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INHIBITION OF PROSTAGLANDIN SYNTHESIS BY SODIUM 2-[4-(2-OXOCYCLOPENTYLMETHYL)PHENYL] PROPIONATE DIHYDRATE (CS-600), A NEW ANTI-INFLAMMATORY DRUG, AND ITS ACTIVE METABOLITE *IN VITRO* AND *IN VIVO*

KEIICHI MATSUDA, YORIHISA TANAKA,* SHIGERU USHIYAMA, KIYOKATA OHNISHI and MITSUO YAMAZAKI†

Biological Research Laboratories, Sankyo Co., Ltd., Hiromachi, Shinagawa-ku, Tokyo 140, Japan

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Abstract—A new anti-inflammatory agent, sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (CS-600), was investigated for its inhibition of prostaglandin (PG) synthesis *in vivo* and *in vitro*. CS-600 caused a marked decrease in the level of urinary PGE_2 and $PGF_{2\alpha}$ in rats. The dose of CS-600 which resulted in a 50% decrease of urinary PGE_2 excretion was 1.9 mg/kg, p.o., and this value agreed well with the ID_{50} of the drug for carrageenin edema (1.2 mg/kg, p.o.). This suggests that CS-600 inhibits prostaglandin synthesis *in vivo*. However, CS-600 had only weak inhibitory activity against *in vitro* prostaglandin synthesis by bovine seminal vesicle microsomes (IC_{50}: 760 μM). A main plasma metabolite of CS-600, which was produced by stereospecific reduction of the cyclopentanone moiety to transhydroxy cyclopentane, exhibited potent inhibitory activity toward the prostaglandin synthetase of bovine seminal vesicle microsomes (IC_{50}: 11 μM). In cell cultures of 3T6 fibroblasts from mice, CS-600 inhibited production of PGE_2 and $PGF_{2\alpha}$ by the cells at low concentrations (IC_{50} for PGE_2: 1.6 μM). The active metabolite exhibited more potent inhibition (IC_{50}: 0.29 μM), and conversion of CS-600 into the active metabolite occurred in the cell system. Inhibition of prostaglandin synthetase in the membrane fraction of the fibroblast cells was also investigated. Available evidence indicates that CS-600 is a prodrug and exerts its pharmacological activities after conversion to the active metabolite.

CS-600 is a new phenylpropionate anti-inflammatory agent with marked analgesic and antipyretic activities and relatively weak gastrointestinal ulcerogenicity [1].

Inhibition of prostaglandin (PG) synthesis has been considered to be the main anti-inflammatory mechanism of nonsteroidal anti-inflammatory drugs [2, 3]. In a previous paper [4], we reported that urinary PGE_2 and $PGF_{2\alpha}$ in rats were decreased by administration of ten typical nonsteroidal anti-inflammatory drugs and that the decrease in the urinary prostaglandins correlated well with the anti-inflammatory potencies of the drugs [4]. Therefore, measurement of urinary prostaglandins is thought to be a suitable assay system for the estimation of *in vivo* prostaglandin synthesis. The previous paper [4] also described the inhibitory effects of nonsteroidal anti-inflammatory drugs on PGE_2 synthesis in a cell culture system of 3T6 fibroblasts.

The present study was undertaken to elucidate the mechanism by which CS-600 exerts its pharmacological activities. Although CS-600 had only weak inhibitory activity toward prostaglandin synthetase in seminal vesicle microsomes, this drug produced a marked decrease in urinary PGE_2 and $PGF_{2\alpha}$ excretion in rats. These results suggested that CS-600 may show its activities after conversion to an active metabolite. Thus, metabolites of CS-600 were examined for their inhibitory activities toward prostaglandin synthetase. Effects of CS-600 and its metabolites on PGE_2 and $PGF_{2\alpha}$ synthesis in the cell culture system of 3T6 fibroblasts and on prostaglandin synthetase in the membrane fraction of the fibroblast cells were also investigated.

MATERIALS AND METHODS

CS-600, its metabolites, and [^{14}C]CS-600 labeled at the methylcarbon of the propionic acid moiety were synthesized in the Chemical Research Laboratories of the Sankyo Co., Tokyo [5]. The *trans*-OH metabolite and the *cis*-OH metabolite of CS-600 were isolated from rat urine [6].

