DISTRIBUTION OF C^{14}-LABELLED ARTEETHER IN KIDNEYS AND LIVERS OF EXPERIMENTAL MICE AFTER INTRAMUSCULAR INJECTION

Cheeraratana Cheeramakara[1], Vasant Khachonsaksumet[2], Nopachai Suthisai[1], Wanyarat Nakosiri[1], Kriyaporn Songmuaeng[1], Channarong Saenghirun[1], Duangduen Phienpicharn[1] and Apichart Nontprasert[3]

[1]Department of Tropical Radioisotopes, [2]Department of Tropical Pathology, [3]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. To study the distribution and localization of oil-soluble arteether in experimental mice, we injected C^{14}-labelled arteether (20 μCi/kg body weight) intramuscularly and measured radioactivity in the blood, kidney, and liver. The labelled arteether distributed and localized more to the kidney (819,180.4 ± 34,134 dpm/cm^3) than the liver (288,628.9 ± 54,954 dpm/cm^3) 4 hours post-injection. The main localization of labelled arteether was in the kidney cortex rather than the medulla ($p < 0.05$). However, the distribution of radioactivity was homogeneous in the liver. The terminal half-life of labelled arteether in the blood was 1.8 hours. The blood : kidney : liver ratio was 1 : 5 : 2. These findings show that labelled arteether was distributed quickly and localized in the cytoplasmic cortex of the kidney and homogeneously in the liver.

INTRODUCTION

Artemisinin is a natural component of the plant *Artemisia annua*, used in traditional Chinese medicine for over 2,000 years to treat fever. Artemether and artesunate are its best-known derivatives. A number of semi-synthetic derivatives have been prepared in recent years, *eg*, arteether, artelinate and dihydroartemisinin. Artemisinin derivatives have been widely used for multidrug-resistant falciparum malaria. In clinical trials, and in general use, artemisinin derivatives have been very well tolerated (Hien and White, 1993; Price *et al,* 1999).

Arteether is an ethyl ether derivative of artemisinin. Arteether and the closely related compound, artemether are oil-soluble derivatives of dihydroartemisinin formulated for intramuscular injection in sesame oil and groundnut oil, respectively. Arteether can be used in the treatment of acute falciparum malaria (Pareek *et al*, 2006). It has a long elimination half life (> 20 hours), and is more stable than the other artemisinin compounds (WHO, 2001). The toxic effects of arteether and artemether in animals are similar. In rodents, dogs, and monkeys, these oil-soluble drugs at high doses have been associated with an unusual pattern of selective damage to auditory and vestibular areas of the brain stem (Brewer *et al,* 1994a,b; Kamchonwongpaisan *et al,* 1997; Petras *et al,* 1997; Nontprasert *et al,* 1998; Nontprasert *et al,* 2000, 2002). Apart from central nervous system toxicity, arteether has been shown to exhibit toxicity in the liver, kidney, bone marrow, heart, and reproductive organs (Davidson, 1994). Although the pharmacokinetics of arteether have been reported (Li *et al*, 1998), there is no information on the

Correspondence: Dr Apichart Nontprasert, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2354 9100 ext 1437; Fax: 66 (0) 2354 9169
E-mail: apichart@tropmedres.ac

distribution or excretion of arteether using a radio-labelled drug. We therefore investigated the distribution and localization of C^{14}-labelled arteether in experimental mice.

MATERIALS AND METHODS

Preparation of C^{14}-labelled arteether for injection

The C^{14}-labelled arteether was manufactured by the Research Triangle Institute, USA. The radio-labelled arteether (radioisotope activity 0.992 mCi, specific activity 28.8 mCi/mmol, M.W. of arteether 312.29) in solid form was dissolved in 50 μl of 50% ethanol and kept refrigerated for stock. The working solution for labelled arteether was further diluted with unlabelled arteether in sesame oil, to obtain a specific activity of 2 μCi/mg arteether.

Animals

The experimental procedures were approved by the Ethics Committee for Research in Animals, Faculty of Tropical Medicine, Mahidol University, Thailand. All animal experiments were performed according to international biomedical-research standards. Adult male Swiss albino mice weighing 23 - 25 g, obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand, were used. The animals were allowed to acclimate to the new environment for 2 weeks before entering the study. They were housed one mouse per cage with access to food and water *ad libitum* and were provided standard care for laboratory animals.

