Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 123–130.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1202

JLCR

Research Article

Synthesis and biological evaluation of ^{131}I-labelled pyronaridine

SANG HYUN PARK*, HUI JEONG GWON and KANNAMPALLI PRADEEP

Radiation Application Research Division, Korea Atomic Energy Research Institute, 1266 Sinjeong-dong, Jeongeup, Jeonbuk 580-185, Republic of Korea

Received 21 November 2006; Accepted 22 November 2006

Abstract: ^{131}I-Labelled pyronaridine, an antimalarial drug was synthesized and evaluated for its stability and biodistribution in rabbits. We observed that there was >99% yield of the labelled compound as determined by HPLC and radio-TLC in our study. Dynamic imaging studies in rabbits showed that ^{131}I-labelled pyronaridine was not concentrated in the thyroid for up to 30 min as that of Na^{131}I. Biodistribution studies in the blood, liver, heart, spleen, kidney and lung of rats revealed that the maximum percentage of the injected dose (% ID) and the percentage of the injected dose per gram organ weight (% ID/g) of ^{131}I-labelled pyronaridine was found in the blood and liver when compared to the other organs even after 6 days of the drug administration. Results suggest that ^{131}I-labelled pyronaridine can be effectively used as an imaging agent to study the metabolism and mode of a response of pyronaridine. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: ^{131}I-labelled pyronaridine; antimalarial drug; biodistribution

Introduction

Malaria is a serious and potentially fatal infectious disease that has been a scourge of mankind for many years. It still remains one of the most intractable human diseases responsible for over 2 million deaths annually, mostly in young children and pregnant women.[1] According to the World Health Organisation's (WHO) latest report, malaria occurs in over 100 countries accounting for nearly half the world population. Malaria primarily affects tropical regions, with over 90% of all cases occurring in sub-Saharan Africa. Symptoms of malaria include fever, chills, sweats and headache and in some instances may progress to jaundice, blood coagulation defects, shock, kidney or liver failure, central nervous system disorders and coma. Malaria is caused by four different species of the *Plasmodium* family of protozoa, *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The malarial parasite is typically transmitted to humans by mosquitoes belonging to the genus *Anopheles*. The human malarial parasite has a complex life cycle that requires both a human host and a mosquito host. In *Anopheles* mosquitoes, *Plasmodium* reproduces sexually (by merging the parasite's sex cells) and in humans, the parasite reproduces asexually (by a cell division), first in liver cells and then, repeatedly, in red blood cells. Hence, most antimalarial drugs are targeted to the liver and blood where the parasite is active and highly concentrated. In spite of the availability of a vast array of antimalarial drugs, malaria still continues to be a threat to mankind. This is mainly attributed to the development of a resistance towards the available drugs by the malarial parasite. This constrain underscores the need for the development of novel compounds with an enhanced potent activity. Hence, the quest for a new and effective antimalarial drug has become inevitable.[2] Various drugs are being tested both alone or as combinations and few have shown to be effective.[3] Pyronaridine is one such antimalarial drug that is currently under investigation. It was first synthesized in China in the late 1970s.[4] Chemically it is 2-methoxy-7-chloro-10-[3,5-bis(pyrolidinyl-1-methyl)-4-hydroxyphenyl-aminol] benzo [b]-1,5-naphthyridine and belongs to the class of an Azacrine type Mannich base.[5] Pyronaridine was found to be highly effective against multidrug-resistant *P. falciparum* both in laboratory strains and clones.[6] It is also reported to be effective against rodent malaria parasite[7] and

*Correspondence to: S. Hyun Park, Radiation Application Research Division, Korea Atomic Energy Research Institute, 1266 Sinjeong-dong, Jeongeup, Jeonbuk 580-185, Republic of Korea.
E-mail: parksh@kaeri.re.kr
Contract/grant sponsor: Korea Ministry of Science and Technology

against simian malaria.[8] Although the clinical efficacy of pyronaridine has been reported by many investigators,[9–10] information regarding its bioavailability and biodistribution has not been well documented.