Arachidonic acid, PGE_2, and $PGF_{2\alpha}$ were obtained from the Sigma Chemical Co., St. Louis, MO. [^{14}C]Arachidonic acid, [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ were obtained from the New England Nuclear Corp., Boston, MA. Radioimmunoassay kits for PGE and $PGF_{2\alpha}$ were purchased from Clinical Assay, Cambridge, MA. Dulbecco modified Eagle's Minimum Essential Medium (Dulbecco-Eagle MEM) was obtained from the Nissui Pharmaceutical Co., Tokyo. 3T6 fibroblast cells, a cultured strain from the Swiss albino mouse, were obtained from the Tissue Culture Center of the Dainippon Pharmaceutical Co., Osaka.

* Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd.

† Author to whom correspondence should be addressed.

Acetylsalicylic acid was obtained from Wako Chemicals, Osaka, Japan; indomethacin from Merck, Rahway, NJ, U.S.A.; and ketoprofen from Rhone-Poulenc, Vinty-sur-Seine, France. Diclofenac sodium and phenylbutazone were from Fujisawa-Ciba-Geigy, Osaka. Mefenamic acid and meclofenamic acid were from Warner Lambert, Detroit, MI.

Inhibition of prostaglandin synthetase in seminal vesicle microsomes. The inhibitory activities of CS-600 and other compounds on prostaglandin synthetase in bovine seminal vesicle microsomes were estimated according to the method described previously [4]. A microsomal fraction of bovine seminal vesicles was prepared according to the method of Takeguchi *et al.* [7], lyophilized, and stored below −70° before use. The reaction mixture contained 20 μmoles Tris–HCl, pH 7.6, 0.2 μmole epinephrine, 0.4 μmole glutathione, 200 μg of the microsomal enzyme, CS-600 or compound to be tested (dissolved in 5 μl ethanol), and 2 nmoles [^{14}C]arachidonic acid (60 nCi), in a total volume of 200 μl. The microsomal preparation was preincubated with the drug at 30° for 5 min, and reaction was started by the addition of [^{14}C]arachidonic acid dissolved in 5 μl ethanol, was performed at 30° for 10 min, and was stopped by the addition of 50 μl of 1 N HCl. Prostaglandins were extracted with 1.5 ml of ethylacetate, and the ethylacetate layer was dried with nitrogen gas, dissolved in 40 μl of methanol, and applied to a thin-layer plate (Merck, Kieselgel 60 F). The solvent of the chromatography was an organic phase of ethylacetate, acetic acid, iso-octane and water (11:2:5:10) [8]. Under these conditions, PGE_2 is the only product from arachidonic acid. The R_f values for PGE_2 and arachidonic acid were 0.47 and 0.81 respectively. The PGE_2 fraction was detected with a radioactive scanner (Berthold Co.) and scraped off for radioactivity determination.

Urinary prostaglandin estimation. PGE_2 and $PGF_{2\alpha}$ levels in 4-hr urine of rats administered CS-600 and other drugs were estimated as described previously [4]. Male Wistar rats (about 200 g) were starved for 16 hr before use. Drugs were suspended in 0.5% tragacanth and administered orally. When administered intravenously, drugs were dissolved in saline. Water (2.5 ml/100 g body wt) was given orally 1 hr after drug administration to get a constant volume of urine. Urine from four rats was collected into one ice-cold container using a urine collection cage for 4 hr after drug administration. The urine was adjusted to pH 3.0 with formic acid, and prostaglandins were extracted twice with an equal volume of chloroform [9]. The chloroform layer was dried under a flow of nitrogen gas, and the extract was chromatographed on a silicic acid column according to the method of Jaffe *et al.* [10]. Column recoveries for PGE_2 and $PGF_{2\alpha}$ were 74 and 88% respectively. PGE_2 was converted to PGB_2 by heating at 80° for 10 min in 0.1 N NaOH and was radioimmunoassayed using anti-PGB rabbit serum. $PGF_{2\alpha}$ was radioimmunoassayed using anti-$PGF_{2\alpha}$ rabbit serum.

