± 30.3	14.7 ± 1.1	23.1 ± 2.3
	Whole blood	220.6 ± 21.0	8.0 ± 0.0	211. ± 51.7	23.1 ± 2.8	38.8 ± 3.7

Figure 5: Mean concentrations of radioactivity/g tissue in female Sprague-Dawley rats after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean$\pm$S.D.).

Figure 6: Cumulative activity excretion profile in the urine and feces of male Sprague-Dawley rats at different time period after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean$\pm$S.D.).

Figure 7: Cumulative activity excretion profile in the urine and feces of female Sprague-Dawley rats at different time period after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean$\pm$S.D.).

than 30 μg eq/g post 48 hours of drug administration (Tables 2 and 3, Figures 4 and 5).

3.3. Mass Balance/Excretion of ^{14}C-PNDP. The main route of excretion for ^{14}C-PNDP was through the urine followed by fecal excretion. About 47% (49.9% in males and 47.6% in females) of the drug were excreted in the urine, whereas excretion of the drug in feces accounted for only about 5-6% of the total drug administered. Radioactivity was observed in urine and feces from 4 hours postdosing. The rate of excretion via urine peaked by 24 hours and thereafter it remained constant with no further decrease (Table 4, Figures 6 and 7).

3.4. $^{14}CO_2$ Breath Test. ^{14}C-PNDP was also excreted in the breath as $^{14}CO_2$. The rate of excretion was minimal for up to 8 hours and it peaked by 24 hours and thereafter there was a gradual increase in the radioactivity until 240 hours. There was no gender difference observed with respect to the excretion of the drug in the breath. The carcass also contained a moderate amount of radioactivity at 240 hours postdosing, and it accounted for about 7.7% of the radioactive dose (Figure 8).
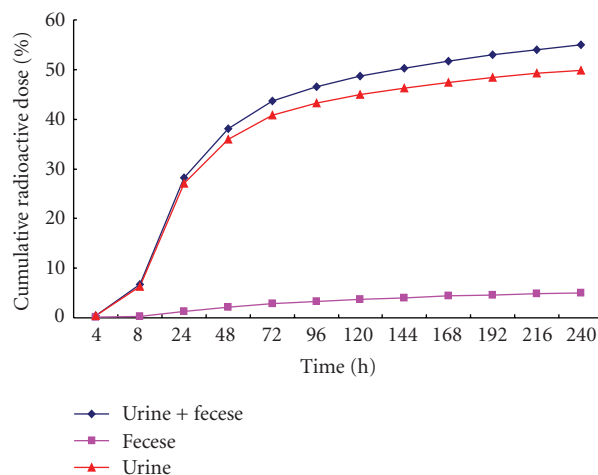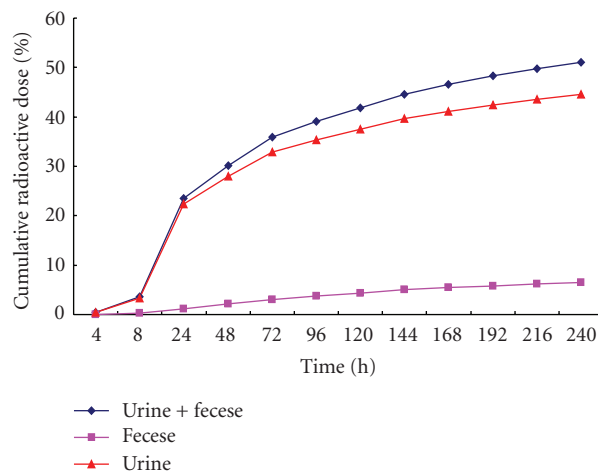
4. Discussion

The control of malaria has been severely compromised by the development of malaria parasites resistant, particularly in *Plasmodium falciparum* to nearly all available antimalarial

TABLE 2: Tissue pharmacokinetic parameters in male Sprague-Dawley rats after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean±S.D.).

Tissues	C_{max} (μg/g)	T_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{240\,hr}$ (μg·hr/g)	AUC_{inf} (μg·hr/g)
Brain	6.7 ± 1.8	31.3 ± 6.5	160.2 ± 165.3	78.1 ± 15.4	171.2 ± 65.5
Lung	36.4 ± 4.6	10.7 ± 6.5	109.0 ± 19.7	2573.6 ± 201.9	3006.6 ± 246.2
Heart	10.1 ± 1.5	10.7 ± 6.5	200.6 ± 37.9	713.8 ± 104.3	901.6 ± 170.4
Liver	186.8 ± 18.9	4.7 ± 1.6	216.4 ± 46.8	4167.5 ± 557.3	8107.2 ± 969.1
Spleen	88.0 ± 14.9	44.0 ± 49.0	125.7 ± 30.3	1005.3 ± 224.1	1367.7 ± 334.7
Kidney	53.6 ± 12.7	21.3 ± 6.5	111.8 ± 17.9	441.6 ± 44.2	557.3 ± 94.8
Stomach	47.9 ± 13.6	1.0 ± 0.0	129.1 ± 37.1	535.3 ± 12.8	700.9 ± 62.5
Small intestine	166.3 ± 15.5	2.0 ± 1.5	142.6 ± 73.6	2181.8 ± 251.3	2532.7 ± 505.5
Large intestine	17.5 ± 4.7	16.0 ± 8.8	105.0 ± 29.8	1063.8 ± 83.5	1265.6 ± 94.9

TABLE 3: Tissue pharmacokinetic parameters in female Sprague-Dawley rats after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean±S.D.).

