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Metabolism of S-1108, a New Oral Cephem Antibiotic, and Metabolic Profiles of Its Metabolites in Humans

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The metabolism and pharmacokinetics of pivalic acid, a major metabolite of S-1108, were studied with three healthy volunteers. Concentrations of S-1006 (the active compound), pivalic acid, and pivaloylcarnitine in plasma and urine were measured after administration of S-1108. Recoveries in urine at the doses of S-1108 given (100 and 200 mg) were 33 to 41% for S-1006, 93% for total pivalic acid, and 89 to 94% for pivaloylcarnitine in 24 h, and maximum concentrations in plasma were 2 μg of S-1006 per ml, 1 μg of total pivalic acid per ml, and 2 μg of pivaloylcarnitine per ml after a 200-mg oral administration of S-1108. More than 90% of the pivalic acid was excreted as pivaloylcarnitine, and no measurable amount of free pivalic acid was present in urine samples, indicating that the pivalic acid liberated from S-1108 was almost quantitatively conjugated with carnitine in the human body. The level of free carnitine in plasma was unaffected by a single 200-mg administration of S-1108, whereas urinary excretion of free carnitine decreased as levels of acylcarnitine increased. The acylcarnitines were excreted primarily in the form of pivaloylcarnitine. This study clearly showed how the pivalic acid was metabolized and excreted in humans. The importance of monitoring carnitine, an essential cofactor in fatty acid metabolism, was also discussed in terms of its utilization by pivalic acid.

S-1108, an oral cephem antibiotic, is a prodrug of S-1006 (4a). S-1108 is hydrolyzed by an esterase in the intestinal tracts of humans to produce S-1006, pivalic acid (PA), and formaldehyde in a manner similar to that of other prodrugs having a pivaloyloxymethyl (POM) ester (3, 6, 18). The metabolic and pharmacokinetic profiles of S-1108 have been intensively studied, including clinical evaluations (12a, 18a). Furthermore, an investigation of the metabolism of PA was needed to confirm the biological profile of the POM ester type of prodrug. The metabolism of PA has been studied by Vickers et al. (19), who found that pivaloylcarnitine (PC) was its major urinary metabolite when the POM ester derivative of methyldopa was given to humans. Since then, PC has been found to be the common metabolite of prodrugs that possess the POM ester group in their chemical structures (5, 9, 10). Carnitine is an essential cofactor in fatty acid β-oxidation, which takes place mainly in the mitochondrial matrix (1, 2). Carnitine acts as a carrier of the acyl groups to transport fatty acids into the mitochondrial inner membrane, which is otherwise impermeable to coenzyme A compounds. It would be clinically important from a toxicological viewpoint to investigate carnitine utilization with a substance containing a fatty acid group, since abnormal consumption of carnitine might induce a metabolic dysfunction (5). In this regard, levels of free carnitine in plasma and urine in patients receiving therapy with the prodrug which liberates PA must be monitored.

This study focused on the metabolism and the pharmacokinetic profile of PA after oral administration of S-1108 in connection with carnitine levels in humans. Concentrations of PA and PC in plasma and recoveries in urine were studied while simultaneously monitoring S-1006 concentrations. Free carnitine and concentrations of acylcarnitine in plasma and urine were also determined as an indicator of free carnitine consumption.

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MATERIALS AND METHODS

Subjects. A group of three healthy males (mean age, 33.7 years; mean body weight, 65.3 kg) was selected according to evaluation criteria. The subjects were administered 100 and 200 mg of S-1108 orally at 2-week intervals. S-1108 was given exactly 30 min after breakfast. Blood samples (8 ml) were collected into heparinized tubes 0.5, 1, 2, 3, 5, 6, 8, 12, and 24 h after oral administration, and the plasma was then separated by centrifugation. Urine samples were collected 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, and 12 to 24 h after administration. Both samples were stored at −20°C until analyzed.

Reagents. S-1006 and PC hydrochloride were synthesized at Shionogi Research Laboratories. PA was obtained from Tokyo Chemical Industry (Tokyo, Japan), and isovaleric acid was obtained from Nacalai Tesque (Kyoto, Japan). Carnitine was purchased from Sigma (St. Louis, Mo.). Solvents used for chromatography were of high-performance liquid chromatography (HPLC) grade, and all other reagents and chemicals used were of reagent grade.

