RELATIONSHIP BETWEEN THE ANTI-INFLAMMATORY EFFECTS AND INTRAPLEURAL ACCUMULATIONS OF ANTI-INFLAMMATORY DRUGS IN RATS CARRAGEENIN PLEURISY

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We simultaneously determined the time course of the anti-exudative effects of three types of anti-inflammatory drug and their concentrations in serum and pleural exudate in carrageenin-induced pleurisy in rats. Dexamethasone (DM, steroidal anti-inflammatory drug, 0.1 mg/kg, *p.o.*) in spite of the gradual decline of DM concentration in the pleural exudate with time and its actual loss at 16 h after carrageenin, showed an anti-exudative effect throughout the period of testing after carrageenin which was more marked in the relatively late phase than in the relatively early phase. Mepirizole (MP, non-acidic, non-steroidal anti-inflammatory drug, 100 mg/kg, *p.o.*) showed an anti-exudative effect only at 3 h after carrageenin and not thereafter, although MP concentration in exudate was the same between 3 and 6 h after carrageenin and then declined gradually. Ketoprofen (KP, acidic, non-steroidal anti-inflammatory drug, 1 mg/kg, *p.o.*) showed an anti-exudative effect for 6 h after carrageenin and not thereafter, while KP concentration in exudate reached the peak at 3 h after carrageenin and then declined with time. However, at the relatively late phase (10 h after carrageenin) the twice administration of KP resulted in an increase in its exudate concentration enough to show an anti-inflammatory effect, but did not result in any more anti-exudative effect than the single administration of KP. Therefore, these differences in the anti-exudative effects of three types of anti-inflammatory drug can not be explained by the change in their concentrations in the inflamed tissue, but may reflect the difference in their intrinsic anti-inflammatory activities. The anti-inflammatory drugs accumulated in the inflamed pleural cavity independently of their ability to bind to plasma proteins, because the ratio of concentrations of three types of drug in exudate to those in serum were similar in spite of marked differences in protein binding capacities among these drugs.

Keywords — dexamethasone; ketoprofen; mepirizole; rats carrageenin pleurisy; drug level; HPLC; radioimmunoassay

INTRODUCTION

It has been reported that the action of some anti-inflammatory drugs was related to their concentration in synovial fluids of rheumatoid arthritis patients.^{1–5)} On oral administration of acetylsalicylic acid,²⁾ indomethacin³⁾ or ketoprofen⁵⁾ to patients with rheumatoid arthritis, the synovial fluid concentrations of three drugs were more than these plasma concentrations after reaching a peak level. Contrary to these results, concentration of DA 2370⁴⁾ or salicylate¹⁾ was consistently less in the joint fluid than in the

plasma.

Recently, it was reported^{6,7)} that the qualitative difference between the anti-exudative activity of steroidal anti-inflammatory drugs and that of non-steroidal anti-inflammatory drugs was observed in the relatively late phase in rat carrageenin pleurisy. Steroidal anti-inflammatory drugs, but not non-steroidal anti-inflammatory drugs, showed an anti-exudative activity on the relatively late phase in rat carrageenin pleurisy while both types of drug showed an anti-exudative activity on the relatively early

phase. It is of interest whether the anti-exudative activity of both types of drug is correlated with their concentration or content in the pleural cavity in rat carrageenin pleurisy. The present work was undertaken to examine in rat carrageenin pleurisy: (1) the time course of the anti-exudative activities of dexamethasone, ketoprofen and mepirizole and their concentration in serum and pleural exudate, (2) the correlation between the anti-exudative activity and the intrapleural concentration of ketoprofen in the relatively late phase when it was administered once or twice, and (3) the correlation between the protein binding affinity of the drug and the permeability of the drug into the pleural cavity.

Dexamethasone was used as a representative of the steroidal anti-inflammatory drug, ketoprofen as that of the acidic non-steroidal anti-inflammatory drug and mepirizole as that of non-acidic non-steroidal anti-inflammatory drug.