Radiotracers are nowadays widely used to study the bioavailability, biodistribution, pharmacokinetics, structure-activity relationship and receptor-specific binding of novel molecules. This non-invasive imaging approach provides a powerful research tool for the discovery and development of new drugs, providing information which cannot be obtained by other means.[11] Radiolabelling of drugs can be accomplished by two methods. Direct labelling method involves a reaction between a drug and a radioisotope to produce a radiolabelled drug whereas, the indirect labelling method employs the use of spacers or linkers to attach a radioisotope to a drug. Although the binding of a radioisotope to a drug is random during a direct labelling, it does not cause any major changes with respect to the structure and molecular weight of a drug as in the case of an indirect labelling. Hence, we preferred to use a direct labelling method by using radio iodine (^{131}I) for the synthesis of ^{131}I-labelled pyronaridine. The aim of our investigation was to synthesis ^{131}I-labelled pyronaridine as an incomplete labelled compound and to study its biological behavior in rabbits and rats. The purity of the ^{131}I-labelled pyronaridine was determined by HPLC and radio-TLC. ^{131}I-labelled pyronaridine was then administered to rabbits to study its bioavailability. The *in vivo* stability of the ^{131}I-labelled pyronaridine was evaluated by administering Na^{131}I to rabbits at the same dose of the drug and it was also monitored for 30 min. In addition, the biodistribution of ^{131}I-labelled pyronaridine was determined in various organs like the blood, liver, heart, lung, spleen and kidney of rats at different time intervals of 30 min, 2, 4 and 6 days.

Results and discussion

Radio iodination of pyronaridine (**1**) with Na^{131}I was carried out as described in Scheme 1. Briefly, a mixture containing pyronaridine (2 mg), ascorbic acid (10 mg), $SnSO_4$ (0.5 mg) and $CuSO_4 \cdot 5H_2O$ (0.15 mg) was mixed with 0.9 ml H_2SO_4 (0.04 M) and stirred well. To this mixture Na^{131}I (pH 7–8) was added and the pH was adjusted to 7.4 with a 0.1 M phosphate buffer (pH 7.4) and heated in a heating block at 110°C for 30 min. ^{131}I-labelled pyronaridine (**2**) thus formed was then separated by using a DEAE sephadex column with a 0.2 μm filter. The labelling yield and radiochemical purity of the ^{131}I-labelled pyronaridine was then checked by HPLC and radio-TLC, respectively. The HPLC data (Figure 1) showed a single peak confirming the presence of >99% of the ^{131}I-labelled pyronaridine with only a few traces of the unlabelled drug and free ^{131}I. Radio chromatograms from radio-TLC (Figure 2) also confirmed the presence of >95% of the ^{131}I-labelled pyronaridine with only a few traces of the free iodine (^{131}I). The dynamic images of rabbits, 30 min post-injection showed that Na^{131}I was mainly concentrated in the thyroid (Figure 3). In contrast to this ^{131}I-labelled pyronaridine was not concentrated in the thyroid (Figure 4) even in trace quantities. The *in vivo* stability of radio iodinated compounds is mainly reflected in their thyroid radioactivity concentration. The relatively low level of radioactivity observed in the thyroid during the ^{131}I-labelled pyronaridine administration is suggestive of the fact that it is highly stable in *in vivo*. Determination of the biodistribution of the drug in various organs in rats from 30 min, 2, 4 and 6 days revealed that maximum percentage of the injected dose (% ID) and the percentage of the injected dose per gram tissue (% ID/g) was found in the blood and liver when compared to the other organs (Figures 5–8). Although the concentration decreased with an increased number of days, the blood and liver maintained a high level of the drug when compared to the other organs throughout the study period. Malarial parasites are mainly concentrated in the blood (RBC's) and liver, and are hence considered to be the effective targets of antimalarial drugs. The above results clearly demonstrate that a complete bioavailability of the ^{131}I-labelled pyronaridine is ensured at the target organs.