Administration of radioisotope, blood sampling and tissue preparation

Experimental mice were placed in a mouse-handling box. Human exposure to radiation was prevented by the use of a plastic shield. The mice were injected intramuscularly with a dose of 20 μCi/kg (10 mg/kg) of C^{14}-labelled arteether (n = 5) or placebo (n = 2). Blood samples were taken at time 0 (before injection), 5′, 15′, 30′, 40′, 50′, 60′, 70′, 80′, 90′, 120′, 150′, 180′, 210′, and 240′ post-injection by cutting and squeezing blood from the tail vein. Ten microliters were taken for each blood sample, to count whole-blood radioactivity. After 240 minutes, the animals were anesthetized by injecting 4% sodium chloral hydrate intraperitoneally. Perfusion and fixation was performed by infusing 0.96% sodium chloride transcardially from the left ventricle through the right atrium, followed by a fixative solution (37% formalin in phosphate buffer). The livers and kidneys were removed for measurement of radioactivity. Tissue sections for autoradiography were frozen by dipping into a hexane-solid CO_2 mixture (-80ºC) and embedded in pre-freezing solution. The embedded tissues were sagitally serial sectioned at 35 μm thickness by a cryostat section machine (Leica). The tissue slices were placed onto transparent plastic sheets and left to dry at room temperature, or dipped in 95% ethanol then absolute ethanol. Three slices of tissue were put into a 1.5 ml Eppendorf tube to measure radioactivity.

Blood and tissue extraction for radioactivity measurement

Ten microliters of whole blood were digested in 100 μl of a mixture of Soluene-350 (Packard) and isopropanol (1 : 1) at room temperature until the solution was clear. The blood sample was bleached with 20 μl hydrogen peroxide (30%) for 10-15 minutes and kept at 40ºC for 10-15 minutes. BetaMax scintillation fluid (Packard Instrument Co) was added to the extracted samples and radioactivity was measured with a Beta counter (LKB).

Tissues were prepared fresh, after removal from the body, weighed, minced finely with scissors, and blended with a mortar. Extraction was performed with 1 ml Soluene-350 for every 100 mg of moist tissue. Digestion was performed at 50ºC for 4 hours. Scintillation fluid was added to the extracted tissue before measuring radioactivity.

Autoradiography of mouse liver and kidney

The tissue slices, on transparent plastic, were put in close contact with a low energy storage phosphor screen (Amersham Biosciences) in a cassette for 12 days. The radioactive signal on the phosphor screen was detected by a Typhoon laser scanned imager (Amersham Biosciences) at 100 μm resolution, high sensitivity, using a red filter. Then, the images were assessed quantitatively for signal density and the tissue area was measured using the NIH ImageJ program. Parts of the kidney and liver in the autoradiogram were identified by comparison with a rat kidney and liver atlas. Unneeded signals on the phosphor screen was deleted by Image eraser (Amersham Biosciences) for 15 minutes.

All radioactive waste, corpses and contaminated materials were sent to the Office of Atomic Energy for Peace, Bangkok, Thailand to dispose of any radioactive waste safely. Radioisotope handling and disposal strictly adhered to the guidelines for radiation safety.

Statistical analysis

Pharmacokinetic characterization was performed by non-compartmental analysis as implemented in WinNonlin version 4.1 (Pharsight Corporation, Mountain View, CA). Non-compartmental analysis was performed using the log-linear trapezoidal method. The terminal elimination half-life, maximal radioactivity concentration (C_{max}), time to maximal concentration (T_{max}), and area under the curve (AUC) was estimated by log-linear regression of 5 observed animals. Radioactivity in the extracted livers and kidneys was compared and the intensities of the autoradiograms for these tissues was also compared using the Student's *t*-test (SPSS, Chicago, IL).

RESULTS

The pharmacokinetics of a single dose of intramuscular C^{14}-labelled arteether in rat blood showed a long terminal half-life of 1.8 hours, T_{max} 36 minutes, C_{max} 5,184.8 dpm/ml, AUC 559,215.9. The labelled arteether was quickly absorbed and extensively distributed to many organs. It persisted for a long time and was eliminated from the body slowly.

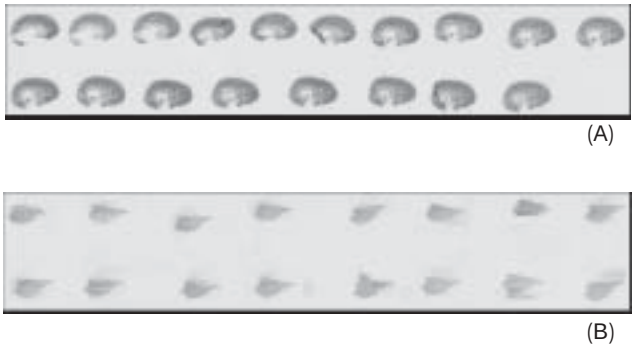

Fig 1–Autoradiography of the sagittal section of mouse liver and kidney after intramuscular administration of C^{14}-labelled arteether at hour 4 of injection (20 μCi/kg body weight). A) radioactivity signal of labelled arteether in the kidney. B) radioactivity signal of labeled arteether in the liver.