EXPERIMENTAL

Materials — The following drugs and reagents were purchased from the respective companies or synthesized in our laboratory. Ketoprofen (KP: 2-[benzoyl-3-phenyl] propionic acid) from Petrochemical Co., Ltd. Dexamethasone (DM: 9-fluoro-11 β , 17, 21-trihydroxy-16 α -methyl-pregna-1, 4-diene-3, 20-dione) from Steraloids Inc., and ^3H -dexamethasone (^3H -DM: 50 Ci/mmol) from New

England Nuclear. Mepirizole (MP: 1-[4-methoxy-6-methyl-2-pyrimidinyl]-3-methyl-5-methoxy-pyrazole) was synthesized and obtained analytically pure in our laboratory. Lamda carrageenin was purified from Viscarin with the previously described method.⁸⁾ Ethylketoprofen (EKP: 2-[benzoyl-3-phenyl] butyric acid) and zolimidine (ZM: 2-[*p*-methoxy-sulfonyl-phenyl] imidazo [1, 2-*a*] pyridine) used as internal standard (I.S.) for ketoprofen and mepirizole analysis were synthesized and obtained analytically pure.

Rabbit anti-dexamethasone 21-hemisuccinate antisera was elicited in the rabbit by immunization with 0.5 ml of complete Freund's adjuvant and 2 mg of hapten-bovine serum albumin (BSA) in 0.5 ml of saline, according to the method described by Hichens and Hogans.⁹⁾ The antibody used in this assay is specific for DM as shown Table I.

DM (0.1 mg/kg), KP (1 mg/kg) and MP (100 mg/kg) were dissolved or suspended in a vehicle consisting 0.5% (w/v) carboxymethylcellulose (CMC) and 2% (v/v) Tween 80, and were administered *p.o.* in a volume of 5 ml/kg.

Carrageenin-induced pleurisy in rats and collection of pleural exudate and serum. Male Wistar rats (body weight 300–400 g) were lightly anesthetized with diethylether and 0.1 ml of 2% (w/v) lamda carrageenin in sterile saline was injected into the right pleural cavity using a procedure similar to that described by Hurley *et*

TABLE I. *Percent Cross-Reaction of Anti-dexamethasone-21-hemisuccinate-BSA Antisera with Related Steroid*

	% cross-reactivity ^{a)}
Dexamethasone	100
Prednisol	3.4
Methyl prednisolone	0.5
Cortisol	1.5
Corticosterone	1.0
Cortisone	<0.1
Triamcinolone	13.2

a) At 50% of inhibition.

al.,¹⁰ except that a No. 26-gauge 1/2 needle was used instead of a No. 22-gauge lumbar puncture needle. The drugs were administered *p.o.* 1 h before carrageenin. At 3, 6, 8 and 16 h after carrageenin, blood was collected from abdominal aortic artery under diethylether anesthesia. The pleural cavity was exposed, the exudate harvested by aspiration into a plastic syringe and exudate volume was determined. The cavity was washed once with 0.2% (w/v) ethylenediaminetetra acetic acid (EDTA) saline. Exudate and washes were pooled and their volume adjusted to 5 ml with 0.2% EDTA saline and stored at -20°C until determination of drugs and measurement of protein. For determination of drugs, diluted exudate was centrifuged at $2500 \times g$ for 10 min and the supernatant was used, because the same values for KP were obtained in the uncentrifuged exudate and the supernatant about the same exudate obtained from KP-treated rats with carrageenin pleurisy.

Dexamethasone Analysis — DM was determined by radioimmunoassay (RIA). Four to forty micro liters of rat serum or 0.04–0.8 ml of pleural exudate diluted with 0.2% EDTA buffer and 15 ml of CH_2Cl_2 were added to a test tube containing 1000 dpm of ^3H -DM which was used as an estimate of recovery. After shaking test tube, organic layer was washed once with H_2O and evaporated *in vacuo*. The residue dissolved with 1 ml of benzene-MeOH (95:5, v/v) was submitted to column chromatography (tuberculin injection tube of 2 ml, 2 ml of bed volume) on Sephadex LH-20 (Pharmacia Fine Chemicals) employing benzene-MeOH (95:5, v/v) as an effluent. After discarding an initial 13 ml of the effluent, 5 ml of the effluent was collected into test tube. Two and a half milliliter aliquots were subjected to radioactivity counting to determine the recovery rate for this separation procedure. The remaining 2.5 ml of this effluent was dried with N_2 gas stream. To the residue was added 10000 dpm of ^3H -DM in 0.1 ml of MeOH and RIA was carried out by the following manner. After removal of MeOH with N_2 gas stream, to all the test tube was added 0.25 ml of

