Experimental Parasitology 161 (2016) 54–61

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Application of SPECT/CT imaging system and radiochemical analysis for investigation of blood kinetics and tissue distribution of radiolabeled plumbagin in healthy and *Plasmodium berghei*-infected mice

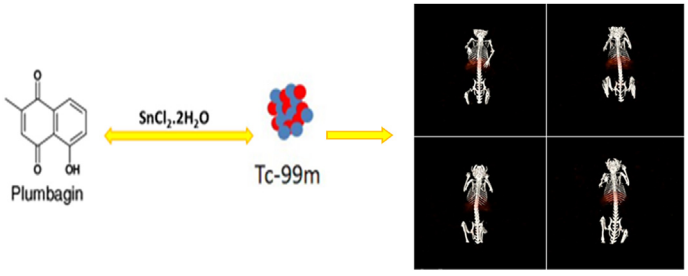
W. Sumsakul [a], J. Karbwang [b], K. Na-Bangchang [c, *]

[a] Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, 12121, Thailand
[b] Department of Clinical Product Development, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan
[c] Center of Excellence for Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Graduate Program in Bioclinical Sciences, Chulabhorn International College of Medicine, Thammasat University, Pathumthani, 12121, Thailand

HIGHLIGHTS

- ^{99m}Tc-plumbagin complex was shown to be stable up to 24 h (80–92%).
- ^{99m}Tc-plumbagin complex was rapidly cleared from blood circulation.
- Major routes of excretion were hepatobiliary and pulmonary routes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 April 2015
Received in revised form
13 September 2015
Accepted 6 December 2015
Available online 20 December 2015

Keywords:
Plumbagin
Technetium-99m
Biodistribution
SPECT/CT imaging system

ABSTRACT

Plumbagin is a derivative of napthoquinone which is isolated from the roots of plants in several families. These compound exhibits a wide range of biological and pharmacological activities including antimalarial, antibacterial, antifungal, and anticancer activities. The aim of the study was to investigate blood kinetics and tissue distribution of plumbagin in healthy and *Plasmodium berghei*-infected mice using Single-Photon Emission Computed Tomography/Computed Tomography (SPECT/CT) and radiochemical analysis by gamma counter. Plumbagin was labeled with ^{99m}technetium and the reducing agent stannous chloride dihydrate (50 μg/ml) at pH 6.5. Blood kinetics and tissue distribution of the radiolabeled plumbagin were investigated in healthy and *P. berghei*-infected mice (2 males and 2 females for each experimental group). *In vitro* and *in vivo* stability of plumbagin complex suggested satisfactory stability profiles of ^{99m}Tc-plumbagin complex in plasma and normal saline (92.21–95.47%) within 24 h. Significant difference in blood kinetics parameters (C_{max}, AUC, $t_{1/2}$, MRT, V_d, and CL) were observed between *P. berghei*-infected and healthy mice. The labeled complex distributed to all organs of both healthy and infected mice but with high intensity in liver, followed by lung, stomach, large intestine and kidney. Accumulation in spleen was markedly noticeable in the infected mice. Plumbagin-labeled complex was rapidly cleared from blood and major routes of excretion were hepatobiliary and pulmonary routes. In *P. berghei*-infected mice, $t_{1/2}$ was significantly decreased, while V_d and CL were increased compared with healthy mice. Result suggests that malaria disease state influenced the pharmacokinetics and disposition

* Corresponding author. Chulabhorn International College of Medicine, Thammasat University (Rangsit Campus), Klongluang, Pathumthani, 12121, Thailand.
E-mail address: kesaratmu@yahoo.com (K. Na-Bangchang).

http://dx.doi.org/10.1016/j.exppara.2015.12.001
0014-4894/

of plumbagin. SPECT/CT imaging with radiolabeled ^{99m}Tc is a viable non-invasive technique that can be applied for investigation of kinetics and biodistribution of plumbagin in animal models.

1. Introduction

The use of natural sources particularly herbal remedies has been viewed as promising approach for the discovery and development of new drug candidates. Many plants are potential sources of anti-infective and anticancer chemotherapeutic agents (Bent and Ko, 2004). The two antimalarial drugs currently widely used for malaria control originally derived from indigenous medicinal plants are quinine from the Peruvian's Cinchona's bark and artemisinins from the Chinese plant *Artemisia annua* Linn. Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products and the plant kingdom has been the most significant source. These include vinca alkaloids, Taxus diterpenes, Camptotheca alkaloids and podophyllum lignans. Lapachol, a derivative of naphthoquinones was demonstrated to exhibit both antimicrobial and anticancer properties. It has been applied as an archetype for the design and synthesis of new antimicrobial and anticancer agents with enhanced activity. Potent antiparasitic activities of ruthenium complexes containing lapachol were shown against *Leishmania amazonensis* and *Plasmodium falciparum* (Barbosa et al., 2014). Plumbagin–ruthenium conjugates showed a more potent activity against resistant cancer cells than free plumbagin (Spoerlein-Guettler et al., 2014). Moreover, lawsone (compounds 2 and 5), derivatives of naphthoquinones showed highest activity against *Plasmodium falciparum* with IC_{50} (concentration that inhibits parasite growth by 50%) in a similar range as the antimalarial drug chloroquine (<5 μM) (de Souza et al., 2014).

