Studies on the Absorption, Distribution, Metabolism, and Excretion of Propionylmaridomycin in Rats

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The absorption, distribution, metabolism, and excretion of propionylmaridomycin was investigated in the rat by using \(^{14}C\)propionylmaridomycin. Propionylmaridomycin was absorbed from the gastrointestinal tract at a relatively rapid rate and was readily distributed into tissues. Among the tissues examined, liver, kidney, and lung showed a remarkably higher level of the radioactivity than plasma, while distribution of the radioactivity into brain was little. However, a significant accumulation of the radioactivity in tissues was not observed. Excretion of the radioactivity was mainly via the fecal route and the high fecal recovery was accounted for by unabsorbed drug and biliary excretion of the drug and/or its metabolites. Tissue distribution of antimicrobial activity was also investigated and lung was found to show the highest antimicrobial activity among any tissues examined. Propionylmaridomycin was completely converted to several metabolites by the rat and the presence of at least three components was confirmed as biologically active metabolites. Of these metabolites, \(^4\)"-depropionyl-9-propionylmaridomycin was identified, which was the major metabolite in tissues, plasma, and urine.

Propionylmaridomycin (PMDM), a new semi-synthetic macrolide antibiotic, is the 9-propionyl ester of maridomycin (MDM) which was isolated from the culture filtrate of *Streptomyces hygroscopicus*. The physico-chemical properties and antimicrobial activities of PMDM have been described in previous papers (1, 5–7). In the clinical trials, PMDM has been proven to be therapeutically effective in the treatment of infections caused mainly by the gram-positive organisms. However, it has been noticed that PMDM, similar to the other macrolide antibiotics, gives a relatively low blood level when administered to man (4), and its clinical efficacy is hardly explainable in relation to its blood level.

A relatively large number of macrolide antibiotics have been developed and used successfully in the treatment of a wide variety of infections. Despite their wide clinical usage, however, the extent of past studies on the metabolic fate of these antibiotics was limited. Recently, Kuriaki et al. (8) have reported on the disposition of josamycin in mice by using the tritium-labeled drug and they have presented an interesting information on the metabolic fate of the antibiotic. These facts led us to investigate the overall biotransformation of PMDM for obtaining information toward a better understanding of its clinical efficacy. The present paper described studies on the metabolic fate of \(^{14}C\)PMDM in the rat.

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MATERIALS AND METHODS

Compounds. \(^{14}C\)PMDM was supplied from our Medicinal Research Laboratories. The structure of PMDM and the labeled positions proposed are shown in Fig. 1, and the specific activity was 0.853 mCi/mmol (11). This labeled PMDM was appropriately diluted with nonradioactive PMDM for the animal studies. The standard reference compounds, \(^4\)"-deacetyl-9-propionylmaridomycin (PMDM-M), MDM, and \(^4\)"-deacetylmridomycin (MDM-M) were also supplied from the Medicinal Research Laboratories (9).

Animal studies. Albino male Sprague-Dawley rats, weighing 200 to 250 g, were divided into groups of three. Each rat in a group was given 200 mg of PMDM per kg (approximately 25 µCi/kg of \(^{14}C\)) by the oral route. Dosage form for the oral administration was 2% suspension in 5% gum acacia, which was infused into stomach by stomach tube. Blood samples were withdrawn by cardiac puncture from each rat of a group at 0.5, 1, 2, 3, 4, 6, 8, 24, and 48 h after dosing, then the rats were sacrificed by decapitation and the main organs, liver, lungs, kidneys, spleen, brain, and muscle were immediately excised in their entirety. These organs were frozen for later analysis. Collection of urine, feces, and expired air was carried out in other groups of rats. The rats were placed in individual metabolism cages after dosing, and urine and feces were collected separately at specified intervals. Carbon dioxide in the expired air was absorbed in 5 N KOH solution. Bile samples were collected from the
common bile duct cannulated rats placed in restraining cages.