Animal experiments

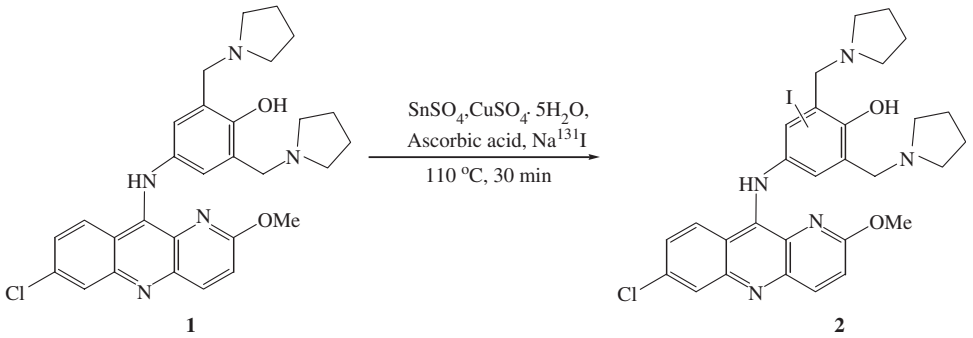
The biological behavior of ^{131}I-labelled pyronaridine was studied in rabbits by an administration of a single dose (33.3 MBq/0.9 ml) of the radiotracer. To check the *in vivo* stability of the drug, Na^{131}I was also administered to another group of rabbits at the same dose. The biodistribution pattern was obtained by using rats at 30 min, 2, 4 and 6 days post-injection of ^{131}I-labelled pyronaridine from the uptake of the drug by the excised organs (blood, liver, kidney, spleen, heart and lung). The biodistribution data showed that the ^{131}I-labelled pyronaridine was mainly accumulated in the blood and liver when compared to the other organs studied.

Experimental

General

Na^{131}I was obtained from the Research reactor, HANARO, KAERI. Unless otherwise stated, all the other chemicals and reagents used were of analytical grade and were used without any further purification.

DOI: 10.1002.jlcr



1 2

Scheme 1 Synthesis of ^{131}I-labelled pyronaridine (**2**) from Na^{131}I and pyronaridine (**1**).

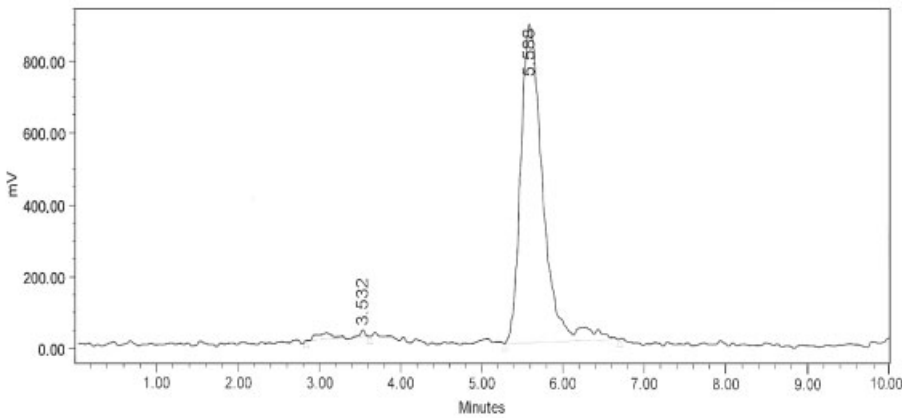


Figure 1 HPLC chromatogram of ^{131} I-labelled pyronaridine. Experimental conditions: μBondapack C-18 column; Injection volume: 2 μl; Mobile phase: ethanol/ethyl acetate/NH_4Cl (20/20/1); flow rate: 1 ml/min; R_t:5.58 min.