the diluted anti-serum (1:25000, v/v) with 0.06 M borate buffer (pH 8.0) containing 0.06% (w/v) BSA and 0.05% (w/v) bovine serum γ -globulin. After incubation for 30 min at room temperature (r.t.), to the tubes was added 0.25 ml of saturated $(\text{NH}_4)_2\text{SO}_4$ solution. The resulting suspension was allowed to stand at r.t. for 10 min and centrifuged at $2500 \times g$ for 10 min at 4°C . After centrifugation, 0.2 ml of each supernatant fraction containing antibody-free ^3H -DM was decanted carefully into a counting vial, added with based scintillation fluid consisting of dioxane (11), naphthalene (112 g), 2,5-diphenyl-oxazole (6 g) and 2, 2-*p*-phenylenebis-[5-phenyl-oxazole] (0.275 g). Radioactivity was counted in an Aloka model 651 liquid scintillation spectrophotometer. Bound % of ^3H -DM was calculated from the following equation:

$$B = \left(1 - \frac{C \times 5/2}{I}\right) \times 100$$

Where B is the bound %, I is the initial dpm and C is the counted dpm in 0.2 ml of supernatant. An amount of DM in sample of serum and diluted pleural exudate was calculated from a standard curve. A standard curve was constructed by plotting the bound % against amount (0–2 ng) of DM added to the blank serum and diluted exudate.

Ketoprofen Analysis — KP in serum and pleural exudate was determined using high performance liquid chromatography (HPLC) with a modified procedure of that described by Farizotti and Mahuzier.¹¹ One hundred microliter aliquots of I.S. (EKP) solution ($3 \mu\text{g}/\text{ml}$) were transferred to 30 ml stoppered tubes and evaporated with N_2 gas stream. Then 0.1 ml of serum or 1 ml of diluted pleural exudate, 4 ml of distilled water, 0.1 ml of 70% perchloric acid and 20 ml of diethylether were added to the residue. After mechanical agitation for 5 min, 18 ml of organic phase was washed once with H_2O and evaporated to dryness with N_2 gas stream. Then, 5 ml of 0.1 M AcOH was added to the residue, and the solution was submitted to Sep-Pak cartridge (Waters Associate Ltd.). The car-

tridge was washed with 5 ml of MeOH-0.1 M AcOH (60:40, v/v) and eluted 5 ml of MeOH-0.1 M AcOH (70:30, v/v). The eluate was evaporated *in vacuo*, and the residue was dissolved with about 3 ml of acetone. After filtration of the acetone solution through filter of 0.45 μm of pore size (FM type; Fuji Photo Film Co., Ltd.), the filtrate was dried with N_2 gas stream. To the residue was added 50 μl of MeOH-AcOH (54:46, v/v) and 2–10 μl of this solution was injected into HPLC system: HPLC was performed on a Waters instrument equipped with a 6000 A pump, model U6K universal injector. Detection was carried out with a Shimadzu model SPD-2A UV photometer fixed at 257 nm, with a sensitivity of 0.01 AUFS. Separation was achieved with a solvent system of MeOH-0.1 M AcOH (54:46, v/v) with a constant flow rate of 1 ml/min, using a Radial Pak A cartridge (Waters Associates). Retention times of KP and I.S. were found at 13.5 and 20.4 min, respectively. Calibration curve was constructed by calculating the ratio of the peak height of KP to that I.S., plotting the ratio against the amount (50–1600 ng) of KP added to the blank serum and diluted pleural exudate.

Mepirizole Analysis — Determination of MP was followed, one hundred microliter aliquots of ZM solution (10 $\mu\text{g}/\text{ml}$) were transferred to 30 ml stoppered centrifuge tubes and evaporated with N_2 gas stream. Then 0.2 ml of serum or 0.5 ml of diluted pleural exudate, 10 ml of distilled water, 1 ml of 1 N HCl and 10 ml of cyclohexane were added to 30 ml tube and shaken for 5 min. The tubes were centrifuged at $2500 \times g$ for 5 min and the organic layer was discarded. To the aqueous solution were added 2 ml of 1 N NaOH and 10 ml of benzene and centrifuged at $2500 \times g$ for 5 min. The obtained organic layer was evaporated *in vacuo* and the residue was dissolved with about 3 ml of acetone. After filtration of the acetone solution through filter of 0.45 μm , the filtrate was dried with N_2 gas stream. To the residue was added 50 μl of MeOH-PIC A reagent (Waters Associates Ltd.) (42:58, v/v) and 2–10 μl of this solution was in-