Tissues	C_{max} (μg/g)	T_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{240\,hr}$ (μg·hr/g)	AUC_{inf} (μg·hr/g)
Brain	8.2 ± 2.7	36.7 ± 28.4	184.3 ± 111.2	107.1 ± 22.5	176.5 ± 28.9
Lung	41.9 ± 5.4	8.0 ± 0.0	120.3 ± 26.9	3033.5 ± 324.6	4123.0 ± 400.3
Heart	7.9 ± 1.0	7.3 ± 1.6	198.5 ± 53.9	700.6 ± 147.8	1267.9 ± 279.8
Liver	189.8 ± 8.1	4.7 ± 1.6	218.4 ± 74.0	4919.1 ± 342.2	8887.8 ± 894.9
Spleen	76.6 ± 7.0	42.3 ± 5.6	118.9 ± 55.1	1257.6 ± 509.9	2483.6 ± 536.8
Kidney	31.0 ± 2.8	8.0 ± 1.2	144.2 ± 17.5	372.5 ± 20.9	572.3 ± 60.9
Stomach	30.7 ± 11.8	1.0 ± 0.4	127.0 ± 27.9	623.2 ± 46.5	835.6 ± 49.7
Small intestine	186.1 ± 12.2	4.0 ± 1.2	166.6 ± 16.3	3479.4 ± 173.6	3793.5 ± 309.8
Large intestine	23.9 ± 2.8	18.0 ± 2.3	99.8 ± 29.6	1556.5 ± 125.6	2078.4 ± 539.9
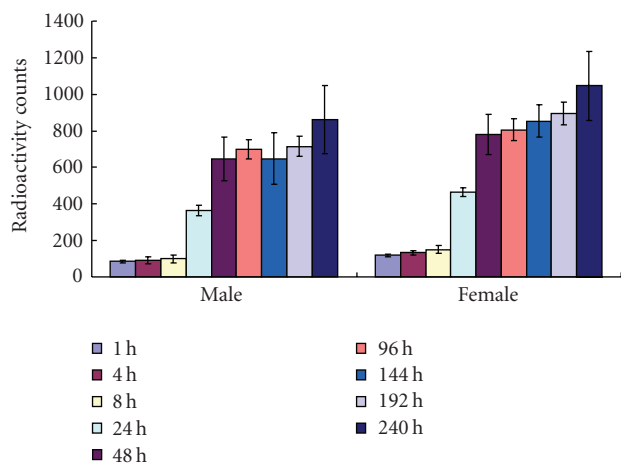
FIGURE 8: Excretion of ^{14}CO$_2$ in the breath of male and female Sprague-Dawley rats at different time period after administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP ($n = 6$, Mean±S.D.).

drugs used for prophylaxis and treatment [17]. The world-wide spread of strains of *Plasmodium* that are resistant to chloroquine and the serious side-effects encountered on using the newer drugs have highlighted the urgent need for developing new antimalarial drugs. Pyronaridine

tetraphosphate, a benzonaphthyridine derivative, has been in use in China for more than 10 years for the treatment of malaria. It is a Mannich base which is reported to exhibit antimalarial blood schizonticidal activity and is currently under investigation for use in the chemotherapy of malaria. Although the effect of pyronaridine on the ultrastructure of malaria parasite has been reported [18], studies on its absorption, distribution, and excretion in rodents have not been previously reported. Lee et al. [19] have reported that in vitro incubation of pyronaridine with rat and human liver microsomes resulted in the formation of 11 metabolites, with pyronaridine quinoneimine as the major metabolite. The synthesis of ^{14}C-PNDP by classical method and also by microwave irradiation technique has been earlier reported [15].

In the present investigation, the absorption of ^{14}C-PNDP after a single oral dose administration followed by its distribution in various tissues like blood, plasma, liver, lung, heart, spleen, kidney, brain, stomach, small intestine and large intestine and its excretion in urine, feces and in breath as ^{14}CO$_2$ were determined in male and female Sprague-Dawley rats for 10 days. After oral administration, close and regular visual monitoring of the animals revealed that there were no severe acute toxicity responses as none of the animals showed any signs of behavioral or neurological toxicity during the entire study period. The results indicate

Table 4: Mean percentage cumulative recovery of total radioactivity over 240 hours post administration of a single oral dose of 10 mg/kg of ^{14}C-PNDP.