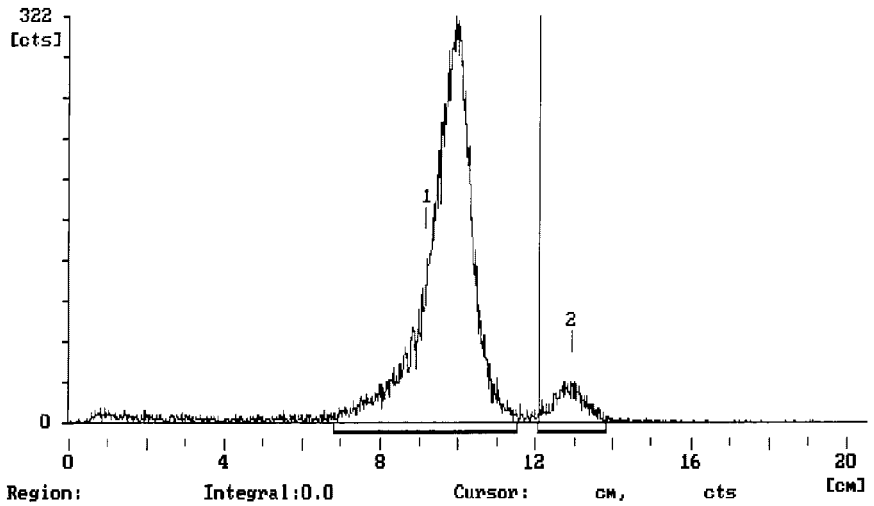


Figure 2 Radio-TLC chromatogram of ^{131} I-labelled pyronaridine. Experimental conditions: mobile phase: ethanol/ethyl acetate/NH_4Cl (20/20/1); R_t: 9 min.

Chemistry

Radio-TLC—The labelling efficiency and stability of the ^{131}I-labelled pyronaridine were measured with a radio thin layer chromatography (radio-TLC) system which consisted of a radio-TLC scanner (EC & G Berthold Linear Analyzer, Germany) and a one-dimensional analysis of the Berthold chroma program. ^{131}I-labelled pyronaridine solution was spotted onto a silica gel coated fiber sheet (Gelman Sciences