In case of intravenous administration, 40 mg of PMDM per kg (approximately 8 μCi/kg of [14C]) was administered to rats through femoral vein. The parenteral solution was prepared by dissolving PMDM in Carbowax 1000 and diluting it with water.

**Measurement of radioactivity.** Appropriate portions (usually 0.2 ml) of plasma, urine, and bile were added directly to a liquid scintillation vial containing 10 ml of scintillation solution. The scintillation solution had the following composition: 0.3 g of 1,4-bis[2-[(5-phenyloxazolyl)]benzene, 12 g of 2,5-diphenyloxazole, 100 g of naphthalene, 45 ml of methanol, 135 ml of toluene, and 720 ml of dioxane. For the assay of radioactivity in tissues and feces, pooled, frozen tissues, and feces were homogenized with a small volume of water, lyophilized, and combusted in an oxygen atmosphere. The resulting radioactive carbon dioxide was trapped in 10 ml of Hyamine 10X solution (Packard Instrumental Co., Inc., Downers Grove, Ill.). Portions of the Hyamine solution were placed in a vial containing 10 ml of scintillation solution. The scintillation solution used for this assay contained 0.1 g of 1,4-bis[2-[(5-phenyloxazolyl)]benzene, 3 g of 2,5-diphenyloxazole, and 1 liter of toluene. For the expired air, the [14C], trapped in 5 N KOH solution was also similarly assayed as described above after regeneration and reabsorption into Hyamine solution. All samples were counted in duplicate in an Aloka liquid scintillation spectrometer (model LSC-502) and the observed count was corrected for quench by the channel ratio method.

**Assay of total antimicrobial activity.** Total antimicrobial activity of samples was determined by the paper disk method with *Sarcina lutea* ATCC 9431 as test organism and PMDM as reference compound. Grove and Randall medium no. 5 (pH 8.0) was used as the assay plate medium (3). Paper disks were impregnated with suitable portions of samples. After drying, the paper disks were placed on an agar plate and the plate was incubated for 16 h. Standards were prepared by impregnating paper disks with the standard solutions of PMDM. Accordingly, the concentration of the total antimicrobial activity was expressed as PMDM equivalent. By this method, as little as 0.3 μg of PMDM per ml could be detected. Portions of plasma, urine, and bile were directly subjected to the paper disk method.

For the assay of tissues and feces, appropriate amounts (10 to 30 mg) of the lyophilized tissues and feces prepared for the measurement of radioactivity were homogenized in 80% aqueous acetone solution (5 to 20 ml) using a Ultra-Turrax homogenizer (Jarke & Kin Co., West Germany) and the resulting acetone extracts were subjected to the paper disk method. It was confirmed that more than 98% of the radioactivity in these samples was transferred into the acetone extracts.

**Assay of antimicrobial activity of metabolites.** Before the assay, PMDM and its metabolites were extracted from the samples of plasma, urine, bile, tissues, and feces, respectively, and their activities were determined by thin-layer chromatography and bioautography according to the method described by Fugono et al. (2).

**Extraction of metabolites.** Portions (1 to 2 ml) of plasma, urine, and bile samples were adjusted to pH 8 with 0.1 N NaOH and extracted three times with 5 volumes of ethyl acetate. The extracts were combined together and concentrated to dryness under reduced pressure. The residue was dissolved with 0.1 ml of methanol and was applied to thin-layer chromatographic plates. The accuracy and reproducibility of the extraction procedure were examined by determining the recoverability of the reference compounds from rat plasma, urine, and bile. The recoveries of PMDM and its metabolites from these samples were found to be more than 95%. For extraction of metabolites in tissues and feces, portions of the aqueous acetone extracts of the lyophilized tissues and feces were concentrated under reduced pressure. After adjusting pH to 8 with 0.1 N NaOH, the resulting concentrate was then treated as described above. The recoveries of PMDM and its metabolites were also tested by adding the reference compounds to the homogenates of these tissues and feces, and approximately 90% of the compound added was always recovered from all these samples.

