Analgesic Action of a Sustained Release Preparation of Diclofenac Sodium in a Canine Urate-Induced Gonarthritis

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Using hard capsules (SR318B) developed as a sustained release diclofenac sodium (DF-Na) preparation for once-daily administration, we investigated the persistence of the analgesic effect after oral administration in the canine urate-induced gonarthritis model. In the control group, injection of 2% urate into the knee joint induced gait disorder due to pain 2 hr after administration and thereafter. Gait disorder peaked 6 hr after urate injection, and gradually recovered after 12 hr. In the treatment group, SR318B at 1.0 mg DF-Na/kg body weight was orally administered 6 hr before urate injection, and the walking score significantly decreased 2 hr after urate injection compared with the control group ($p < 0.05$). Although the analgesic effect was not observed at the peak of urate-induced pain, the walking score significantly decreased 14, 16, and 18 hr after urate injection compared with the control group ($p < 0.05$). The plasma diclofenac (DF) concentration peaked 6 hr after SR318B administration, and decreased to about 1/3–1/5 12–18 hr after administration (peak of urate-induced pain), and the plasma level was below the quantification limit in three of five animals 24 hr after administration. DF was detected in the synovial fluid 24 hr after administration in all animals and the concentration was 0.03 ± 0.01 μg/ml (mean ± standard error). The above findings showed that the SR318B exhibits a persistent analgesic effect in a canine urate-induced gonarthritis model. Not only DF in the plasma but also the DF that transferred to the synovial fluid may be involved in this persistent analgesic effect.

Key words —— diclofenac sodium, urate-induced gonarthritis, sustained release preparation, dog

INTRODUCTION

Diclofenac sodium (DF-Na) is a phenylacetate non-steroidal anti-inflammatory agent synthesized by Ciba-Geigy of Switzerland in 1965 (Fig. 1). In Japan, sustained release capsules for twice-daily administration have been developed by SS Pharmaceutical Co., Ltd. (Japan) and used to treat rheumatoid arthritis, osteoarthritis, and lumbago.

In order to reduce adverse effects, persistent drug effect, and improve patient compliance, our aim was to develop a preparation for once-daily administration by inhibiting a rapid increase in the blood DF-Na concentration and maintaining the blood level for a prolonged time. We designed four preparation methods: 1) hard capsules containing rapid release granules and granules coated with a combination of enteric and water-insoluble polymers; 2) hard capsules containing rapid release granules and enteric film-coated plain granules combined with an organic acid; 3) hard capsules containing granules coated with a combination of water-insoluble and water-soluble polymers; and 4) hard capsules containing granules double-coated with water-insoluble and enteric films. We prepared 3–4 prototypes using each method and performed pharmacokinetic studies using beagles, and selected one preparation from those prepared from each method.[1] Next, we administered the four preparations to healthy adult men and investigated the safety and pharmacokinetics. The hard capsules containing rapid release granules and enteric film-coated plain granules combined with an organic acid at a ratio of DF-Na of 3 : 7 were the most appropriate for the sustained release preparation for once-daily administration based on the pharmacokinetics of diclofenac (DF).

Thus, we orally administered this sustained re-

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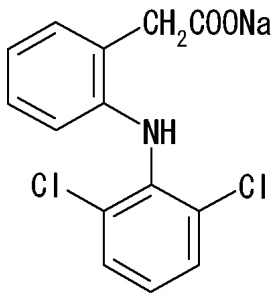

Fig. 1. Chemical Structure of Diclofenac Sodium

lease preparation containing diclofenac (SR318B) to a canine urate-induced gonarthritis model[2] and investigated the persistence of the analgesic effect.

MATERIALS AND METHODS

Materials —— The test substance SR318B contains 89.98 mg of rapid release granules and 52.68 mg of sustained release granules per capsule, and 7.5 mg and 17.5 mg of DF-Na were contained in the rapid release and sustained release granules, respectively. The capsules were stored at room temperature until use.