DOI: 10.1002.jlcr

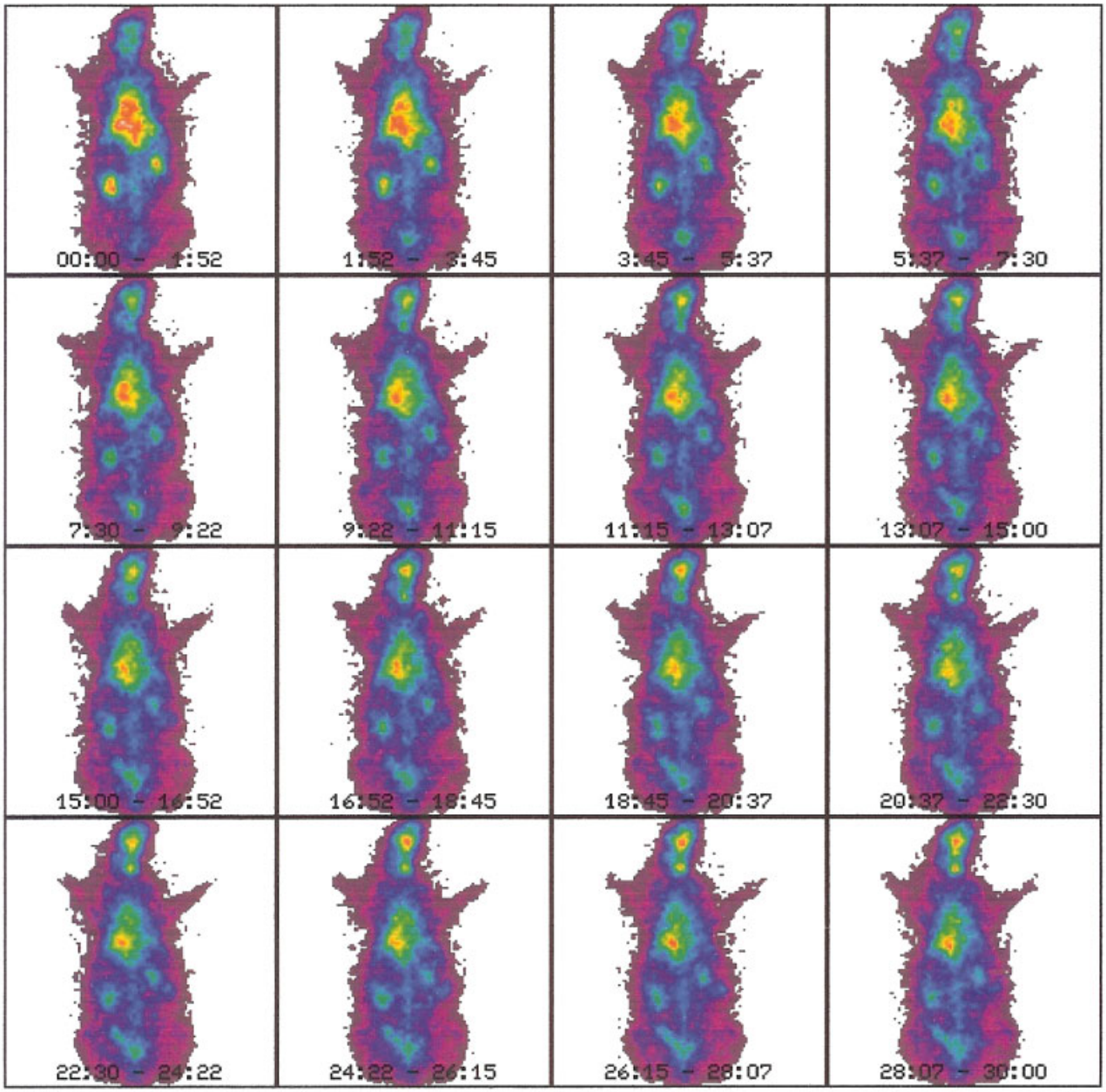


Figure 3 Dynamic image scans of a rabbit for 30 min after a single intraperitoneal administration of $Na^{131}I$. Figure available in colour online at www.interscience.com

Inc., Ann Arbor, MI, USA) and the sheet was eluted with a mixture of ethanol: ethyl acetate: NH_4Cl (20:20:1). The labelling efficiency of the ^{131}I-labelled pyronaridine was calculated by comparing the radioactivity of ^{131}I-labelled pyronaridine (at the origin) and the free iodine peaks (at the solvent front). Labelling yield of the ^{131}I-labelled pyronaridine was checked by HPLC (Waters, USA), coupled with a μBondapak C-18 column (3.9×300 mm, Waters, USA). A mixture of ethanol: ethyl acetate: NH_4Cl (20:20:1) was also used as a mobile phase, Isocratic (100%) with a flow rate of 1 ml/min and an injection volume of 2 µl.

Radioimaging studies

Imaging studies were carried out using 6-week-old male New Zeland white rabbits (2731 ± 52.9 g; n=3) obtained from Gyeryong Science Inc. (Daejon, Korea). Rabbits were injected with 33.3 MBq/0.9 ml in saline with the test radio labelled complexes (^{131}I-labelled pyronaridine and $Na^{131}I$) through the left ear vein. All