jected into HPLC (instruments were described above) on Radial Pak C_8 cartridge (Waters Associates Ltd.: i.d., 5 mm) and MeOH-PIC A reagent (42:58, v/v) as eluate solvent. Detection was carried out with a Shimadzu model SPD-2A UV photometer fixed at 250 nm, with a sensitivity of 0.01 AUFS. Retention times of MP and ZM were found at 13.7 and 16.8 min, respectively. Calibration curve was constructed by calculating the ratio of the peak height of MP to that of I.S., plotting the ratio against the amount (0.5–10 μg) of MP added to the blank serum and diluted pleural exudate.

Protein Binding — Determination of protein-MP and -DM binding was performed by equilibrium dialysis at 37°C. Dialysis sacs were made from Visking tube (i.d.: 6.4 mm), boiled in 0.5% (v/v) acetic acid, and thoroughly washed and equilibrated with 1/15 M phosphate buffer in saline, pH 7.4. Rat serum was diluted to 20% (v/v) with buffer. A half milliliter aliquots of the sample (DM: 0.2 and 5 $\mu\text{g}/\text{ml}$ containing $2 \times 10^5 \text{dpm}$ ^3H -DM or MP: 1 and 12.5 $\mu\text{g}/\text{ml}$) dissolved with 20% rat control serum (1 ml) was placed inside a dialysis sac. The sacs were dialyzed in 5 ml of 1/15 M phosphate buffer, pH 7.4 for 48 h at 4°C. The levels of DM radioactivity or MP inside and outside the dialysis sacs were measured by scintillation counting or HPLC (instruments were described above) after extraction, respectively.

Determination of Protein Concentration — Protein concentration in serum and pleural exudate was determined according to the method of Lowry *et al.*¹²⁾ with bovine serum albumin as standard.

RESULTS

All drugs were administered *p.o.* 1 h before the intrapleural injection of carrageenin. At 3, 6, 8 and 16 h after carrageenin, pleural exudate and blood were simultaneously collected. DM (0.1 mg/kg) markedly reduced exudate volume and protein content throughout the period of testing after carrageenin (Fig. 1). These inhibitory effects of DM were marked in the relatively late phase

(between 8 to 16 h after carrageenin) than in the relatively early phase (between 3 to 6 h after carrageenin). KP (1 mg/kg) significantly reduced exudate volume for 16 h after carrageenin, but it did not inhibit the rate of exudate accumulation since after 6 h. The time course of protein content in the pleural cavity was similar to that of exudate volume (Fig. 2). MP (100 mg/kg) significantly reduced exudate volume and protein content only at 3 h after carrageenin. Between 6 to 16 h after carrageenin, these were rather augmented with MP (Fig. 3).

Concentration of DM in the exudate was peak at 3 h after carrageenin and then declined with time (Table II). DM concentration in serum was the same as that in exudate throughout the period of testing. The peak content of DM in the pleural cavity was 70.74 ± 8.47 ng at 6 h after carrageenin, and DM content at 16 h

after carrageenin was about 65-fold lower than the peak content (Table III). Content of KP in the pleural cavity and concentration of KP in serum and exudate are shown in Table III. Concentration of KP in exudate was peak at 3 h after carrageenin and then declined with time. Concentration of KP in the exudate was slightly higher than in serum at 3 h, but thereafter was similar to that in serum. At 6 h after carrageenin, KP concentration in serum was 2.23 ± 0.21 $\mu\text{g/ml}$ and that in exudate was 2.25 ± 0.27 $\mu\text{g/ml}$. Content of KP in pleural cavity had a peak at 6 h after carrageenin and then declined gradually.

MP concentration in serum and exudate and MP content in the pleural cavity after carrageenin are shown in Table IV. Concentration of MP

FIG. 1. Time Course of the Inhibitory Effects of Dexamethasone on Exudate Accumulation and Protein Content in the Pleural Cavity after the Intrapleural Injection of Carrageenin in Rats

Dexamethasone (0.1 mg/kg: ●) or vehicle (○) was given p.o. 1 h before carrageenin injection. Each point represents mean $\pm$ S.E.M. obtained from 7 rats.