Apparatus. The gas chromatograph system used for the determination of PA concentrations consisted of a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector and a capillary column (liquid phase, Bonded FFAP) with the following dimensions: length, 25 m; inner diameter, 0.32 mm; and film thickness, 1 μm (Quadrex Corp., New Haven, Conn.). Operating conditions were as follows: column oven temperature, 150°C; injection port and detector temperature, 200°C; helium carrier gas flow rate, 1.0 ml/min; make-up gas flow rate, 30 ml/min; hydrogen flow rate, 30 ml/min; air flow rate, 370 ml/min. The split-injection mode was employed with a split ratio of 20:1. The HPLC system employed for the PC assay was the same as that used in previous work (7a).

Assay methods. S-1006 concentrations were determined by a microbiological assay described previously (7). No compounds interfered with this assay. Detection limits of S-1006 were 0.01 μg/ml for both plasma and urine. Coefficients of

variation at the assay ranges were less than 4 and 7% for plasma and urine, respectively. For the assay determining total PA, 0.2 ml of sample plasma, 50 μl of water, and 1 ml of 1 N NaOH were added to a 12-ml glass test tube, and the mixture was then allowed to stand for 2 h at room temperature. To acidify the solution, 0.5 ml of 10% HCl was added, and the mixture was extracted with a reciprocal shaker for 3 min with 5 ml of methylene chloride containing 50 μg of isovaleric acid as an internal standard. The emulsion was centrifuged, the organic layer was transferred to a test tube containing 50 μl of dimethylformamide, and then the solution was concentrated to a volume of approximately 50 μl with a rotary evaporator at room temperature. To the residual solution 100 μl of acetone was added, and a 1-μl aliquot of the solution was injected onto the gas chromatograph. For free PA, 1.05 ml of water, 0.5 ml of 10% HCl, and 5 ml of methylene chloride containing 50 μg of isovaleric acid were added to 0.5 ml of sample plasma, and the mixture was then extracted as described above. Hereafter, the assay was performed according to the total PA assay. Detection limits of PA in plasma and urine were 0.2 and 0.5 μg/ml, respectively. Coefficients of variation at the assay ranges were less than 8 and 3% for plasma and urine, respectively. PC concentrations in plasma and urine were determined by HPLC with fluorescence detection (7a). Detection limits of PC in plasma and urine were 0.02 and 1 μg/ml, respectively. Coefficients of variation at the assay ranges were less than 4% for both plasma and urine. Amounts of free carnitine and total carnitine in plasma and urine were determined by an enzyme radioassay with modifications of the published method (4). Detection limits of carnitine were 6 and 25 nmol/ml with coefficients of variation of 5% for both plasma and urine. The concentration of acylcarnitines was estimated by subtracting the free carnitine concentration from the total carnitine concentration.

Pharmacokinetic analysis of data. Concentrations of S-1006, total PA, and PC in plasma were fitted to a one-compartment model with first-order absorption and absorption lag time by using nonlinear least-squares regression and the NONLIN program (11), with each concentration weighted equally. The elimination half-life ($t_{1/2}$) was calculated by using the estimated first-order elimination rate constant as follows. Area under the concentration time curve (AUC) was calculated from the plasma drug concentration data of 0 to 24 h after administration by the trapezoidal rule. Maximum concentration in plasma and its corresponding time were shown as mean values of individual data. $t_{1/2} = \ln 2/k_{el}$, where k_{el} is the elimination rate constant.

RESULTS

Concentrations in plasma. Concentrations of S-1006, total PA, and PC in plasma after oral administration of S-1108 are shown in Fig. 1, and the pharmacokinetic parameters of each metabolite are listed in Table 1. The mean level of S-1006 in plasma reached a maximum at 5 and 2 h, and the mean maximum concentrations in plasma were 0.8 and 2.1 μg/ml at 4.3 and 2.3 h after 100- and 200-mg oral administrations of S-1108, respectively. The apparent $t_{1/2}$s were 1.5 and 2.2 h and the AUCs were 5.3 and 10.1 μg · h/ml for each dose. The level of total PA in plasma reached a maximum concentration of 0.4 μg/ml at 3.7 h for the 100-mg regimen and a maximum concentration of 1.0 μg/ml at 2.3 h for the 200-mg regimen. The $t_{1/2}$s were 4.2 and 6.1 h, and the AUCs were 2.7 and 5.1 μg · h/ml, respectively. No measurable amounts of free PA were found in plasma after either dose. Peak concentrations of PC in plasma were 0.9 and 2.1 μg/ml, obtained 4.3 and 2.3 h after administration for the 100- and 200-mg regimens, respectively. The mean $t_{1/2}$s were 2.6 and 3.0 h and the AUCs were 7.6 and 13.7 μg · h/ml for the 100- and 200-mg regimens, respectively.