**Thin-layer chromatography.** Ascending thin-layer chromatography on silica gel plate (Spot film, Tokyo Kasei Co., Japan) was carried out by using the lower layer from a mixture of chloroform-ammonia water-methanol (40:20:3) as solvent. To the plate, 10 to 50 aliters of the methanol solution of the extracts was applied using a micropipette and developed for 2 h at room temperature.

**Bioautography.** The dried thin-layer-chromatogram strips were placed on the agar plate seeded with *S. lutea* ATCC 9431 for 20 min. After removing the strips, the agar plate was incubated for 16 h at 37 C. The spots, the zones of inhibition, were located by spraying 0.6% methylene blue solution. Under these conditions, the *Rf* values of standard reference compounds on the bioautogram were 0.89 for PMDM, 0.77 for PMDM-M, 0.55 for MDM and 0.37 for MDM-M, respectively. The concentrations of metabolites were calculated from the diameters of corresponding inhibition zones, as compared to the parent compound, and expressed as PMDM equivalents.

**RESULTS**

**Tissue distribution of radioactivity.** The appearance of radioactivity in tissues of rats after oral administration of [14C]PMDM was relatively rapid. Concentrations of radioactivity in plasma and in several tissues are illustrated.

![FIG. 1. Position of labels in [14C]PMDM.](image-url)
in Fig. 2. A peak plasma level of radioactivity was attained at 3 h after dosing and most of the tissues also reached a maximal level within 4 h after drug administration. The concentrations of radioactivity in liver, kidney, and lung were much higher than in plasma. Of the tissues examined, liver had the highest concentration of radioactivity and kidney and lung showed approximately equal levels of radioactivity, whereas a very small amount of radioactivity was detected in brain. At the end of 24 h, the radioactivity in liver and kidney remained to an appreciable extent, but the radioactivity in the other tissues declined to a negligible level by this time.

**Urinary, fecal, and biliary excretion of radioactivity.** The urinary, fecal, and biliary recoveries of radioactivity after oral administration of [14C]PMDM are shown in Table 1. The 48-h urine of rats contained approximately 16% of the administered radioactivity and the excretion of radioactivity was almost complete within 24 h. On the other hand, approximately 76% of the administered radioactivity was detected in feces in 48 h. Thus, more than 90% of the dose was accounted for by urinary and fecal radioactivity. When [14C]PMDM was administered orally to rats with bile duct cannulae, approximately 8% of the administered radioactivity was excreted in the bile during 24 h. Table 1 also indicates the excretion of radioactivity after intravenous dosing of [14C]PMDM. Additionally, no radioactivity was detected in the expired air of rats after either oral or intravenous administration of [14C]PMDM.

**Antimicrobial activity in plasma and tissues.** Total antimicrobial activity levels in plasma, liver, kidney, and lung were biologically determined and expressed as the concentration of PMDM. Figure 3 illustrates the time course of the activity levels. All the counts obtained in these tissues are also shown in Fig. 3 in terms of PMDM equivalents. In plasma, liver, and kidney, only 20 to 25% of the total radioactivity was present as biologically active substances and the remainder was occupied by inactive metabolites of PMDM. In contrast to these tissues, the majority of the radioactivity present in lung consisted of active metabolites of the antibiotic.

**Biologically active metabolites of PMDM in plasma and tissues.** Biologically active metabolites of PMDM were examined in plasma, liver, kidney, and lung taken at 3 h after oral administration of [14C]PMDM. It was found that at least three components were present in the ethyl acetate extracts of these tissues. The chromatographic identification of these components revealed that PMDM-M, 4'-deacetyl PMDM, was the major active metabolite of PMDM and that no PMDM could be detected in all the tissues examined. It became evident from the results of quantitative assay

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**Table 1. Urinary, fecal and biliary recoveries of 14C by intact or bile duct-cannulated rats in 48 h after oral and intravenous administration of [14C]propionylmaridomycin**