FIG. 2. Time Course of the Inhibitory Effects of Ketoprofen on Exudate Accumulation and Protein Content in the Pleural Cavity after the Intrapleural Injection of Carrageenin in Rats

Ketoprofen (1 mg/kg: ●) or vehicle (○) was given p.o. 1 h before carrageenin injection. Each point represents mean $\pm$ S.E.M. obtained from 7 rats.

in exudate was the same between 3 and 6 h after carrageenin and declined gradually. MP concentration in serum declined more gradually with time than in exudate. MP content in the pleural cavity was increased up to 8 h ($71.24 \pm 12.09 \mu\text{g}$).

FIG. 3. Time Course of the Inhibitory Effects of Mepirizole on Exudate Accumulation and Protein Content in the Pleural Cavity after the Intrapleural Injection of Carrageenin in Rats

Mepirizole (100 mg/kg: ●) or vehicle (○) was given *p.o.* 1 h before carrageenin injection. Each point represents mean $\pm$ S.E.M. obtained from 7 rats.

To determine the apparent permeability of three drugs to the inflamed space, the ratio of concentration in exudate to that in serum was calculated. As illustrated in Table V, these ratios of all three drugs ranged narrowly from 1.016 to 1.393 at 3 h after carrageenin.

KP did not inhibit the rate of exudate accumulation since 6 h after carrageenin (the relatively late phase), and its concentration in serum and exudate decreased gradually with time. Therefore, it is possible that the failure of KP to show the anti-exudative effect on the relatively late phase may be due to the decrease of KP concentration in exudate below the effective concentration. To determine this possibility, we examined the anti-exudative effect and KP concentration in exudate on twice administration (1 h before and 6 h after carrageenin) of KP in the relatively late phase (at 10 h after carrageenin). Concentration of KP in serum and exudate following twice administration was about 3-fold higher than those after single administration (Table VI) and this concentration was enough to show an anti-exudative effects as judged from the concentration of KP in serum and exudate at 3 h after carrageenin when KP was administered *p.o.* at the anti-inflammatory dose (Table III). However, the exudate volume after twice administration of KP was equal to that by single administration. This result suggests that the reason why KP did not inhibit exudate accumulation in the relatively late phase of carrageenin pleurisy

TABLE II. Dexamethasone (DM) Concentration in Serum and Exudate and Its Content in the Pleural Cavity after the Intrapleural Injection of Carrageenin in Rats

Time after carrageenin (h)	Concentration of DM		Content of DM
	Serum (ng/ml)	Exudate (ng/ml)	Pleural cavity (ng/cavity)
3	119.15 ± 19.17	117.11 ± 9.41	70.03 ± 2.80
6	86.37 ± 12.89	76.67 ± 11.88	70.74 ± 8.47
8	49.67 ± 8.64	30.03 ± 8.88	36.72 ± 6.51
16	2.83 ± 0.83	4.57 ± 1.24	1.19 ± 0.38

Dexamethasone (0.1 mg/kg) was given *p.o.* 1 h before the intrapleural injection of carrageenin. Each value represents mean $\pm$ S.E.M. obtained from 7 rats.

TABLE III. *Ketoprofen (KP) Concentration in Serum and Exudate and Its Content in the Pleural Cavity after the intrapleural Injection of Carrageenin in Rats*

Time after carrageenin (h)	Concentration of KP		Content of KP
	Serum ($\mu\text{g/ml}$)	Exudate ($\mu\text{g/ml}$)	Pleural cavity ($\mu\text{g/cavity}$)
3	2.83 ± 0.27	3.73 ± 0.61	0.72 ± 0.08
6	2.23 ± 0.21	2.25 ± 0.27	2.18 ± 0.23
8	1.78 ± 0.12	1.46 ± 0.02	2.05 ± 0.05
16	0.69 ± 0.07	0.49 ± 0.04	1.23 ± 0.16

Ketoprofen (1.0 mg/kg) was given p.o. 1 h before the intrapleural injection of carrageenin. Each value represents mean $\pm$ S.E.M. obtained from 7 rats.