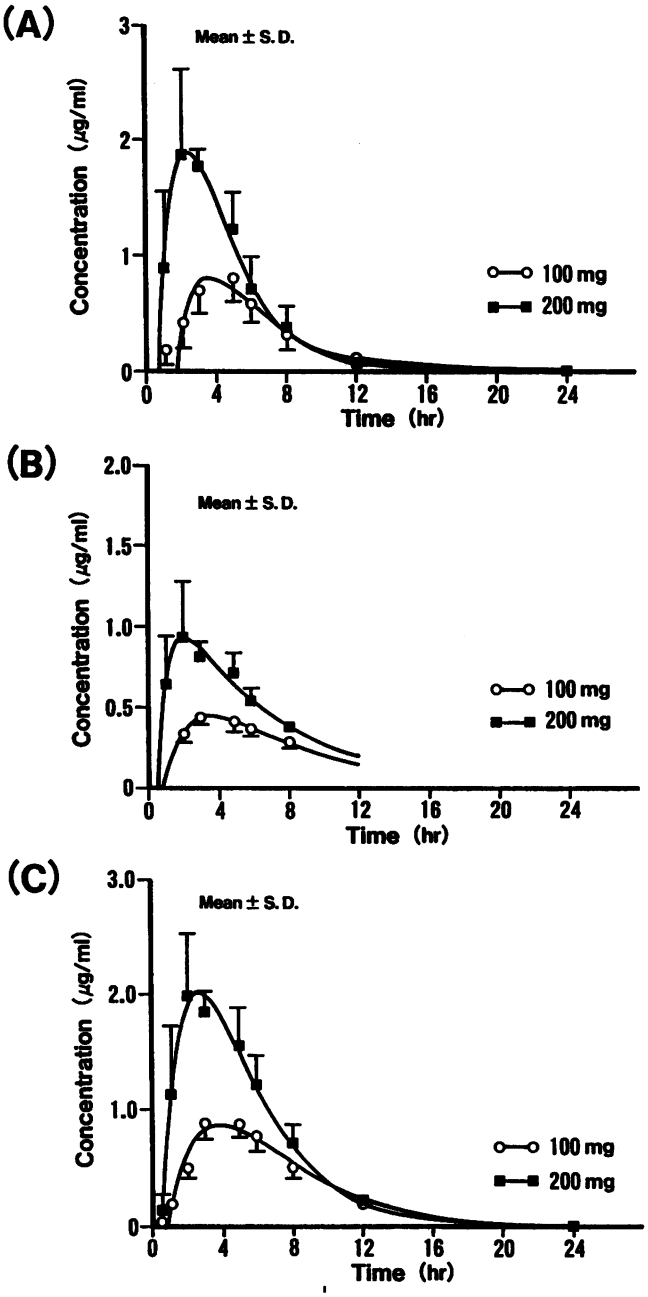

FIG. 1. Concentrations of S-1006 (A), total PA (B), and PC (C) in plasma after 100- and 200-mg administrations of S-1108 to healthy volunteers; values are mean concentrations from three subjects, and bars represent standard deviations.

Urinary excretion. Urinary excretion of S-1006, total PA, and PC is shown in Fig. 2. More than 90% of that excreted in 24 h was excreted within the first 12 h for these metabolites. Recoveries of S-1006 in urine were 34% for the 100-mg regimen and 41% for the 200-mg regimen at 24 h, whereas 93% of total PA was excreted within 1 day for both regimens.

TABLE 1. Pharmacokinetic parameters of S-1006, total PA, and PC after administration of S-1108[a]

Dose (mg)	Compound	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg · h/ml)
100	S-1006	0.83 ± 0.21	4.33 ± 1.15	2.24 ± 0.75	5.28 ± 1.02
	TPA	0.44 ± 0.04	3.67 ± 1.15	6.07 ± 0.51	2.66 ± 0.16
	PC	0.94 ± 0.08	4.33 ± 1.15	2.64 ± 0.31	7.62 ± 0.84
200	S-1006	2.06 ± 0.46	2.33 ± 0.58	1.53 ± 0.16	10.08 ± 0.77
	TPA	1.00 ± 0.27	2.33 ± 0.58	4.16 ± 0.56	5.10 ± 0.95
	PC	2.07 ± 0.42	2.33 ± 0.58	3.01 ± 0.84	13.69 ± 2.02

[a] All values are means ± standard deviations (n = 3 for all trials). TPA, total PA; C_{max}, maximum concentration in plasma; T_{max}, time to reach maximum concentration in plasma.