Urate crystals were provided by SSP Co., Ltd (Japan). Heparin sodium solution for injection (Shimizu Pharmaceutical Co., Ltd., Japan), pentobarbital sodium (Tokyo Kasei Kogyo Co., Ltd., Japan), water for injection, and physiological saline (Otsuka Pharmaceutical Factory, Inc., Japan) were used.

Animals —— Ten male beagles aged six months were purchased from Nalc Co. (body weight: 8.57–10.03 kg). The animals were individually housed in metal bracket cages under conditions at 21–26°C temperature, 44–66% humidity, and 12-hr lighting (8:00–20:00). Animals were given about 300 g of solid food (LABO D STOCK, Nihon Nosan Kogyo, Co., Japan) per day and tap water *ad libitum*, and quarantined and acclimatized for 10 days. Animals that showed no abnormalities during the quarantine/acclimation period were used.

Methods ——

Establishment of dosage and timing of urate administration: In the preliminary study in which 0.3 and 1.0 mg DF-Na/kg body weight were orally administered to dogs, the analgesic effect was observed at a dose of 1.0 mg/kg, and a small amount of DF was detected in the plasma and synovial fluid 24 hr after administration. Therefore, 1.0 mg/kg was selected for the dose. Since 2% urate crystal suspension in physiological saline caused severe persistent gait disorder, we considered that to evaluate the analgesic effect of SR318B, it is necessary to inject urate into the joint when the plasma DF concentration is high, and selected 6 hr after administration of the test substance for the time of administering urate.

Test substance administration and judgment of analgesic effect: Using five dogs per group, SR318B was orally administered at a dose of 1.0 mg/kg body weight between 21:00 and 22:00, and about 30 ml of water was immediately administered orally. The amounts of the rapid release and sustained release granules contained in the capsule were adjusted by body weight for each dog. 6 hr after administration of SR318B, 0.5 ml of 2% urate crystal suspension in physiological saline was injected into the knee joint to induce inflammation, and walking was observed 2, 6, 12, 14, 16, and 18 hr after injection of urate. Walking was evaluated by five steps: normal walking (0 point), mild claudication (1), moderate claudication (2), walking on tiptoe (3), and walking on three legs (4), and the mean score was obtained in the control and SR318B treatment groups.

Measurement of plasma and synovial diclofenac concentrations: About 2 ml of blood was collected from the cephalic vein using a heparinized syringe 2, 6, 12, 18, and 24 hr after administration in five animals in the SR318B treatment group. At the final observation, synovial fluid was collected from the knee joint in all animals.

The collected blood and synovial fluid were centrifuged at 4°C, 3000 rpm for 10 min, and the obtained plasma and supernatant of synovial fluid were stored at –20°C until analysis.

The plasma and synovial DF concentrations were measured by high performance liquid chromatography.[1]

Statistical analysis: The mean and standard error of the measured values were calculated in each group. For analysis of significance in comparison of the walking score between the SR318B treatment and control groups, Wilcoxon rank sum test was used.[3] A difference with a significance level less than 5% was regarded as significant.

RESULTS AND DISCUSSION

The walking scores after injection of 2% urate suspension into the knee joint in the SR318B treat-

Table 1. Effects of SR318B in the Walking Scores after Injection of 2% Urate Suspension into the Knee Joint of Beagle Dogs

Group	Time after injection of 2% urate suspension (hr)								
	2	4	6	8	10	12	14	16	18
Control	2.8±0.4	3.4±0.2	3.6±0.2	3.6±0.2	3.4±0.2	3.2±0.4	2.8±0.2	2.4±0.4	2.0±0.3
SR318B 1 mg/kg	1.6±0.4*	3.2±0.4	3.4±0.4	3.4±0.4	3.2±0.4	2.4±0.2	1.6±0.4*	1.4±0.2*	0.8±0.4*

Each value represents the mean ± S.D. ($n = 5$). SR318B was administered at 6 hr before urate injection. *$p < 0.05$, Significantly different from control group by using Wilcoxon rank sum test.