TABLE IV. *Mepirizole (MP) Concentration in Serum and Exudate and Its Content in the Pleural Cavity after the Intrapleural Injection of Carrageenin in Rats*

Time after carrageenin (h)	Concentration of MP		Content of MP
	Serum ($\mu\text{g/ml}$)	Exudate ($\mu\text{g/ml}$)	Pleural cavity ($\mu\text{g/cavity}$)
3	46.55 ± 9.93	58.44 ± 14.97	23.62 ± 6.39
6	38.48 ± 6.39	56.22 ± 13.12	50.48 ± 7.16
8	42.46 ± 5.04	28.05 ± 2.15	71.24 ± 12.09
16	15.72 ± 4.71	10.42 ± 2.55	30.12 ± 11.46

Mepirizole (100 mg/kg) was given p.o. 1 h before the intrapleural injection of carrageenin. Each value represents mean $\pm$ S.E.M. obtained from 7 rats.

TABLE V. *Concentration Ratios of Each Drug in Exudate to that in Serum at 3, 6, 8 and 16 h after the Intrapleural Injection of Carrageenin in Rats*

Time after carrageenin (h)	Dexamethasone (0.1 mg/kg)	Ketoprofen (1 mg/kg)	Mepirizole (100 mg/kg)
	Exudate/serum ^{a)}	Exudate/serum ^{a)}	Exudate/serum ^{a)}
3	1.016 ± 0.109	1.215 ± 0.085	1.393 ± 0.336
6	0.885 ± 0.049	0.907 ± 0.025	1.798 ± 0.606
8	1.646 ± 0.480	1.030 ± 0.084	0.679 ± 0.050
16	1.564 ± 0.230	0.715 ± 0.084	0.749 ± 0.073

Each drug was administered p.o. 1 h before the intrapleural injection of carrageenin. Drug concentration in exudate and serum at 3, 6, 8 and 16 h after the intrapleural injection of carrageenin in rats was determined as described manner in methods.
a) Concentration ratio of each drug (concentration in exudate/that in serum) was calculated for individual rats and the ratios shown represented mean $\pm$ S.E.M. obtained from 7 rats.

is not its disappearance from the pleural cavity.

We determined the protein binding of DM and MP by the method of equilibrium dialysis.

Bound percents of DM ($0.2 \mu\text{g/ml}$) and MP ($1 \mu\text{g/ml}$) were 28.8 and 8.5%, respectively (Table VII).

TABLE VI. *Exudate Volume and Ketoprofen Levels in Serum and Exudate after Single or Twice Oral Administration of Ketoprofen in Rat Carrageenin Pleurisy*

Number of treatment with ketoprofen	Exudate volume ml	Ketoprofen levels		
		Serum	Exudate	
		$\mu\text{g/ml}$	$\mu\text{g/ml}$	$\mu\text{g/cavity}$
0 (Vehicle)	1.82 ± 0.14	—	—	—
Single	1.18 ± 0.11	1.47 ± 0.14	1.61 ± 0.14	1.80 ± 0.14
Twice	1.18 ± 0.14	4.18 ± 0.22	4.68 ± 0.38	5.35 ± 0.33

Ketoprofen (1 mg/kg) was singly or twice administered p.o. to rats at 1 h before and 6 h after the intrapleural injection of carrageenin in rats.

Detailed timetable was followed:

Each value represents mean $\pm$ S.E.M. obtained from 7 rats.

TABLE VII. *Protein Binding of Dexamethasone and Mepirizole by Equilibrium Dialysis Using 20% Rat Serum Solution in 1/15 M Sodium Phosphate Buffer (pH 7.4) at 4°C*

Compound	Concentration ($\mu\text{g/ml}$)	% binding
Dexamethasone	0.2	28.8 ± 2.8
	5.0	30.4 ± 1.4
Mepirizole	1.0	8.5 ± 2.8
	12.5	9.3 ± 3.1

Results are given as the mean $\pm$ S.E.M. obtained from four or five replicates.

DISCUSSION

Some differences are seen in the clinical effect of steroidal and non-steroidal anti-inflammatory drugs. Fukuhara and Tsurufuji¹³⁾ have reported a substantial difference in the mode of action of these two types of drug on the rat carrageenin granuloma. In carrageenin-induced pleurisy in rats, the qualitative difference was reported to exist between the anti-exudative activity of steroidal anti-inflammatory drugs and that of non-steroidal anti-inflammatory drugs on a relatively late phase.^{6,7)} Steroidal anti-inflammatory drugs,

but not non-steroidal anti-inflammatory drugs, showed an anti-exudative activity on the relatively late phase in carrageenin pleurisy while both types of drug showed an anti-exudative activity on the relatively early phase.