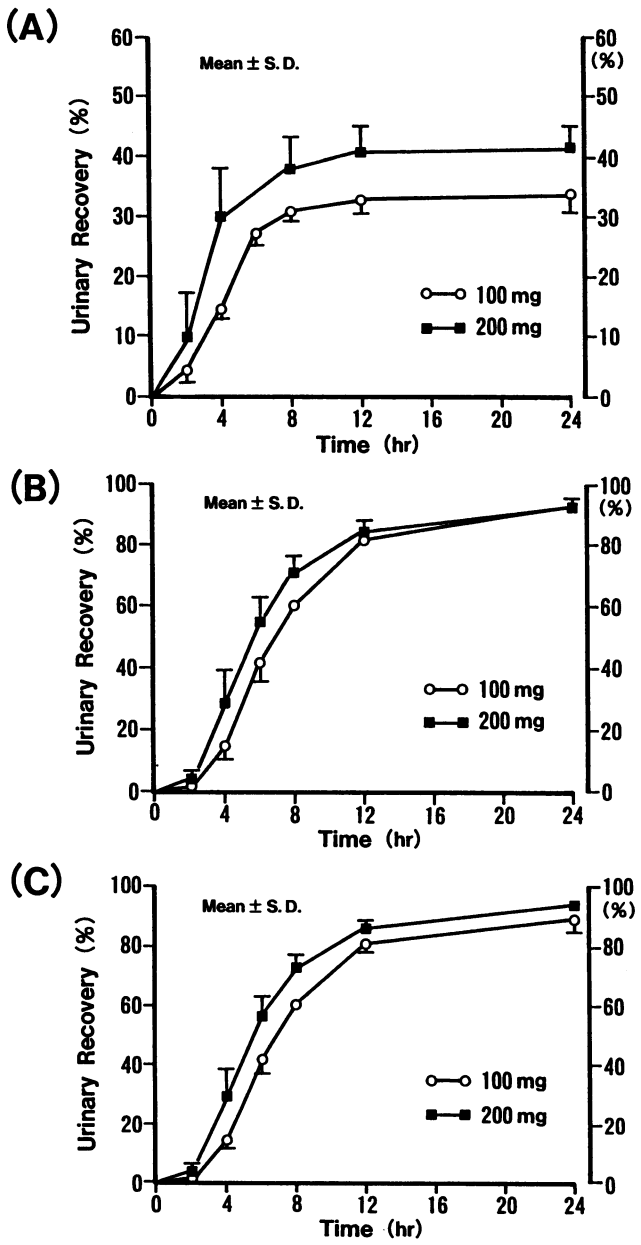

FIG. 2. Recoveries of S-1006 (A), total PA (B), and PC (C) in urine after 100- and 200-mg administrations of S-1108 to healthy volunteers; values represent mean recoveries from three subjects, and bars represent standard deviations.

No measurable amounts of free PA were found in urine after either dose. Recoveries of PC at 24 h were 89% for the 100-mg regimen and 94% for 200-mg regimen. The urinary excretion profile of PC was similar to that of total PA.

Profiles of carnitine levels in plasma and urine in humans. The time course of levels of free carnitine in plasma after the 100- and 200-mg oral administrations of S-1108 is shown in Fig. 3. The level of free carnitine in plasma before S-1108 treatment was 60 nmol/ml, and the concentration remained at approximately the same level during the 24-h period after administration of S-1008. Urinary excretion of carnitine and acylcarnitine after the 200-mg administration of S-1108 is shown in Fig. 4. The excretion of free carnitine decreased from 23 μmol in the urine sample taken 0 to 2 h after administration of S-1108 to 6 μmol in the urine sample taken at 6 to 8 h, and the excretion of acylcarnitine increased from 33 μmol in the urine sample taken at 0 to 2 h to 118 μmol in the urine sample taken at 2 to 4 h for the 200-mg regimen of S-1108. The excretion profile of acylcarnitine was similar to that of PC in each individual time period.