Table 2. Plasma and Synovial DF Concentrations after Injection of 2% Urate Suspension into the Knee Joint of Beagle Dogs

Group	Plasma concentration (μg/ml)					Synobial concentration (μg/ml)
	Time after injection of 2% urate suspension (hr)					
	−4 hr (2)	0 hr (6)	6 hr (12)	12 hr (18)	18 hr (24)	18 hr (24)
SR318B 1 mg/kg	0.36±0.16	0.53±0.08	0.20±0.04	0.10±0.01	0.03±0.02	0.03±0.01

Each value represents the mean ± S.D. ($n = 5$). (): Time after administration of SR318B (1 mg/kg).

ment and control groups are shown in Fig. 2 and Table 1.

In the control group, mild claudication (2) or severer gait disorder was observed in all animals 2 hr after urate injection and thereafter. The peak of gait disorder was 6 hr after the injection, and the mean score was 3.6 (walking on tiptoe-walking or on three legs). At 12 hr after urate injection, the walking score gradually decreased, but gait disorder of a mean score of 2.0 was still observed 18 hr after the injection.

In the group that received oral administration of SR318B at a DF-Na dose of 1.0 mg/kg 6 hr before urate injection, the mean walking score was 1.6 2 hr after urate injection (8 hr after SR318B administration), showing a significant decrease in the score compared to the control group ($p < 0.05$). The score tended to decrease 6 and 12 hr after urate injection compared with the control group, and the decreases 14, 16, and 18 hr after urate injection were significant compared with the control group ($p < 0.05$).

Time-course changes in the plasma DF level and the measurement results of the synovial DF concentration 24 hr after administration in the SR318B treatment group are shown in Table 2. In this study, SR318B was orally administered 6 hr before injection of inflammatory substance urate based on the results of the preliminary study to investigate the persistence of the effect of DF-Na preparation for once-daily administration (SR318B). As shown in Table 2, the plasma DF level reached a peak: 0.53 ± 0.08 μg/ml (mean ± standard error) 6 hr after administration of SR318B, then decreased to about 1/3–1/5 12–18 hr after administration (peak of urate-induced pain). Variation among animals was observed 24 hr after administration and the plasma DF level was below the quantification limit in three of five animals. In contrast, DF was detected in the synovial fluid 24 hr after administration in all animals, and the concentration was 0.03 ± 0.01 μg/ml (mean ± standard error).

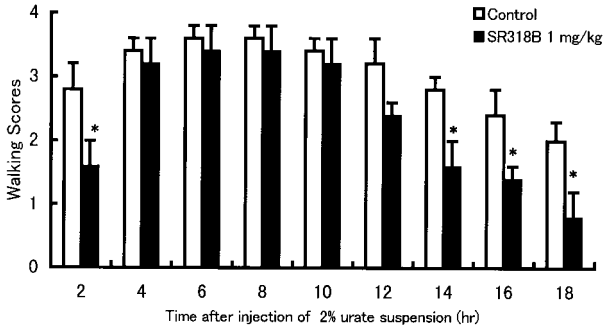

Fig. 2. Effects of SR318B in the Walking Scores after Injection of 2% Urate Suspension into the Knee Joint of Beagle Dogs

Each value represents the mean ± S.D. ($n = 5$). SR318B was administered at 6 hr before urate injection. *$p < 0.05$, Significantly different from control group by using Wilcoxon rank sum test.

Based on the above findings, oral administration of SR318B at 1.0 mg/kg of DF-Na significantly reduced gait disorder induced early after urate injection and the residual gait disorder after 14 hr (20–

24 hr after SR318B administration), and tended to reduce gait disorder at the peak of urate-induced pain in the canine urate-induced gonarthritis model was reduced. Therefore, the analgesic effect of orally administered SR318B persisted for 24 hr.

As described above, oral administration of sustained release diclofenac sodium-containing preparation SR318B at 1.0 mg DF-Na/kg body weight maintained a significant analgesic effect 24 hr after administration, and even in animals in which DF was not detected in the plasma at this time point, DF was still detected in the synovial fluid. Therefore, not only the drug component in the plasma but also the presence in the local pain region may contribute to the analgesic effect of the preparation.

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