Metabolism of a New Bactericidal Antiseptic, OPB-2045, in Rats following Subcutaneous Administration

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Summary: To investigate the metabolism and disposition of OPB-2045, 1-(3,4-dichlorobenzyl)-5-octylbiguanide, ^{14}C -labeled OPB-2045 was subcutaneously injected to male Sprague-Dawley rats at a dose of 1 mg/kg and the resulting radioactivity in the urine and bile was analyzed using a high-performance liquid radiochromatography. The radioactivity retained at the injection site was also analyzed.

1. The metabolites excreted in the urine included 6-[5-(3,4-dichlorobenzyl)-1-biguanidino] hexanoic acid (DM-210), 4-[5-(3,4-dichlorobenzyl)-1-biguanidino] butanoic acid (DM-212), 5-[5-(3,4-dichlorobenzyl)-1-biguanidino] pentanoic acid (DM-213) and 3,4-dichlorobenzoic acid. Within 72 hr after the dosing, the metabolites DM-210, DM-212, DM-213, and 3,4-dichlorobenzoic acid excreted in the urine accounted for 4.6, 1.6, 1.5 and 1.4% of the administered dose, respectively. No excretion of the unchanged compound was detected in the urine.

2. The metabolites excreted in the bile included DM-210, DM-212 and DM-213. The combined excretion rate of these metabolites accounted for 15.6% of the dose within 24 hr postdosing. No excretion of 3,4-dichlorobenzoic acid or the unchanged OPB-2045 was detected in the bile.

3. The radioactivity retained at the injection site at 1, 8 and 24 hr postdosing accounted for 51.5%, 34.6% and 13.0% of the administered dose, respectively. The unchanged compound was found to account for at least 80.8% of the radioactivity, and no metabolites including DM-210, DM-212, DM-213 and 3,4-dichlorobenzoic acid, were detected. These results indicated that OPB-2045 was not metabolized by the skin/tissues at the injection site, but instead by other organ(s).

Key words: OPB-2045, Metabolism, Subcutaneous administration, Rat

Introduction

OPB-2045 is a newly synthesized antimicrobial agent¹⁾ and has potent bactericidal activity toward fungi (yeast), gram-negative and gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) at low concentrations.²⁾ The chemical structure of the compound is shown in **Fig. 1**.

The absorption, distribution and excretion of OPB-2045 in rats and dogs following a single subcutaneous administration have been previously reported using ^{14}C -labeled OPB-2045.^{3,4)} The disappearance of radioactivity from the circulation was relatively slow in either animal with half-lives of 58.6-63.3 hr and the radioactivity retained at the injection site at 168 hr postdosing accounted for 2 % of the dose, showing slow elimination from the site.

In dogs, the urinary and fecal excretion of radioactivity within 168 hr postdosing following the subcutaneous injection was 59.4% and 23.5% of the administered dose, respectively, demonstrating that most radioactivity was excreted in the urine.⁴⁾ Four OPB-2045 metabo-

lites have been structurally identified using synthetic standards and/or spectral data as 6-[5-(3,4-dichlorobenzyl)-1-biguanidino] hexanoic acid (DM-210), 4-[5-(3,4-dichlorobenzyl)-1-biguanidino] butanoic acid (DM-212), 5-[5-(3,4-dichlorobenzyl)-1-biguanidino] pentanoic acid (DM-213) and 3,4-dichlorobenzoic acid (**Fig. 1**).⁵⁾ The predominant radioactive metabolites in the excreta were DM-212 and DM-213 in dogs.

The urinary and fecal excretion of radioactivity within 168 hr postdosing was 17.2% and 68.4% of the dose, respectively, in rats given by the subcutaneous administration of ^{14}C -OPB-2045. However, no information on the metabolism of OPB-2045 in rats following subcutaneous administration is available. In the current study, the extent of the excretion of the unchanged OPB-2045 and metabolites in the urine and bile was studied in male Sprague-Dawley rats following a single subcutaneous administration of ^{14}C -OPB-2045 at a dose level of 1 mg/kg. The radioactivity retained at the subcutaneous injection site was also analyzed using synthetic standards.

Fig. 1 Chemical structures of ¹⁴C-labeled OPB-2045 and synthetic standards for OPB-2045 metabolites. Asterisk indicates the ¹⁴C-labeled position.