Determination of CS-600 and its metabolites in rat plasma. [^{14}C]CS-600 (sp. act. 43 μCi/mg) was given orally to rats at a dose of 2 mg/kg body weight. Plasma (1.5 to 2.0 ml) was taken from the rats and was adjusted to pH 3 by addition of 2 N HCl, and then was extracted twice with 3 vol. of ethylether. Most of the radioactivity was recovered in the ethylether layer. The extract was evaporated under a stream of nitrogen gas, and the residue was dissolved in a small amount of ethanol and subjected to thin-layer chromatography (TLC) on a silica gel plate (Kieselgel 60 F, Merck) with benzene–acetone–acetic acid (87:10:3) as the developing solvent. Under these conditions, the R_f values for CS-600, *cis*-OH metabolite and *trans*-OH metabolite were 0.66, 0.58, and 0.53 respectively. Authentic CS-600 and the metabolites were developed simultaneously on the same plate, and bands corresponding to each compound were scraped off for radioactive determination.

Inhibition of prostaglandin synthesis in cultured cells. Inhibition of PGE_2 and $PGF_{2\alpha}$ synthesis in 3T6 fibroblast cells by CS-600 and other compounds was carried out as reported previously [4]. 3T6 fibroblast cells (3×10^5 cells/well) were seeded and grown in 1 ml of Dulbecco-Eagle MEM supplemented with 10% calf serum in a multidish plate (Nunc Co., 24 wells, 14 mm in diameter) at 37° under 5% CO_2 in air. After 48 hr of incubation the fibroblast cells (8×10^5 cells/well) were washed with 2 ml of the MEM medium, and 0.5 ml of fresh MEM medium supplemented with calf serum containing CS-600 or the compound to be tested was added. After the cell monolayers were incubated for an additional 2 hr, the medium was removed. The cells were washed a second time, and 0.25 ml of the MEM medium supplemented with calf serum containing CS-600 or the tested compound plus 5 μg/ml arachidonic acid was introduced [11]. One hour later, the medium was removed and saved for PGE_2 and $PGF_{2\alpha}$ measurement by radioimmunoassay. Measurement of the prostaglandin content of the culture medium was carried out without solvent extraction. Radioimmunoassay was carried out using the anti-PGB and anti-$PGF_{2\alpha}$ rabbit serum as described above.

Determination of CS-600 metabolites in fibroblast cells. Metabolites of CS-600 in the 3T6 fibroblast cells were determined by the following procedure: 3.2 μM [^{14}C]CS-600 (sp. act. 104 nCi/μmole) was incubated with 3T6 fibroblast cells (5×10^5 cells) in 0.25 ml of Dulbecco-Eagle MEM containing 10% calf serum for 6 hr. After various intervals of time, 0.20 ml of the medium was taken and dried under a flow of nitrogen gas. The residue was dissolved in a small amount of ethanol and subjected to TLC with benzene–acetone–acetic acid (87:10:3) as the developing solvent. CS-600 and its metabolites were determined as described above.

Inhibition of prostaglandin synthetase in fibroblast cell membrane. 3T6 fibroblast cells were suspended in 0.1 M Tris–HCl, pH 7.6, at 5×10^7 cells/ml and sonicated by a Branson sonicator for 30 sec. The mixture was centrifuged at 100,000 *g* for 60 min at 4°. The supernatant fraction was discarded, and the precipitate was suspended in 0.1 M Tris–HCl, pH 7.6. This was used as the membrane fraction of 3T6 cells. The reaction mixture contained, in a total volume of 200 μl, 10 μmoles Tris–HCl, pH 7.6, 0.2 μmole epinephrine, 0.4 μmole glutathione, 4 μg

Table 1. Effect of CS-600 on urinary PGE_2 and $PGF_{2\alpha}$ excretion in rats*

	Dose (mg/kg)	4-hr Urine volume (ml)	PGE_2 in 4-hr urine (ng)	ID_{50} for PGE_2 (mg/kg)	$PGF_{2\alpha}$ in 4-hr urine (ng)	ID_{50} for $PGF_{2\alpha}$ (mg/kg)
Expt. 1						
No-drug control		4.0	3.88		1.05	
CS-600	p.o. 1	4.0	2.76		0.82	
	3	3.3	1.54	1.9	0.46	2.3
	10	3.3	0.90		0.25	
	i.v. 1	4.3	3.66		0.83	
	3	4.0	1.45	2.4	0.37	2.4
	10	3.7	0.89		0.19	
Expt. 2						
No-drug control		3.4	4.80		1.35	
Indomethacin	p.o. 1	2.8	3.90		1.11	
	3	3.8	2.60	3.5	0.65	2.9
	10	3.0	1.63		0.40	
	i.v. 1	3.0	3.48		0.89	
	3	2.8	2.10	2.7	0.42	1.8
	10	3.0	0.49		0.16	