DOI: 10.1002.jlcr

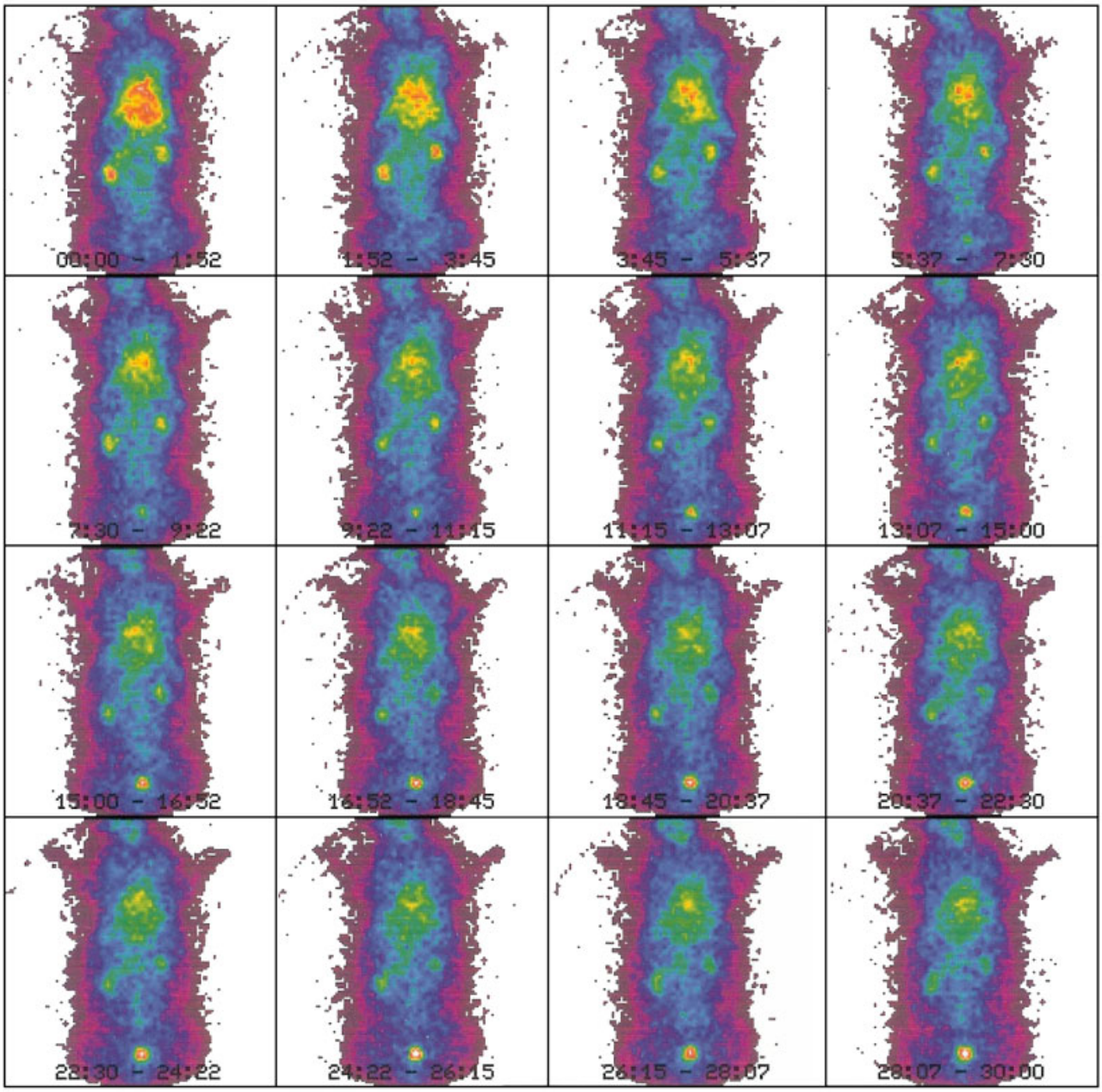


Figure 4 Dynamic image scans of a rabbit for 30 min after a single intraperitoneal administration of ^{131}I-labelled pyronaridine. Figure available in colour online at www.interscience.com

the rabbits were placed in a posterior position. To confirm the dynamic kinetics of the injected compounds, whole body dynamic images for 30 min and 16 dynamic images at predetermined time intervals were obtained by using a gamma camera fitted with a low energy all-purpose collimator. Image data was analyzed under a dynamic procedure of the Micro delta system (Siemens, USA). The dynamic images were taken at 1.52, 3.45, 5.37, 7.30, 9.22, 11.15, 13.07, 15.00, 16.52, 18.45, 20.37, 22.30, 24.22, 26.15, 28.07, and 30 min post administration with a Microdot imager (Siemens, USA).

Biodistribution

Studies were carried out by using healthy female Sprague-Dawley rats (155 ± 3.8 g) obtained from Gyeryong Science Inc. (Daejon, Korea). Animals were randomly divided into four groups with three animals in each group. ^{131}I-labelled pyronaridine was injected

DOI: 10.1002.jlcr

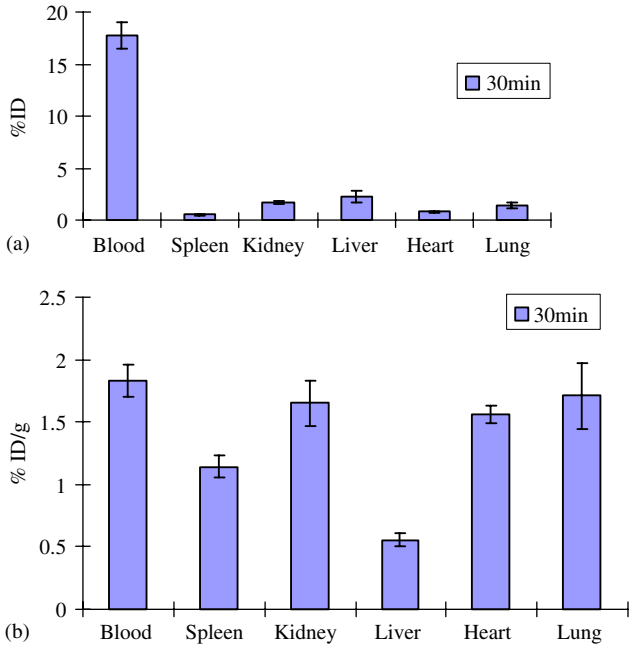


Figure 5 Biodistribution of ^{131}I-labelled pyronaridine in different organs of rats 30 min after an administration: (a) expressed as % ID (*n*=3); (b) expressed as % ID/g tissue (*n*=3). Figure available in colour online at www.interscience.com