Four hours after injection, labeled arteether was distributed to the liver and kidneys, as shown in Fig 1. Autoradiograms of tissue exposure to labelled arteether indicated that the density of the radioactive signal in the kidney ($8.2 \times 10^5 \pm 3.41 \times 10^4$ dpm/cm^3) was significantly higher than the liver ($2.88 \times 10^5 \pm 5.49 \times 10^4$ dpm/cm^3). There were no significant differences in total radioactivity count per cm^3 in serial sections of liver and kidney tissues. The ratio of labelled arteether distribution in the blood : kidney : liver was 1 : 5 : 2. Higher kidney radioactivity was noted in the cytoplasmic cortex than the medulla ($p = 0.001$). The radioactivity signal was homogeneous in the liver. When compared with fresh tissue preparation, labelled arteether was distributed to the kidneys ($1.24 \times 10^6 \pm 5.02 \times 10^5$ dpm/g wet wt) and liver ($3.45 \times 10^5 \pm 2.33 \times 10^5$ dpm/g wet wt), at 3% and 0.1% of the injecting dose, respectively.

DISCUSSION

The liver and kidneys are the major organs protecting the intrusion of xenobiotics, either by preventing their absorption or by detoxifying and eliminating them from the body. Drug transport in the liver and kidneys is similar, with movement across epithelial cells by simple diffusion or by specific transport processes. In this study, C^{14}-labelled arteether was extensively distributed to the liver and kidneys. The labelled arteether was quickly absorbed and persisted for a long time before being slowly eliminated from the mice. A higher level of labelled arteether was found in the tenal cortex than the medulla of the kidney but homogeneous distribution was found in the liver 4 hours after injection. These findings of absorption, distribution, and elimination of labelled arteether are comparable to previous reports in experimental animals.

Early Chinese studies on pharmacokinetics and drug distribution in organs showed that H^3-labelled qinghaosu was absorbed and excreted rapidly when given orally or subcutaneously to mice (Qinghaosu-antimalaria Coordinating Research Group, 1979). Radioactivity in the blood and organs peaked in 1 hour, and dropped rapidly thereafter. The half-life of the drug was 4 hours. Approximately 80% of total radioactivity was excreted via urine and stool within 24 hours post-administration. The distribution of oral artemisinin showed that the highest level of drug was found in the liver, followed by the brain, plasma, and lungs, but lower levels in the kidneys, muscle, heart, and spleen. The radioactivity content of mice tissues after intramuscular administration of H^3-labelled artemether decreased considerably within 24 hours; the liver was the most exposed organ, but radioactivity in the brain was very low (China Co-operative Research Group, 1982).

The drug with the greatest percentage of conversion for oral, intramuscular and intravenous routes in rats was artesunate, followed by arteether, artemether, and artelinate (Li *et al*, 1998). Differences between the metabolism and tissue distribution of the lipid- and water-soluble analogs may provide a possible explanation for this observation. Artesunate is hydrolyzed by ubiquitous plasma esterase, rather than by hepatic enzymes, and is rapidly converted into metabolized dihydroartemisinin. The conversion of artemether and arteether to dihydroartemisinin is limited by their distribution into peripheral tissues because of their lipid solubility (Li *et al*, 1998). Therefore, the radioactivity of labeled arteether detected in this study was more likely to be the parent drug. C^{14}-labelled artesunate in rats was widely distributed, mostly to the intestinal tract, brain, kidneys, and liver within the first hour post-intravenous administration, and rapidly declined after 24 hours (Zhao and Song, 1989; Li *et al*, 2006). In rats, the excretion and distribution of intravenous C^{14}-labelled artemether into the bile, liver, kidney, and intestinal tract, was rapid during the first hour after injection. Small amounts of the drug were found in the brain, heart, lungs, and spleen. After 5 hours, plasma samples were devoid of quantifiable radioactivity (Maggs *et al*, 2000).

Toxicity studies showed that intramuscular artemether (100 mg/kg for 28 days) caused neuropathological damage in mice (Nontprasert *et al*, 2002). High-dose artemether (400 mg/kg) administered orally to rats once every 2 weeks resulted in transient focal degeneration of the liver (Xiao *et al*, 2002). Due to its lipid solubility and low molecular mass (288), the labeled arteether can penetrate into the cerebrospinal fluid and accumulate in the lipid-rich brain (Kearney and Aweeka, 1999), liver, and kidneys. A long elimination half-life may exacerbate organ toxicity in mice.