* CS-600 or indomethacin was administered orally or intravenously. Water (2.5 ml/100 g body wt) was given 1 hr after drug administration. Urine from four rats was collected into one container for 4 hr after drug administration, and PGE_2 and $PGF_{2\alpha}$ contents in the 4-hr urine were determined by radioimmunoassay as described in Materials and Methods. Urine volume (ml) and PGE_2 and $PGF_{2\alpha}$ contents (ng) in the 4-hr urine are the average of four rats and are expressed per one rat.

hemoglobin, the membrane fraction of 3T6 cells (protein, 33μg), 3.2 nmoles [^{14}C]arachidonic acid (165 nCi), and CS-600 or the compound to be tested. The reaction was started by the addition of [^{14}C] arachidonic acid dissolved in 5 μl ethanol, was performed at 37° for 20 min, and was stopped by the addition of 50 μl of 0.2 M citric acid. The radioactive materials were extracted with 1.5 ml of ethylacetate and analyzed by TLC in the same manner as inhibition of prostaglandin synthetase in seminal vesicle microsomes.

RESULTS

Decrease in urinary prostaglandin excretion in rats. To investigate the inhibitory activity of CS-600 on prostaglandin synthesis *in vivo*, changes in urinary PGE_2 and $PGF_{2\alpha}$ excretion were measured in rats administered CS-600. Urine was collected for 4 hr after drug administration, and urinary PGE_2 and $PGF_{2\alpha}$ were determined by radioimmunoassay. Urinary PGE_2 and $PGF_{2\alpha}$ were decreased similarly by oral and intravenous administration of CS-600, and

Table 2. Inhibitory effects of CS-600 and its metabolites on prostaglandin synthetase in bovine seminal vesicle microsomes*

Compounds	IC_{50} values for PGE_2 production (μM)
CS-600	760
Indomethacin	0.4
Ketoprofen	0.5
Metabolites of CS-600	
cis-OH metabolite	340
trans-OH metabolite	11
Isomers of *trans*-OH metabolite (Configuration of three asymmetric carbons)	
2S, 1'R, 2'S	9
2S, 1'S, 2'R	90
2R, 1'R, 2'S	>1000
2R, 1'S, 2'R	>1000

* The metabolites of CS-600 were isolated from rat urine. The isomers of the *trans*-OH metabolite were synthesized chemically: positions of the asymmetric carbons are shown in Fig. 1. The inhibition activity was determined as described in Materials and Methods. Each value is the average of three independent experiments.

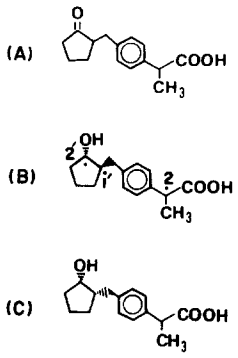

Fig. 1. Structures of CS-600 and its main plasma metabolites. (A) CS-600 (free acid), (B) *trans*-OH metabolite, and (C) *cis*-OH metabolite. Asterisks (*) and numbers attached to the structur of the *trans*-OH metabolite indicate asymmetric carbons and their positions.

this decrease was dose dependent (Table 1). As a comparison, indomethacin was administered to rats under the same conditions. CS-600 was as potent as indomethacin in inhibiting urinary prostaglandin excretion.

Inhibition of prostaglandin synthetase in seminal vesicle microsomes. CS-600 was examined for its inhibitory effect on PGE_2 synthesis by bovine seminal vesicle microsomes. CS-600 showed only a weak inhibition of prostaglandin synthetase. Its inhibition activity was about one-thousand of indomethacin and ketoprofen (Table 2). Therefore, plasma metabolites of CS-600 were examined for their abilities to inhibit prostaglandin synthetase in this assay system. The plasma metabolites of CS-600 were products reduced at the cyclopentanone moiety, and *trans*-OH and *cis*-OH metabolites were produced from CS-600 (Figs. 1 and 2). The *trans*-OH metabolite was a major metabolite and its plasma levels were high and comparable with those of CS-600 (Fig. 3). The *trans*-OH metabolite showed much more potent inhibition of prostaglandin synthetase than CS-600 (Table 2). Other plasma and urine metabolites including the *cis*-OH metabolite showed slight undetectable inhibition.