Materials and Methods

1. Compound

¹⁴C-labeled OPB-2045 monohydrochloride (lot No. C-921201) was synthesized at Nemoto & Co., Ltd., (Tokyo, Japan). Specific radioactivity was 3.47 MBq/mg and radiochemical purity, 99.3% as determined by a thin-layer chromatography using a silica gel 60 F₂₅₄ plate in the solvent system, CHCl₃ : methanol : CH₃COOH (8 : 3 : 0.2), or 98.4% determined by HPLC using a column of YMC A-302 (4.6 mm i.d. × 150 mm; YMC Ltd., Corp., Kyoto, Japan) and mobile phase of methanol : water (85 : 15) containing 2 g CH₃(CH₂)₅SO₃Na per one liter at a flow rate of 0.7 ml/min. Non-radiolabeled OPB-2045 (lot No. 92K85M), DM-210 (lot No. 3K11M), DM-212 (lot No. 4H03M), and DM-213 (lot No. 4H02M) were provided by Otsuka Pharmaceutical Co., Ltd. 3,4-dichlorobenzoic acid was purchased from Wako Pure Chemical Industries Ltd., (Osaka, Japan). All other reagents used were of analytical grade.

2. Experimental Animals

Male Crj:CD® Sprague-Dawley rats at 6 weeks old were obtained from Charles River Japan Inc., and were acclimatized for more than one week in a controlled animal area maintained at 23 or 24 ± 2°C for temperature, 55 or 60 ± 10% for relative humidity and 13 times/hr ventilation during 12-hr light/dark cycles. Rodent Chow MF (Oriental Yeast Co., Ltd., Japan) and water were available *ad libitum* under acclimatization. The animals at 7–8 weeks old (187–276 g) were used in the experiments.

3. Dose Preparation and Administration

¹⁴C-OPB-2045 was dissolved in 5 (w/v) % D-mannitol (lot No. I21942; Kyowa Hakko Kogyo Co., Ltd.) aqueous solution for subcutaneous dosing at 3.47 MBq/1 mg/kg/2.5 ml and injected into the dorsal part. The radiochemical purity of the dose preparation was more than 95.5% as judged by radio-HPLC.

4. Sample Collection

Following subcutaneous administration of ¹⁴C-OPB-2045, the rats were housed individually in metabolic cages and the excreted urine was collected at intervals of 24 hr up to 72 hr postdosing. The bile ducts of three rats were cannulated with polyethylene tubes for bile collection under ether anesthesia and the animals were then subcutaneously administered with ¹⁴C-OPB-2045 at 1 mg/kg, and placed in Bollman's cages. Bile was collected during 0–4, 4–8, and 8–24 hr after dosing. Skin/tissues at the injection site were sampled at 1, 8, and 24 hr postdosing.

5. Analysis of Urinary and Biliary Radioactivity

The urinary samples were centrifuged at 140 × g for 5 min, and 5 to 20 ml of the supernatant was evaporated to dryness. The residue was suspended in 150 μl of methanol and centrifuged at 140 × g for 5 min; 30 μl of the resulting supernatant was analyzed by HPLC.

A total of 200 μl of bile was evaporated to dryness, and the residue was suspended in 150 μl of methanol. The suspension was centrifuged at 140 × g for 5 min, and 30 μl of the resulting supernatant was analyzed by HPLC.

6. Analysis of Radioactivity at the Injection Site

The skin/tissues at the injection site were cut into small pieces with scissors, mixed with 20 ml of methanol, and homogenized at 18000 rpm for 5 min in a high-flex homogenizer (HF-92; Iuchi Corp., Japan). The homogenate was centrifuged at $140 \times g$ for 5 min, and the supernatant was obtained. This procedure was repeated three times and the extracts were combined. A portion (0.5 to 2 ml) of the extracted methanol fraction was dried, and the residue was then dissolved in 150 μ l of methanol, and 30 μ l of the resultant solution was analyzed by HPLC.

7. High Performance Liquid Chromatography

The following devices were used for HPLC: a Model 510 high-pressure pump (Waters), Model U6K universal injector (Waters), Model 484 tunable UV/VIS detector (Waters), Model 680 automatic gradient controller (Waters), and Model C-R7A chromatopak (Shimadzu).