ACKNOWLEDGEMENTS

We are very grateful to Dr R Scott Miller,

Armed Forces Research Institute of Medical Sciences (AFRIMS), for kindly providing C^{14}-labelled arteether. We thank Dr Sumalee Kamchonwongpaisan and the staff of the National Center for Genetic Engineering and Biotechnology (BIOTEC) for their helpful technical advice and use of instruments. This study was supported by the Wellcome Trust of Great Britain. Thanks to the Faculty of Tropical Medicine, Mahidol University, for underwriting the publication fee.

REFERENCES

Brewer TG, Peggins JO, Grate SJ, Petras JM, Levine BS, Weina PJ. Neurotoxicity in animals due to arteether and artemether. *Trans R Soc Trop Med Hyg* 1994a; 88: 33-6.

Brewer TG, Grate SJ, Peggins JO, Weina PJ, Petras JM, Levine BS. Fatal neurotoxicity of arteether and artemether. *Am J Trop Med Hyg* 1994b; 51: 251-9.

China Cooperative Research Group. Studies on the toxicity of qinghaosu and its derivative. *J Tradit Chin Med* 1982; 2: 31-8.

Davidson DE Jr. Role of arteether in the treatment of malaria and plans for further development. *Trans R Soc Trop Med Hyg* 1994; 88: S51-2.

Hien TT, White NJ. Qinghaosu. *Lancet* 1993; 341: 603-8.

Kamchonwongpaisan S, McKeever P, Hossler P, Ziffer H, Meshnick SR. Artemisinin neurotoxicity : neuropathology in rats and mechanistic studies *in-vitro*. *Am J Trop Med Hyg* 1997; 56: 7-12.

Kearney BP, Aweeka FT. The penetration of anti-infectives into the central nervous system. *Neurol Clin* 1999; 17: 883-900.

Li QG, Peggins JO, Fleckenstein LL, Masonic K, Heiffer MH, Brewer TG. The pharmacokinetics and bioavailability of dihydroartemisinin, arteether, artemether, artesunic acid and artelinic aid in rats. *J Pharm Pharmacol* 1998; 50: 173-82.

Li Q, Xie LH, Haeberle A, Zhang J, Weina P. The evaluation of radiolabeled artesunate on tissue distribution in rats and protein binding in humans. *Am J Trop Med Hyg* 2006; 75: 817-26.

Maggs JI, Bishop LPD, Edwards G, O' Nell PM, Winstanley PA, Park KB. Biliary metabolites of β-artemether in rats: biotransformations of an antimalarial endoperoxide. *Drug Met Disp* 2000; 28: 209-17.

Nontprasert A, Nosten-Bertrand M, Pukrittayakamee S, Vanijanonta S, Angus BJ, White NJ. Assessment of the neurotoxicity of parenteral artemisinin derivatives in mice. *Am J Trop Med Hyg* 1998; 59: 519-22.

Nontprasert A, Pukrittayakamee S, Dondorp AM, Clemens R, Looareesuwan S, White NJ. Neuropathological toxicity of artemisinin derivatives in a mouse model. *Am J Trop Med Hyg* 2002; 67: 423-9.

Nontprasert A, Pukrittayakamee S, Nosten-Bertrand M, Vanijanonta S, White N J. Studies of the neurotoxicity of oral artemisinin derivatives in mice. *Am J Trop Med Hyg* 2000; 62: 409-12.

Pareek A, Nande A, Kochar D, Patel KH, Mishra SK, Mathur PC. Efficacy and safety of β-arteether and α/β-arteether for treatment of acute *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 2006; 75: 139-42.

Petras JM, Kyle DE, Gettayacamin M, *et al*. Arteether: risks of two week administration in *Macaca mulatta*. *Am J Trop Med Hyg* 1997; 56: 390-6.

Price R, Van Vugt M, Phaipun L, *et al.* Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am J Trop Med Hyg* 1999; 60: 547-55.

Qinghaosu-antimalaria coordinating research group. Antimalaria studies on Qinghaosu. *Chin Med J* 1979; 2: 81-6.

World Health Organization. The use of antimalarial drugs. Report of an informal consultation. Geneva: World Health Organization, 2001.

Xiao SH, Yang Y, You Q, *et al*. Potential long-term toxicity of repeated orally administered doses of artemether in rats. *Am J Trop Med Hyg* 2002; 66: 30-4.

Zhao KC, Song ZY. Distribution and excretion of artesunate in rats. *Proc Chin Acad Med Sci Peking Union Med Coll* 1989; 4: 186-8.