The *trans*-OH metabolite has three asymmetric carbons, and thus four stereo-isomers of the *trans*-OH metabolite exist. These stereo-isomers of the *trans*-OH metabolite were synthesized chemically, and their inhibitory effects on prostaglandin synthetase were investigated. One of the isomers, (2S)-

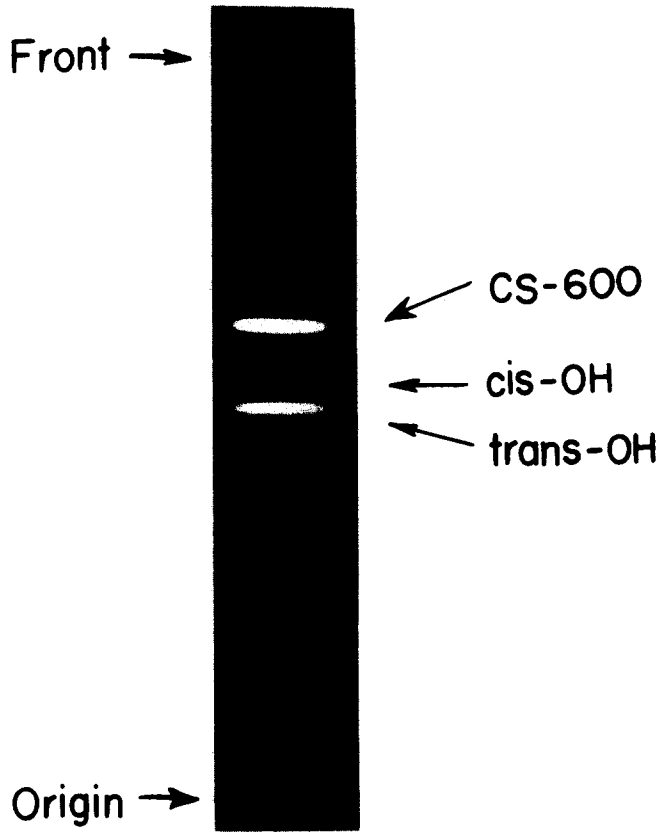

Fig. 2. Thin-layer chromatography radiochromatogram of the plasma metabolites after administration of [^{14}C]CS-600 to rats. [^{14}C]CS-600 (sp. act. 43 μCi/mg) was given orally at a dose of 2 mg/kg body weight; 30 min later rat plasma was taken and extracted with ethylether and subjected to TLC with benzene–acetone–acetic acid (87:10:3) as the solvent.

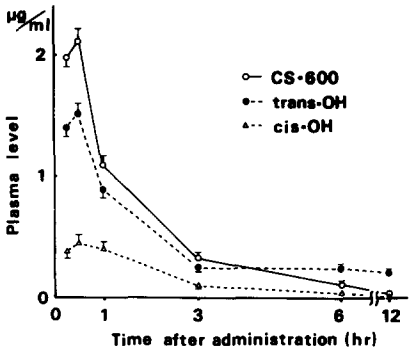

Fig. 3. Plasma levels of CS-600 and its metabolites in rats. [^{14}C]CS-600 (sp. act. 43 μCi/mg) was administered orally to rats at a dose of 2 mg/kg body weight, and plasma was taken at various intervals. CS-600 and its metabolites were extracted from the plasma and separated by TLC, and the radioactivity of each fraction was determined as described in Materials and Methods. Key; (○) CS-600; (●) *trans*-OH metabolite; and (△) *cis*-OH metabolite. Each point is the mean of four rats, and the bar represents its standard error.