	Mean percentage radioactive dose	
	Male SD-rat (%)	Female SD-rat (%)
Faeces	5.0 ± 0.7	6.5 ± 1.6
Urine	49.9 ± 3.5	47.6 ± 6.2
Cage rinse	0.5 ± 0.1	0.4 ± 0.1
CO_2	1.0 ± 0.2	1.1 ± 0.1
Carcass	12.7 ± 1.0	13.1 ± 2.0
Tissues	17.1 ± 1.5	18.9 ± 1.2
Blood	13.2 ± 1.3	12.2 ± 1.1
Total	99.4 ± 2.9	99.8 ± 2.5

that the drug is readily absorbed from the small intestine within 1 hour following oral administration and was widely distributed in most of the tissues investigated as determined from the observed radioactivity in the tissues. Peak value of the drug in the blood was reached at around 8 hours post administration and radioactivity was detected in most of the tissues from 4 hours onwards. ^{14}C-PNDP showed poor permeability across the blood-brain barrier characterized by low levels of radioactivity in the brain tissue. It was observed that the T_{max} in the liver was earlier than that of blood, indicating a faster absorption of the drug from the intestine and rapid transport to the liver. The overall absorption, distribution, and excretion of ^{14}C-PNDP were found to be gender-independent as both male and female rats showed a similar pattern of radioactivity. Excretion of the drug was predominantly through the urine which started around 10 hours post administration and peak excretion was observed from 24 hours onwards. A small amount of the drug was also excreted in the feces and also in the breath.

Phase II clinical trials on the pharmacokinetics, clinical and safety outcome of Pyramax (pyronaridine/artesunate) carried out in 16 patients in Uganda with acute falciparum malaria revealed that Pyramax was well tolerated and showed a very pronounced distribution and elimination phase [20]. The C_{max} values in patients receiving pyronaridine/artesunate (12 + 4 mg/Kg once a day for 3 days) were 226.1±157.5 ng/mL which is close to those found in rats in this study. The $t_{1/2}$ of ^{14}C-PNDP in this study was found to be significantly lower compared with that of Phase II clinical trials which is possibly due the multiple dose regimens. Administration of a single dose of ^{14}C-PNDP resulted in the AUC (0-inf) and T_{max} values which were almost similar to those observed in Phase II clinical trial data. The slight variation in these values can be explained by the fact that multiple dose regimens (once a day for 3 days) were used in the clinical trials whereas in the present investigation the rats were administred with only a single dose of ^{14}C-PNDP.

The life cycle of the malarial parasites in the human host occurs mainly in the blood and liver and hence, they are considered to be the main targets of any antimalarial drug. The result of the study shows that ^{14}C-PNDP is rapidly absorbed and distributed in the tissues and the drug was mainly concentrated in the liver and blood than other organs which is evident by the high C_{max} and $t_{1/2}$. The rapid absorption and slow elimination of PNDP from the target organs might provide effective antimalarial activity to the drug. According to the WHO, all uncomplicated *P. falciparum* infections should be treated with an artemisinin-based combination therapy and currently there are four ACTs that are recommended for use in the treatment of malaria: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate-sulfadoxine-pyrimethamine. The choice of ACT is based on the efficacy of the partner medicine in the country or area of intended deployment [1]. Although artemisinins are potent and rapidly acting antimalarial drugs, their widespread use for treating patients with *Plasmodium falciparum* malaria raises the question of emerging drug resistance [21, 22]. A recent study conducted along the Thai-Cambodian border reports of high failure rates associated with ACT, as well as in vitro drug-susceptibility data, suggesting the possibility of clinical artemisinin resistance [23, 24]. Based on their study, Noedl et al. [25] reported that although artemisinin resistance does not seem to be a widespread epidemiologic phenomenon currently, the prolonged parasite-clearance times during artesunate therapy are a matter of concern. These factors underscore the need for the development of improved ACTs and a better understanding of the pharmacokinetics and mechanism of action of the components of ACTs. In such a scenario, pyronaridine tetraphosphate is being further explored for its use in the treatment of malaria either alone or in combination with artemisinin. With Phase II and a controlled Phase III [26, 27] clinical trial on Pyramax of which pyronaridine is a major component showing positive results, its is believed that Pyramax will be available for treating both falciparum and vivax malaria by 2010 in Asia and Africa.

5. Conclusion

The results of the investigation showed that PNDP was readily absorbed and distributed to the tissues following an oral administration. The drug was well tolerated and

effectively eliminated in the urine. Phase II and a controlled Phase III clinical trial on Pyramax of which pyronaridine is a major component has shown positive results. With resistance being reported for ACTs, pyronaridine tetraphosphate seems to be a promising candidate for developing new ACTs.

Acknowledgments

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