A TSKgel ODS-80_{TM} column (4.6 mm i.d. $\times$ 150 mm; Tosoh, Tokyo, Japan) was used for the analysis of radioactivity in urine, bile and extracts of skin/tissues at the injection site. The unchanged OPB-2045 and metabolites were analyzed using a linear gradient developed from 10% CH₃CN in water containing 0.1% acetic acid to 80% CH₃CN in water containing 0.1% acetic acid over a period of 60 min at a flow rate of 1.0 ml/min. The eluent was collected every 30 sec into more than 120 fractions, and the radioactivity counted. The radioactivity peaks were identified by comparison with the elution time of the authentic standards, i.e.; unlabeled OPB-2045, DM-210, DM-212, DM-213, and 3,4-dichlorobenzoic acid.

8. Radiochemical Analysis

The samples were mixed with 0.5–1.0 ml water and 8 ml ACS-II scintillant solution (Amersham, UK), and radioactivity was counted for one min with a liquid scintillation counter (LSC-1050 and -3500; Aloka, Tokyo, Japan). The counting efficiency was assessed by an external standard method.

Results

1. Radioactivity in Urine

Fig. 2 shows a representative HPLC radiogram of the urine, and **Table I** shows the urinary excretion rates of the unchanged OPB-2045 and metabolites at 72 hr after subcutaneous injection of ¹⁴C-OPB-2045 at a dose of 1 mg/kg.

In the radiochromatogram, several radioactive metabolites including DM-210, DM-212, DM-213 and 3,4-dichlorobenzoic acid appeared in the urine. The unidentified radioactive metabolites were also detected in the urine, however, the unchanged OPB-2045 did not appear in any samples.

Within 72 hr of the subcutaneous administration of ¹⁴C-OPB-2045 at 1 mg/kg, 14.2% of the administered

Fig. 2 Representative radio-HPLC chromatogram of a typical urine (0–24 hr) extract from a rat containing OPB-2045 metabolites.

Table I Urinary excretion of OPB-2045 and its metabolites in rats following a single subcutaneous administration of ¹⁴C-OPB-2045 at 1 mg/kg

OPB-2045 or metabolite	Excretion rate (% of dose)
OPB-2045	n.d.
DM-210	4.6 $\pm$ 0.6 (32.4)
DM-212	1.6 $\pm$ 0.1 (11.3)
DM-213	1.5 $\pm$ 0.3 (10.6)
3, 4-dichlorobenzoic acid	1.4 $\pm$ 0.8 (9.9)
Others	5.0 $\pm$ 0.6 (35.2)
Total	14.2 $\pm$ 1.0 (100)

The urinary sample was collected up to 72 hr postdosing.

Values represent mean $\pm$ SD of three animals.

Values in parentheses are expressed as the percent content of each substance to the total excreta of radioactivity in urine.

n.d., not detected.

dose of radioactivity was excreted in the urine. The rate of DM-210 excretion was particularly high (4.6%) in relation to the administered dose and accounted for 32.4% of the urinary radioactivity. The metabolites DM-212, DM-213, and 3,4-dichlorobenzoic acid excreted in the urine accounted for 1.6%, 1.5%, and 1.4%, respectively, of the administered dose.

2. Radioactivity in Bile

Fig. 3 shows a representative HPLC radiogram of the bile, and **Table II** shows biliary excretion rates of radioactive substances.

Within 24 hr of the subcutaneous administration of ¹⁴C-OPB-2045, 34.1% of the administered dose of radioactivity was excreted in the bile. The metabolites excreted in the bile included DM-210, DM-212, and DM-213. The combined excretion rate of these metabolites accounted for 15.6% of the administered dose and

Fig. 3 Representative radio-HPLC chromatogram of a typical bile (4-8 hr) extract from a rat containing OPB-2045 metabolites.

Table II Biliary excretion of OPB-2045 and its metabolites in rats following a single subcutaneous administration of ^{14}C -OPB-2045 at 1 mg/kg

OPB-2045 or metabolite	Excretion rate (% of dose)
OPB-2045	n.d.
DM-210+DM-212+DM-213	15.6 $\pm$ 3.3 (45.7)
3, 4-dichlorobenzoic acid	n.d.
Others	18.5 $\pm$ 5.7 (54.3)
Total	34.1 $\pm$ 9.0 (100)

The biliary sample was collected up to 24 hr postdosing. Values represent mean $\pm$ SD of three animals. Values in parentheses are expressed as the percent content of each substance to the total excreta of radioactivity in bile. n.d., not detected

45.7% of the biliary radioactivity. No excretion of 3,4-dichlorobenzoic acid or the unchanged OPB-2045 was detected.