DISCUSSION

Metabolism of S-1108 and PA. A metabolic pathway of S-1108 including metabolism of PA in humans is postulated in Fig. 5. S-1108 given orally is hydrolyzed by an esterase in the intestinal tract to form S-1006 (the active form), pivalic acid, and formaldehyde. Approximately 40% of S-1006 was excreted in urine, whereas more than 93% of PA was excreted as the conjugated form. No measurable amounts of

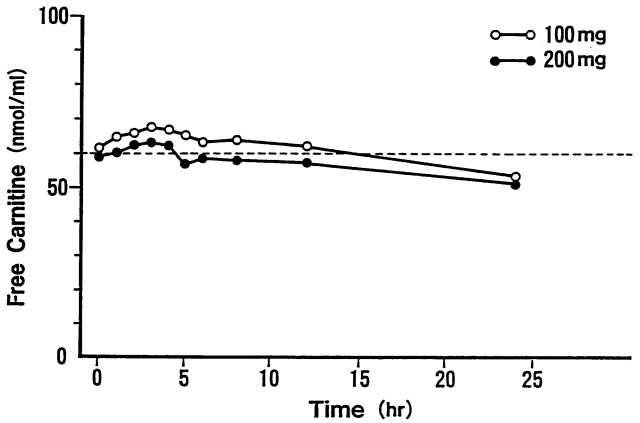

FIG. 3. Concentrations of free carnitine after 100- and 200-mg administrations of S-1108 to healthy volunteers; values represent mean concentrations from three subjects, and the dotted line represents the average carnitine concentration before administration.

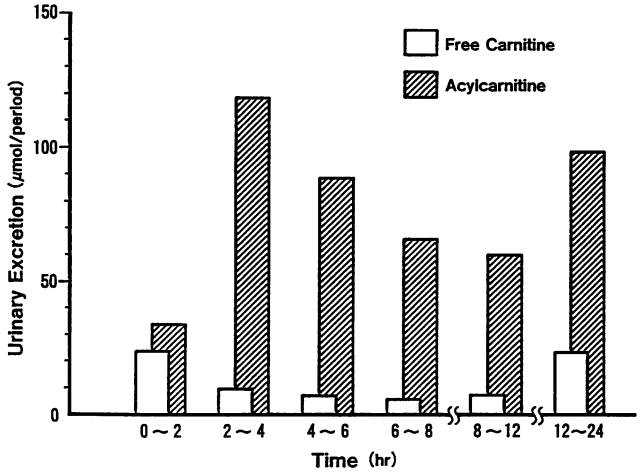

FIG. 4. Excretions of free and acylcarnitine in urine after 200-mg administrations of S-1108 to healthy volunteers.

free PA were found in plasma or urine throughout the study (detection limits of PA are 0.2 μg/ml for both plasma and urine samples). Table 2 shows a comparison of urinary excretion rates of total PA and PC within 24 h after the administrations of S-1108. In the two regimens, the excretion ratios of PC to total PA were 1.0. The results clearly indicate that the total PA excreted in urine was recovered as PC and that the major metabolic pathway of PA is conjugation with carnitine. In the metabolism of pivampicillin (5, 9, 10), pivmecillinam (5), or the POM ester derivative of methyldopa (19), PC is recognized as a major metabolite in human urine. Recently, a carnitine conjugate has been recognized as a common urinary metabolite for drugs in which the chemical structure is related to fatty acids (13, 17). Carnitine conjugation with cyclopropanecarboxilic acid in rats (14),

TABLE 2. Urinary recoveries of S-1006, total PA, and PC after administration of S-1108[a]

Dose (mg)	Average urinary recovery at 0–24 h			
	S-1006	TPA	PC	PC/TPA ratio
100	33.7 ± 2.6	92.5 ± 1.0	89.1 ± 4.3	1.0 ± 0.0
200	41.3 ± 3.9	92.5 ± 3.2	93.8 ± 0.6	1.0 ± 0.0

[a] All values are means ± standard deviations (n = 3 for all trials). Values are expressed as the percent of the original dose recovered. TPA, total PA.

bovines (15), and dogs (16) was first shown by Quistad et al., and valproylcarnitine has been isolated from the urine of patients treated with valproic acid (12). These results clearly indicate that carnitine plays an important role in detoxification of xenobiotics with carboxylic acid in animals and humans.