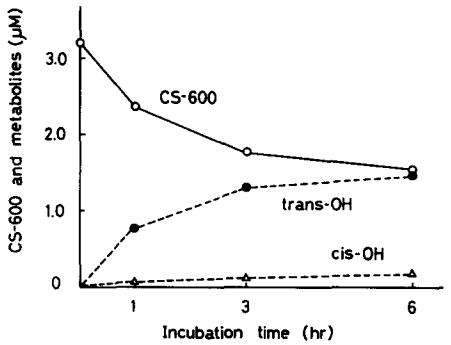

Fig. 4. Time course of the production of *trans*-OH and *cis*-OH metabolites from CS-600 by 3T6 fibroblast cells. [^{14}C] CS-600 (0.42 μCi) at a concentration of 3.2 μM was incubated with cultured 3T6 fibroblasts (5×10^5 cells) in 0.25 ml of Dulbecco-Eagle MEM supplemented with 10% calf serum at 37° for 6 hr. The *trans*-OH and *cis*-OH metabolites and CS-600 were separated by TLC, and the radioactivity was determined as described in Materials and Methods.

2-{4-[trans-(1R, 2S)-2-hydroxycyclopentylmethyl] phenyl}propionic acid, which has the configuration (2S, 1'R, 2'S) at the three asymmetric carbons, showed essentially the same inhibitory activity as the *trans*-OH metabolite isolated from urine (Table 2). Other isomers showed little or no inhibition on prostaglandin synthetase.

Inhibition of prostaglandin synthesis in cultured fibroblast cells. Inhibitory activity of CS-600 on PGE_2 and $PGF_{2\alpha}$ production by mouse cultured fibroblast cells of 3T6 was investigated. CS-600 inhibited PGE_2 and $PGF_{2\alpha}$ production at relatively low concentrations (Table 3). The *trans*-OH metabolite showed much more potent inhibitory activity than its parent compound; it was as active as indomethacin and more potent than ketoprofen.

To explain the inhibitory activity of CS-600 on cellular prostaglandin synthesis, the metabolism of CS-600 in 3T6 cells was investigated. [^{14}C]CS-600 was incubated with 3T6 cells and metabolites were analyzed by TLC. CS-600 was converted to the *trans*-OH metabolite; a small amount of the *cis*-OH metabolite was also produced, but no other metabolites were detected. After 1 hr of incubation, 25% of CS-600 was converted to the *trans*-OH metabolite; 40% was converted in 3 hr (Fig. 4).

Inhibition of prostaglandin synthetase in fibroblast cell membrane. When the membrane fraction of fibroblast cells was incubated with [^{14}C]arachidonic acid in the presence of epinephrine, hemoglobin and glutathione, arachidonic acid was converted to PGE_2 but no other products were detected. CS-600 and the *trans*-OH metabolite were examined for their inhibitory effect on PGE_2 synthesis in this system.

Table 3. Inhibition of prostaglandin synthesis by CS-600 in 3T6 fibroblast cells and in the membrane of fibroblast cells*

Compounds	IC_{50} values for prostaglandin production (μM)		
	Cell		Membrane fraction
	PGE_2	$PGF_{2\alpha}$	PGE_2
CS-600	1.6	0.82	1,400
trans-OH metabolite	0.29	0.20	12
Indomethacin	0.18	0.12	1.1
Ketoprofen	2.8	4.0	ND†

* 3T6 cells were incubated with the drug for 2 hr, and, after washing, the cells were further incubated with the drug plus 5 μg/ml arachidonic acid for 1 hr. Formation of PGE_2 and $PGF_{2\alpha}$ during the 1-hr incubation period was determined by radioimmunoassay. Under these conditions, the cells released 37 ± 1.7 ng PGE_2 and 2.3 ± 0.6 ng $PGF_{2\alpha}$, per 8×10^5 cells, into the medium in 1 hr in the absence of inhibitor. Prostaglandin synthetase activity in the fibroblast cell membrane was determined by estimation of [^{14}C]PGE_2 synthesis from [^{14}C]arachidonic acid as described in Materials and Methods. Each value is the average of three independent experiments.

† ND, not determined.