<table>
<thead>
<tr>
<th>Route</th>
<th>Recovery</th>
<th>Intact</th>
<th>Bile duct-cannulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. oral</td>
<td>Urine</td>
<td>15.8 ± 0.9*</td>
<td>6.1 ± 0.8</td>
</tr>
<tr>
<td>(200 mg/kg)</td>
<td>Feces</td>
<td>75.6 ± 6.8</td>
<td>74.7 ± 10.5</td>
</tr>
<tr>
<td></td>
<td>Bile</td>
<td>8.0 ± 1.9*</td>
<td>8.0 ± 1.9*</td>
</tr>
<tr>
<td>Intravenous</td>
<td>Urine</td>
<td>30.1 ± 1.4</td>
<td>23.4 ± 8.0</td>
</tr>
<tr>
<td>(40 mg/kg)</td>
<td>Feces</td>
<td>58.5 ± 5.1</td>
<td>44.9 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>Bile</td>
<td>21.1 ± 2.9*</td>
<td>21.1 ± 2.9*</td>
</tr>
</tbody>
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*Percent of dose, mean ± standard deviation.

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**Fig. 2. Tissue distribution of 14C after oral administration of [14C]PMDM (200 mg/kg).**

**Fig. 3. Concentration of total antimicrobial activity in tissues after oral administration of [14C]PMDM (200 mg/kg).** Symbols: (—○—), total antimicrobial activity; (—■—), radioactivity expressed as PMDM equivalent. Each point represents mean ± standard deviation of three rats.
that the total antimicrobial activity of metabolites expressed as PMDM equivalent was due mainly to that of PMDM-M (Fig. 4). Two minor components were also examined chromatographically, but their structures were not identified because of their low recoveries. However, a very small portion of the total activity was supposed to be MDM from its Rf value in chromatography. Another minor component with an Rf value less than 0.2 differed from the reference compounds used on the chromatograms and the amount of this substance was slightly larger in liver and kidney than in plasma and lung.

Biologically active metabolites of PMDM in urine and bile. Antimicrobial activity found in the pooled 24-h urine accounted for approximately 5% of the dose given. The chromatographic observation of biologically active metabolites showed a similar result to that in tissues. Three components were detected on the bioautograms and the presence of PMDM-M was readily confirmed as the major urinary metabolite. However, the other components which corresponded to a very small portion of the total activity could not be identified. Figure 5 illustrates the fraction of PMDM-M to the total antimicrobial activity in the urine samples collected between the 0- to 2-, 2- to 5-, and 5- to 24-h period.

In the bile, approximately 5% of the total antimicrobial activity was excreted during 24 h by rats with bile duct cannulae. Bioautography of the bile sample demonstrated it to be composed of at least three biologically active components. The composition of biliary metabolites, however, slightly differed from the results observed in tissues and urine. The results of bioautography showed that a small amount of PMDM-M was also found in the bile and most of the activity was occupied by unidentified metabolites.

**DISCUSSION**

When [14C]PMDM was orally administered to rats, the tissue level data indicated that the antibiotic was absorbed from the gastrointestinal tract at a relatively rapid rate and was readily distributed into several tissues. Furthermore, tissue distribution studies demonstrated that the concentration of radioactivity was much higher in tissues such as liver, kidney, and lung than in plasma and the notable exception of this was the brain which showed a considerably low level of radioactivity. From these results, it can be said that PMDM is not ruled out by other macrolide antibiotics in its tissue distribution behavior. Among the tissues examined, it is particularly noteworthy that most of the radioactivity in lung consists of biologically active substances. This finding gives us an evidence that PMDM is especially effective against the respiratory tract infections in the clinical trials. High localization of the antimicrobial activity in lung is likely to be due to either functional abundance of blood vessels in lung or preferable binding of PMDM and/or its metabolites to tracheo-bronchial secretions, though no direct evidence is obtainable for the explanation of these two possibilities other than the suggestion that basic antibiotics form complexes with deoxyribonucleic acid contained in the pulmonary secretions (12). The tissue distribution studies also exclude the possibility of accumulation of the drug and/or its metabolites, since very small amounts of radioactivity were detected only in the liver and kidney at 24 h after dosing.