Metabolites other than the carnitine conjugate were glucuronide and glycine conjugates, as reported in the cycloproate studies of animals (14–16). In our related study of S-1108 metabolism in dogs, these three conjugates were identified as the major metabolites of PA in urine. In humans, glucuronide and glycine conjugates are potential metabolites of PA, as found by Melegh et al. (9). In our study on the identification of other metabolites of PA derived from S-1108 in humans, we also found trace amounts of pivaloylglycine in urine (7b). Conjugation with glycine could be a minor route of excretion for PA. As glucuronide would also be a possible metabolite of PA in human urine, further investigations are needed to identify and quantitate it. Consequently, we conclude that PA liberated from S-1108 is excreted quantitatively in urine as PC.

In addition to PA, the excretion profile of another metabolite, formaldehyde, should be investigated. The formaldehyde liberated in the metabolism of cefteram pivoxil has

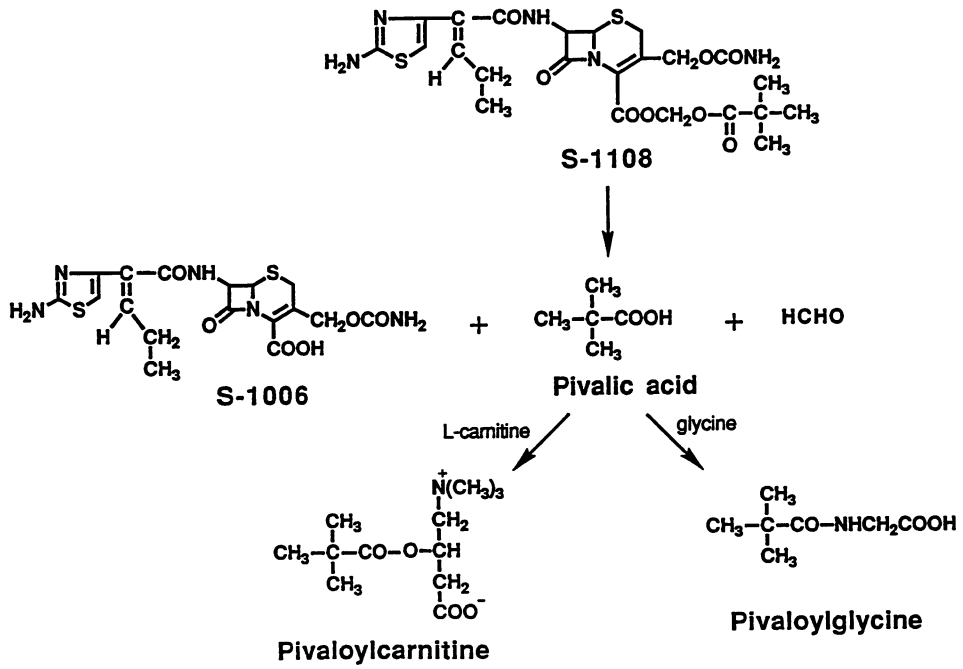

FIG. 5. Probable metabolic pathway of S-1108 in humans.

been hypothesized to be absorbed in the intestinal tract in a manner similar to that of PA and to be metabolized to carbon dioxide via the C_1 metabolic cycle. The carbon dioxide formed was found to be excreted mainly in respiratory air (18).

Effect of PA on the carnitine profile. The biological importance and roles of carnitine are reported as an essential cofactor in fatty acid oxidation. There is, indeed, a debate regarding the risk of adverse effects from prodrugs that give rise to PA which competitively utilize carnitine against endogenous fatty acid conjugation. Carnitine deficiencies induced by long-term therapy with pivampicillin and pivmecillinam in patients under metabolic stress caused by abnormal fatty acid metabolism have been discussed by Holme et al. (5). Although they suggested that there is a potential risk associated with use of these prodrugs, they also pointed out that there were no reports of symptoms or adverse effects indicating carnitine deficiency in patients treated with them. It should be emphasized that no clinical evidence of adverse effects was observed despite the profound changes in carnitine concentration in plasma and excretion in urine (9, 10). Also, in multiple-dose studies of S-1108 (daily dose of 600 mg for 8 days) conducted with healthy volunteers, no significant consumption of endogenous carnitine and no clinical findings of adverse effects were observed (12a). Moreover, carnitine supplement therapy has been recommended for carnitine deficiency syndrome in order to eliminate the potential risks associated with long-term treatment with the POM ester prodrugs at higher doses (8, 10). Recently, several studies have reported on the safety of the drugs that liberate PA, by monitoring profiles of endogenous carnitine and acylcarnitine concentration in plasma and excretion in urine in conjunction with the formation of carnitine conjugates of the drugs during therapy (5, 8, 10).

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