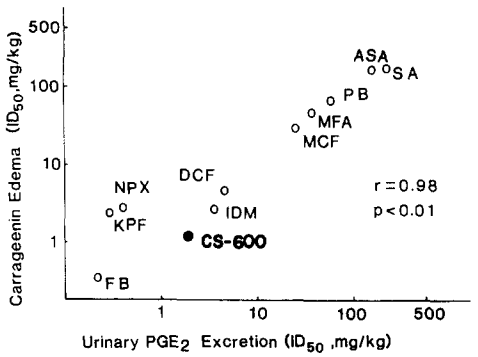

Fig. 5. Correlation between anti-carrageenin edema activity of nonsteroidal anti-inflammatory drugs and their suppression of urinary PGE_2 excretion in rats. Abbreviations: ASA, acetylsalicylic acid: DCF, diclofenac sodium; FB, flurbiprofen; IDM, indomethacin; KPF, ketoprofen; MCF, meclofenamic acid; MFA, mefenamic acid; NPX, naproxen; PB, phenylbutazone; and SA, salicylic acid.

The IC_{50} values obtained in this system were higher than those obtained with the cell system but were essentially the same with the bovine seminal vesicle microsomes (Table 3).

DISCUSSION

Although inhibition of prostaglandin synthesis is generally characteristic of nonsteroidal anti-inflammatory drugs [2, 3], CS-600 showed only a weak inhibition of prostaglandin synthetase. However, this drug produced a marked decrease in urinary PGE_2 and $PGF_{2\alpha}$ excretion in rats, and its main plasma metabolite, the *trans*-OH metabolite, had a potent inhibitory activity on microsomal prostaglandin synthetase and on PGE_2 and $PGF_{2\alpha}$ production in cultured cells. These results suggest that CS-600 is a pro-drug which shows its anti-inflammatory activity after conversion to the active metabolite. The relatively weak gastrointestinal ulcerogenic action of CS-600 (stomach lesion, ED_{50}:16.1 mg/kg; intestinal ulcer, UD_{50}:11.5 mg/kg; in rats) [1] may be due to its weak inhibitory activity on prostaglandin synthetase of gastrointestinal mucosa.

In our previous paper [4], we reported that urinary PGE_2 and $PGF_{2\alpha}$ in rats decreased after administration of nonsteroidal anti-inflammatory drugs and that the decrease in urinary prostaglandins correlated well with the anti-inflammatory potencies of ten typical nonsteroidal anti-inflammatory drugs. In the present study, the ID_{50} of CS-600 for reduction of urinary PGE_2 excretion after oral administration was 1.9 mg/kg. This was lower than those of indomethacin (3.5 mg/kg), diclofenac sodium (5.5 mg/kg), and mefenamic acid (40 mg/kg) [4]. The ID_{50} of CS-600 corresponded well to its pharmacological potency: anti-carrageenin edema (ID_{50}, 1.2 mg/kg) and analgesic activity on scald-pain (ID_{50}, 0.8 mg/kg) [1]. Similar correlation between the reduction of urinary PGE_2 excretion and anti-inflammatory activity was found when CS-600 was compared with the ten typical nonsteroidal anti-flammatory drugs (Fig. 5) [4]. These findings support the view that CS-600 exerts its pharmacological activities by inhibition of prostaglandin synthesis *in vivo*. Estimation of urinary prostaglandins seems to be a useful method for the study of the mode of action of anti-inflammatory drugs, particularly the action of drugs like CS-600.

The initial step in CS-600 metabolism was a reduction of the cyclopentanone moiety to hydroxypentane and the *trans*-OH metabolite was a main plasm metabolite. Naruto *et al.* [6] reported that the *trans*-OH metabolite isolated from urine has the stereochemically pure (2S, 1'R, 2'S)configuration. The present study showed that the *trans*-OH metabolite was a potent inhibitor of prostaglandin synthetase and that the chemically synthesized *trans*-OH metabolite with configuration of 2S, 1'R, 2'S was more potent than other stereo-isomers of the *trans*-OH metabolite. These results indicate that CS-600 is preferentially metabolized to the active metabolite which has potent inhibitory activity toward prostaglandin synthetase.

In the fibroblast cell system, CS-600 was a potent inhibitor of PGE_2 and $PGF_{2\alpha}$ production, but the active metabolite showed more potent inhibition; it was as potent as indomethacin. Inhibition of prostaglandin synthesis in the fibroblasts by CS-600 seems to be due to the formation of the active metabolite because the efficient conversion of CS-600 to the active metabolite occurred in the cell system. In the membrane fraction of the fibroblast cells, CS-600 only weakly inhibited prostaglandin synthetase but the active metabolite exhibited much more potent inhibition. These results also support the view that CS-600 exerted its inhibition activity after conversion to the active metabolite.

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