In the urinary and fecal excretion studies,
high fecal radioactivity after oral administration of the labeled antibiotic could be explained by poor absorption of the drug from the gastrointestinal tract or by biliary secretion of the drug and/or its metabolites. To examine this, the urinary recoveries were compared between oral and intravenous administration. The urinary recovery of the radioactivity was greater after intravenous administration than after oral administration by a factor of about 2 (Table 1). Accordingly, it is not so unreasonable to assume that more than 50% of the dose could be absorbed from the gastrointestinal tract.

In addition, the biliary excretion study revealed that when [14C]PMDM was administered intravenously to the rat with bile duct cannula the radioactivity was secreted in the bile to a considerable extent and the fecal recovery of radioactivity became lower, as compared with the intact rat (Table 1). These results suggest that larger amounts of radioactivity found in the feces of rats given an oral dose of [14C]PMDM may be due mainly to the unabsorbed drug and partly to the radioactivity excreted in the bile. Biliary excretion of PMDM is considered to be the result of physicochemical factors favoring biliary excretion, as well as other macrolide antibiotics such as erythromycin (13) and josamycin (8). Furthermore, a discrepancy in the urinary recoveries between the cannulated and the intact rat also suggested that reabsorption of PMDM and/or its metabolites from the intestinal tract may occur.

Biologically active metabolites of PMDM in tissues, blood, urine, and bile were examined and the results show that no unchanged drug was detected but at least three active metabolites were present in these specimens. One of them was identified by thin-layer chromatography and bioautography as PMDM-M, which was the major metabolite in tissues, blood, and urine but a minor component of the biliary metabolites. The antibacterial spectrum and minimal inhibitory concentration for PMDM-M were examined by Nakazawa et al. (10), and they concluded that PMDM-M yet retained the desirable antimicrobial activity though its antimicrobial activity was slightly lower as compared with PMDM and MDM.

This metabolic finding is interesting compared with the results of Stephens et al. (14) who showed that when propionyl erythromycin was administered orally to rats the propionyl ester in addition to erythromycin was present in the plasma. The reason for the absence of PMDM is probably due to the rapid hydrolysis of PMDM by rats. This is conceivable from the results of Fugono et al. (unpublished data) who found that PMDM was readily hydrolyzed to PMDM-M by in vitro rat plasma, liver, and kidney preparations. Although PMDM has the three acyl groups at position 3, 9, and 4", respectively, the predominant route of metabolism by the rat was via hydrolysis of the ester bond at position 4", and hydrolysis of the ester group at position 9 which was introduced into MDM by chemical modification was little if any. Considering the purpose of the chemical modification of MDM, it is also interesting that the parent antibiotic was not liberated from PMDM. On the other hand, Hara et al. (4) have found that when PMDM was administered orally to man, MDM and MDM-M were the major metabolites in the plasma. Comparing the results in the rat and man, it is evident that there is a marked species difference in the metabolism of PMDM. Two other biologically active metabolites which were the more abundant products in the bile have remained unidentified in the present study.

As other metabolic pathways, oxidative N-dealkylation and hydroxylation are known for some macrolide antibiotics. Welles et al. (15) have found the N-demethylated erythromycin in rats as a metabolite of erythromycin, and Shomura et al. (T. Shomura, I. Komiya, K. Kojima, H. Kadosawa, and K. Umemura, Abstr. 92nd Annu. Meet. Pharm. Soc. Jap., IV, p. 75, 1972) have confirmed hydroxylation of the lactone ring in the metabolism of mydecamycin by rats. Although PMDM has a dimethylamino group in its molecule similar to erythromycin, N-demethylation of PMDM would be unlikely, since the radioactive carbon dioxide derived from the radioactive methyl groups was not detected in the expired air. The possibility for hydroxylation of the lactone ring in PMDM can not be excluded by the present data and further investigation is required for elucidating this.

In conclusion, it can be said through the present study that the clinical efficacy of PMDM is explainable based on the ready distribution of antimicrobial activity into most tissues.

LITERATURE CITED