3. Radioactivity at the Injection Site

The radiochromatograms of the extracts from the injection site are shown in Fig. 4.

The radioactivity retained at the injection site at 1, 8 and 24 hr postdosing accounted for 51.5%, 34.6% and 13.0% of the administered dose, respectively. The unchanged compound was found to account for at least 80.8% of the radioactivity, and no metabolites including DM-210, DM-212, DM-213 and 3,4-dichlorobenzoic acid were detected.

Discussion

In our previous findings, the urinary and fecal excretion of radioactivity following the subcutaneous administration of ^{14}C -OPB-2045 was 17.2% and 68.4%, respectively, in rats and 59.4% and 23.5%, respectively, in

Fig. 4 Representative radio-HPLC chromatograms of a typical extract from the injection site following a single subcutaneous injection of ^{14}C -OPB-2045 at 1 mg/kg to rats.

dogs within 168 hr postdosing, showing species differences due to the different route of excretion of the radioactivity.^{3,4)} The metabolic fate of OPB-2045 in dogs following subcutaneous administration has been previously reported that OPB-2045 was extensively biotransformed into various metabolites and the excretion of the unchanged OPB-2045 was quite low in the feces, and was not observed in the urine.⁵⁾ Four metabolites of OPB-2045 have been structurally identified from the dog urine, i.e.; DM-210, DM-212, DM-213 and 3,4-dichlorobenzoic acid.⁵⁾ With this background, this study was conducted to investigate whether the metabolic fate of OPB-2045 in rats and dogs was similar or not.

Following the subcutaneous administration of OPB-2045 to rats, it was extensively biotransformed into the various metabolites, and no urinary and biliary excretions of the unchanged OPB-2045 was observed. This demonstrated that OPB-2045 was eliminated from the body due to the metabolic clearance, as was the case in dogs.

The excreted metabolites in the rats included DM-

210, DM-212, DM-213 and 3,4-dichlorobenzoic acid in the urine, and the same metabolites except 3,4-dichlorobenzoic acid were found in the bile. In the dogs, the same four metabolites were detected in the urine, and the excretion of these metabolites except 3,4-dichlorobenzoic acid was observed in the feces.⁵⁾ This evidence indicated that the metabolic profile of OPB-2045 in the rats following subcutaneous administration was similar to that in the dogs, and additionally 3,4-dichlorobenzoic acid was probably not excreted in the bile.

The predominant metabolite in the urinary excreta was DM-210 in the rats, accounting for 32.4% of the urinary radioactivity, but it was DM-213 followed by DM-210 in dogs, accounting for 33.3% and 31.8% of the radioactivity in the urine, respectively.⁵⁾ DM-210 was thus one of the major metabolites of OPB-2045 in either animals.

The biotransformation of OPB-2045 to 3,4-dichlorobenzoic acid is considered to occur by several metabolic processes, and the enzymes involved in the process are considered to be a cytochrome P450 system and/or flavin monooxygenase, and aldehyde dehydrogenase.⁵⁾ On the other hand, the octyl group of OPB-2045 was predominantly metabolized and biotransformed to butanoic (DM-212), pentanoic (DM-213) and hexanoic acid (DM-210) in rats as well as in dogs. These metabolites were all a methylene chain shorter with a carboxyl end and thus produced by C-C bond cleavage followed by subtraction of carbon fragments. The mechanism(s) of production of these C-C bond cleavage metabolites was still unclear, but, it was clear in this study that rats could metabolize OPB-2045 to these degraded products as was the case in the dogs. Investigation regarding the mechanism(s) of the C-C bond cleavage of the octyl group is now in progress.

The elimination of the radioactivity retained at the injection site was relatively slow, however, the radioactivi-

ty decreased time-dependently. Radioactivity at the injection site originated from the unchanged OPB-2045 at any time postdosing and no metabolites including DM-210, DM-212, DM-213 and 3,4-dichlorobenzoic acid were detected. This evidence indicated that OPB-2045 was not metabolized by the skin/tissues at the injection site, but instead by the other organ(s).

In conclusion, OPB-2045 following subcutaneous administration was eliminated from the body by the metabolic clearance in rats and the metabolic profile of the compound in the animal was fundamentally similar to that in dogs. Furthermore, it suggested that species difference between these animals due to the different route of excretion of the radioactivity following subcutaneous administration was probably based on the excretory ability of each metabolite into urine or bile.

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