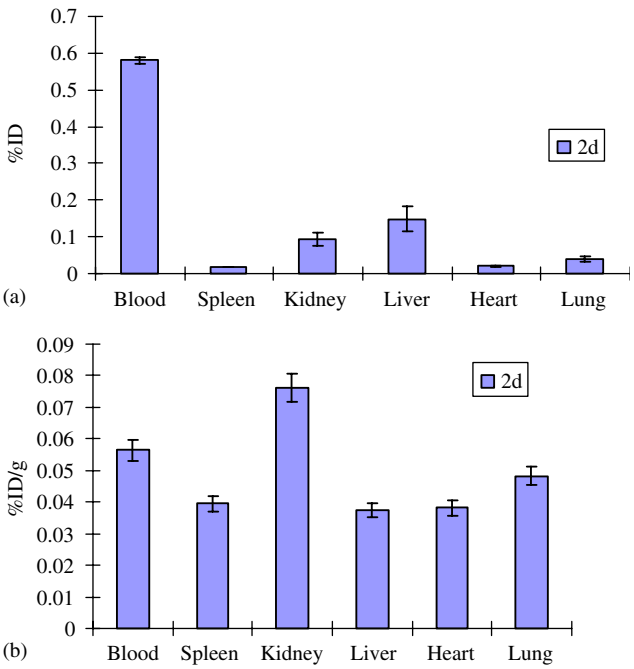


Figure 6 Biodistribution of ^{131}I-labelled pyronaridine in different organs of rats 2 days after an administration: (a) expressed as % ID (*n*=3); (b) expressed as % ID/g tissue (*n*=3). Figure available in colour online at www.interscience.com

into the tail vein under mild isofluorane anesthesia at a dose of 33.3 MBq/0.9 ml in saline. Animals were maintained on a normal diet and water *ad libitum*. Animals in all the groups were sacrificed by an excess anesthesia at different time intervals of 30 min, 2, 4 and 6 days post injection with the radiotracer. Tissue samples (0.1 g) of the main organs were removed, weighed and the activity measured in a γ-counter (Beckman model). The radioactivity in various organs were expressed as percent of the injected dose (% ID)

DOI: 10.1002.jlcr

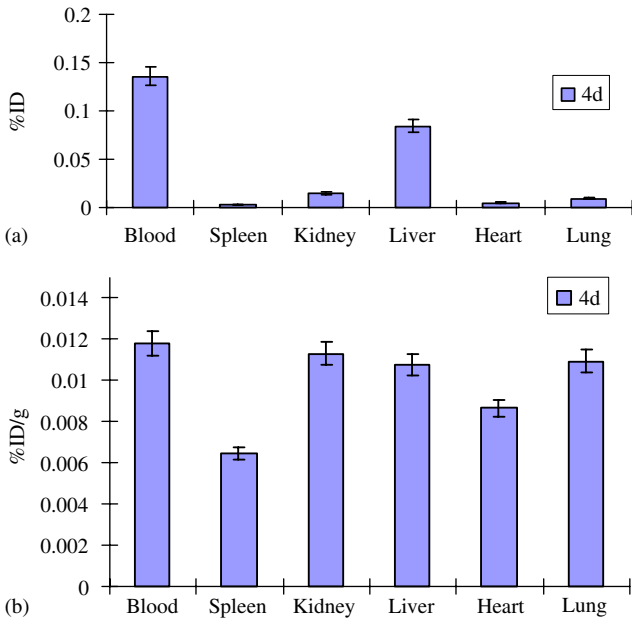


Figure 7 Biodistribution of ^{131}I-labelled pyronaridine in different organs of rats 4 days after an administration: (a) expressed as % ID (*n*=3); (b) expressed as % ID/g tissue (*n*=3). Figure available in colour online at www.interscience.com