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) is one of the napthoquinone derivatives that is found in root, leaf and stem bark of several plants, e.g., Plumbaginaceae, Droseraceae, Dioncophyllaceae and Ancestrocladaceae families. Crude extract of plumbagin from Plumbaginaceae and Droseraceae has long traditional use in folk medicines for treatment of various diseases. Plumbagin has been demonstrated to exhibit a wide range of biological and pharmacological activities including antimalarial, antibacterial, antifungal, and anticancer activities (Bhargava, 1984; Fournet et al., 1992; Itoigawa et al., 1991; Paiva et al., 2003; Premakumari et al., 1977; Sandur et al., 2006; Simonsen et al., 2001; Sumsakul et al., 2014; Suraveratum et al., 2000; Thiengsusuk et al., 2013; Tilak et al., 2004). The antimalarial potential of the ethanolic extract of *Plumbago zeylanica* and *Plumbago indica* Linn. have been demonstrated *in vitro* against chloroquine-sensitive (3D7) and chloroquine-resistant (K1) clones of *P. falciparum* (Simonsen et al., 2001; Thiengsusuk et al., 2013). In our more recent study, promising antimalarial activity of plumbagin was demonstrated with *in vitro* IC_{50} (concentration that inhibits parasite growth by 50%) of 580 (270–640) and 370 (270–490) nM against 3D7 and K1 *P. falciparum*, respectively. Acute and subacute toxicity testing indicated relatively low toxicity at the dose levels up to 100 (single oral dose) and 25 (daily doses for 14 days) mg/kg body weight, respectively. Nevertheless, the compound at the oral dose of 25 mg/kg body weight given for 4 days was shown to exhibit moderate to weak antimalarial activity in mice infected with *Plasmodium berghei* strain ANKA (Sumsakul et al., 2014). The discrepancy between *in vitro* and *in vivo* observations is likely to be due to pharmacokinetic characteristic of the compound. The aim of the present study was to investigate blood kinetics and tissue distribution of plumbagin in healthy and *P. berghei*-infected mice using gamma counter and Single-Photon Emission Computed Tomography/Computed Tomography (SPECT/CT). SPECT/CT imaging system with radiolabeled technetium-99m (^{99m}Tc) is a sensitive non-invasive technique that has well been applied to investigate the kinetics and bio-distribution of drugs or compounds of interest in target tissues (Aboagye et al., 2001).

2. Materials and methods

2.1. Chemicals

Plumbagin (purity 98.2%) was obtained from Apin chemicals Co. Ltd. (Oxford, UK). Methanol, chloroform, chromato sheet, stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) acetone, ethyl acetate, and ammonium hydroxide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Pooled human liver microsomes (50 donors) were purchased from Gibco BRL Life Technologies (Grand Island, NY, USA). Other reagents used were purchased from local market and were of analytical grade. Technetium-99m (^{99m}Tc) freshly eluted from ^{99}Mo by solvent extraction method was purchased from Nihon Mediphysics (Tokyo, Japan).

2.2. Animals

ICR (Imprinting Control Region) mice (5–7 weeks of age, weighting 20–40 g) of both genders were used in the study. All were purchased from Japan SLC Inc., Japan. Animal experiments were housed under controlled light (12 h light and 12 h dark) and temperature (22–24 °C) in animal house facility at Nagasaki University Radioisotope Center (Priyadarshani et al., 2010). All were fed with a stock diet and water *ad libitum*. Approval of the study protocol was obtained from the Ethics Committee for Animal Research, Nagasaki University, Japan (approval date 27/11/2013, certification number 131104-1).

2.3. Plasmodium berghei-infected mice model

P. berghei (ANKA), the rodent *Plasmodium* species was kindly provided by Animal Research Center for Tropical Infections, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan. The donor mice were infected with 200 μl of *P. berghei* suspension to increase parasite inoculum and were sacrificed by cervical dislocation after 7 days of infection (5–10% parasitemia). The parasitized blood of each donor mouse was collected *via* heart puncture and blood was smeared and stained with 10% Giemsa stain solution. Blood film was examined on light microscope under immersion oil at ×100 magnification (Esume et al., 2011). The parasitemia was determined by counting the number of infected erythrocytes and expressed as percentage of total number of cells in approximately 5–10 fields of blood film. Suspension (200 μl in normal saline) of 1×10^7 parasitized erythrocytes was infected to each mouse by intraperitoneal injection.

2.4. Radiolabeling of plumbagin with technetium-99m (^{99m}Tc)

^{99m}Tc-Plumbagin was prepared by dissolving 0.5 mg of