In the present study, we investigated whether this difference in the anti-exudative activities of two types of drug was ascribed to the change of drug concentration in exudate or the difference in their intrinsic anti-inflammatory activities. In the preliminary experiment, the doses of drugs used here were determined in such a way as to

show submaximal anti-exudative activities at 3 h after carrageenin in rat carrageenin pleurisy. KP and MP did not inhibit the rate of exudate accumulation until 6 and 3 h after carrageenin, respectively. DM, however, reduced exudate volume throughout the period of testing after carrageenin, and its anti-exudative effect was marked in the relatively late phase than in the relatively early phase. Furthermore, ^{125}I -BSA was administered *i.v.* 6 h after the intrapleural injection of carrageenin, DM, but neither KP nor MP, reduced vascular permeability of ^{125}I -BSA into the pleural cavity at 9 h after carrageenin (the relatively late phase, unpublished work; data not shown).

In the present study, MP showed an anti-exudative effect at 3 h after carrageenin but not at 6 h, although the same concentration of MP was achieved in the pleural exudate at 3 and 6 h after carrageenin (Fig. 3). Therefore, that MP did not show any anti-exudative effect at 6 h after carrageenin is not due to the decrease of its concentration in exudate below the effective concentration.

KP showed an anti-exudative effect for 6 h after carrageenin, but not thereafter. Concentration of KP in serum and exudate decreased gradually with time. However, at 10 h after carrageenin (the relatively late phase), the twice administration of KP resulted in an increase in its exudate concentration enough to show an anti-inflammatory effect, but did not result in any more anti-exudative effect than the single administration of KP. This result suggests that the failure of KP to show the anti-exudative effect on the relatively late phase is not due to the decrease of KP concentration in the exudate below the effective concentration, and supports the previous inferences that a qualitative differences between anti-inflammatory activity of steroidal anti-inflammatory drug and that of non-steroidal anti-inflammatory drug is present in the relatively late phase of carrageenin pleurisy.

On the other hand, in spite of the gradual decline of DM concentration in the pleural exudate with time and its actual loss at 16 h after carra-

geenin, DM showed an anti-exudative effect throughout the period of testing after carrageenin which was more marked in the relatively late phase than in the relatively early phase. This phenomenon in the relatively late phase may be explained by the hypothesis that the anti-exudative action of DM in the relatively late phase of carrageenin pleurisy may be at least in part mediated by endogenous substance(s) formed through gene expression.⁷⁾

Graf *et al.*¹⁴⁾ reported that acidic non-steroidal anti-inflammatory drugs accumulated specifically in the inflamed tissue, but non-acidic congeners did not show such behavior. They speculated that this phenomenon might be due to the two common physico-chemical characteristics of acidic non-steroidal anti-inflammatory drugs, namely their unusually high degree of binding to plasma protein *in vivo* and their low pK_a value. The ability of drugs to bind to plasma proteins may be participated in elevated concentrations of drugs in the inflamed tissue because extravasation of plasma proteins and so concomitant extravasation of drugs bound to these proteins occurred in inflammation.

In the present study, however, in spite that the values of protein binding were varying widely from 8.5 to 69.6% in three anti-inflammatory drugs based on our results and the report that protein binding of KP was 69.6%,¹⁵⁾ the ratios of the drug concentrations in exudate to those in serum ranged narrowly from 1.016 to 1.393 at 3 h after carrageenin. Therefore, in this inflammatory model anti-inflammatory drugs accumulated in the inflamed pleural cavity independently of their ability to bind to plasma proteins and the drug concentrations were essentially identical to the serum and pleural exudate, implying complete equilibrium.

It is concluded that the qualitative difference in the anti-exudative effect observed between KP and MP in the relatively early phase of carrageenin pleurisy, and between steroidal anti-inflammatory drug and non-steroidal one in the relatively late phase. These differences in the

anti-exudative effects can not be explained by the change in the drug concentration in the inflamed tissue, but reflect the difference in their intrinsic anti-inflammatory activities.

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