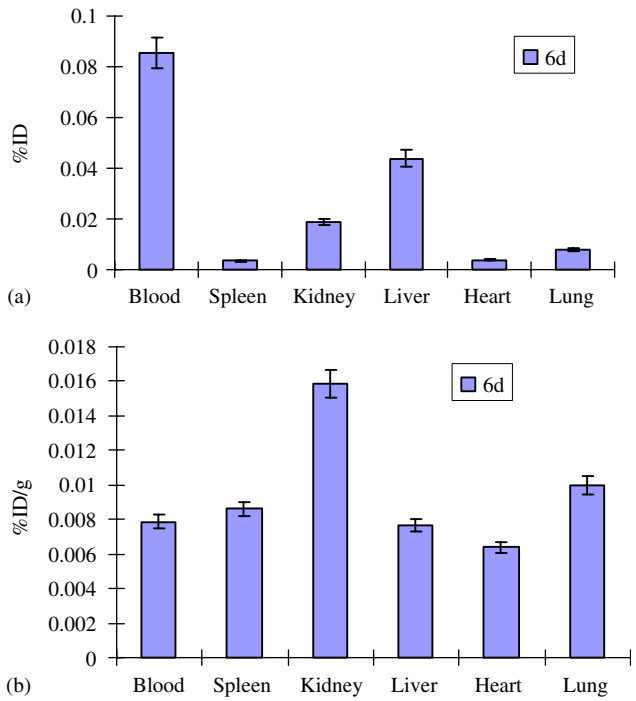


Figure 8 Biodistribution of ^{131}I-labelled pyronaridine in different organs of rats 6 days after an administration: (a) expressed as % ID (*n*=3); (b) expressed as % ID/g tissue (*n*=3). Figure available in colour online at www.interscience.com

and also as percent of the injected dose per gram of the organ (% ID/g organ) and presented as mean ± SD for three of the animals at each time period. In the case of the blood, this value was calculated by assuming that it constitutes 6% of the total weight.

Conclusions

In this paper, we have demonstrated the synthesis, *in vivo* stability and biodistribution of ^{131}I-labelled pyronaridine. We found that ^{131}I-labelled pyronaridine

DOI: 10.1002.jlcr

was stable in an *in vivo* condition during the study period, as it was not deposited in the thyroid unlike $Na^{131}I$. Biodistribution studies on rats revealed that it was mainly concentrated in the blood and liver which are considered to be the prime targets of antimalarial drugs. Hence, we conclude that ^{131}I-labelled pyronaridine can be effectively used as a radiotracer to study the metabolism and mode of a response of pyronaridine.

Acknowledgements

This work was supported by the nuclear research development project from Korea Ministry of Science and Technology.

REFERENCES

1. Sachs J, Malaney P. *Nature* 2002; **415**: 680–685.
2. Mather MW, Darrouzet E, Valkova-Valchanova M, Cooley JW, McIntosh MT, Daldal F, Vaidya AB. *J Biol Chem* 2005; **280**: 27458–27465.
3. Robert A, Benoit-Vical F, Dechy-Cabaret, Meunier B. *Pure Appl Chem* 2001; **73**: 1173–1188.
4. Zeng XY, Xia Y, Gao FH, Guo HZ, Chen C. *Acta Pharmacol Sinica* 1979; **14**: 736–737.
5. Elueze EI, Croft SL, Warhurst DC. *J Antimicrob Chemother* 1996; **37**: 511–518.
6. Childs GE, Hausler B, Milhous W, Chen C, Wimonwattrawatee T. *Am J Trop Med Hyg* 1988; **38**: 24–29.
7. Peters W, Robinson BL. *Ann Trop Med Parasitol* 1992; **86**: 455–465.
8. Kotecka BM, Barlin GB, Edstein MD, Rieckmann KH. *Antimicrob Agents Chemother* 1997; **41**: 1369–1371.
9. Ringwald P, Bickii J, Same-Ekobo A, Basco LK. *Antimicrob Agents Chemother* 1997; **41**: 2317–2319.
10. Pradines B, Tall A, Parzy D, Spiegel A, Fusia T, Hienne R, Trape JF, Doury JC. *J Antimicrob Chemother* 1998; **42**: 333–339.
11. Marathe PH, Shyu WC, Humpreys WG. *Curr Pharm Design* 2004; **10**: 2991–3008.