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Rectal Delivery of Antiinflammatory Drugs. IV.[1] Effect of Amino Acids on the Change in the Rectal Mucosa induced by Diclofenac Sodium

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The effect of the concurrent administration of amino acid(s) with diclofenac sodium on mucosal damage was investigated in rats. An administration of the base alone (Witepsol H-15) induced an only slight desquamation of epithelial cells at the luminal border, without any other changes in the mucosa. However severe histological damage was observed after administration of diclofenac sodium in Witepsol H-15. On the other hand, the concurrent administration of diclofenac sodium with L-form amino acids decreased the damage to the rectal mucosa. The L-forms of methionine and phenylalanine were the most effective, but in the protective effect of their D-forms on the rectal mucosa was much less.

Rectal absorption of diclofenac sodium was investigated when it was administered with amino acids in rabbits, dogs and rats. The absorption was not influenced by the concurrent administration of amino acid(s).

Keywords——diclofenac sodium; amino acids; suppository; histology; rectal absorption; amino acid analyzer; species differences

It is well known that acidic non-steroidal antiinflammatory (NSAI) drugs may damage the mucosa of the gastrointestine as well as the rectal mucous membrane in man and experimental animals.[3] Recently, extensive efforts to reduce the undesirable effects of NSAI drugs have made, especially for oral delivery preparations. Among them, the concomitant administration of some amino acids has successfully reduced the gastric irritation induced by NSAI drugs.[4] In a series of studies on rectal delivery of drugs, [1,5] we also showed that the salts of diclofenac with some kinds of basic amino acids were less irritative to the rectal mucosa than the parent drug or its sodium salt. Thus, their salts of basic amino acids may be useful for the clinical application of acidic NSAI drugs as suppositories without severe side effects on the rectal mucosa. The present investigation was undertaken to study the effects of several kinds of amino acids incorporated in various amounts into the suppository on the inhibitory activity against the irritative damage induced by diclofenac sodium (DCNa) in the rectal mucosa. The species difference in these effects was also investigated.

Experimental

Materials——DCNa was obtained from Nippon Bulk Yakuhin Co., Ltd. and recrystallized from water. Glycine (Gly), L-alanine (L-Ala), L-leucine (L-Leu), L-isoleucine (L-Ile), L-methionine (L-Met), D-methionine (D-Met), L-asparagine (L-Asp·NH_2) L-glutaminic acid (L-Glu), L-glutamine (L-Glu·NH_2), L-arginine (L-Arg) and its hydrochloride (L-Arg·HCl), L-lysine (L-Lys) and its hydrochloride (L-Lys·HCl), and L-phenylalanine (L-Phe) were obtained from Yashima Pure Chemicals Industries Co., Ltd. and D-Arg·HCl, D-Lys·HCl, D-Phe, and D-Ile were from Sigma Chemicals Co., Ltd. (Saint Louis, Missouri, U.S.A.). Other reagents and solvents of analytical reagent grade were also obtained commercially and used without further purification.

Preparation of Suppositories——Suppositories were prepared by the fusion method[5] and used within 1 or 2 d. The suppositories were formulated so as to contain 5% DCNa and, if necessary, various amounts of amino acids in Witepsol H-15 (Dynamit Nobel A.G., Chemishe Werke, West Germany) per one gram.

Study of Rectal Absorption——Male beagle dogs weighing 10—12 kg, male albino rabbits weighing 2.5—3.0 kg, and male Wistar rats weighing 230—250 g were used. The procedures for rectal administration, collection of blood sample, and assay of DC in the plasma were reported in the previous paper.[5]

Histological Study of Rectal Mucosa——Male Wistar rats were used. Fifty milligrams of a suppository were administered into the rectum, and the anus was glued shut with Alon Alpha (Toa Gosei Kagaku Co., Ltd.). At one h after the administration, animals were decapitated. The rectum (2.0 cm length) including the anus was removed and preparations of rectal segments for microscopic observations were made by the same methods as described in the previous paper.[4]

Measurements of Free Amino Acids in the Rectal Tissue——Male Wistar rats were used. Animals were fasted for 24 h prior to drug administration, and the bowel contents that remained in the lower rectum were evacuated by pressing the abdomen. Fifty milligrams of a suppository were administered into the rectum, then the anus was lightly moistened with water, and glued with Alon Alpha (Toa Gosei Kagaku Co., Ltd.). At one h after the administration, animals were sacrificed by decapitation. The rectum (2.5 cm length) including the anus was removed, incised, and carefully spread on a silicone plate. The mucosa was rinsed with 10 volumes of 75% ethanol. Then the sphincter ani was removed and the residual tissue was carefully dried by pressing it between filter papers. The tissue sample was weighed, placed in an aliquot of 60% aqueous trichloroacetic acid solution and homogenized for 1 min at 0°C in an Ultra-Tarrax homogenizer (Junke and Kunkel GmbH K.G. IKA-Werk, West Germany). The homogenate was centrifuged for 10 min at 3000 rpm. After the extraction of trichloroacetic acid in the supernatant with ethyl ether three times, the aqueous layer was freeze-dried. An aliquot of the freeze-dried preparation was dissolved in 1 N HCl solution and the content of free amino acids was analyzed by employing a Hitachi high performance amino acid analyzer model 835 (Hitachi Seisakusho Co., Ltd.).

Results and Discussion

Effect of Amino Acids on the Damage to the Rectal Mucosa induced by Diclofenac Sodium

In the previous paper,[5] it was demonstrated that DCNa induced strong irritation at the rectal mucosa after administration as a suppository. In the present investigation, the concurrent administration of amino acid (s) with DCNa was tried in the hope of reducing mucosal damage (s) in rats. Suppositories were prepared with Witepsol H-15 as a base so as to contain 0.157 mmol (50 mg) of DCNa and 0.628 mmol (4-fold molar excess over DCNa) of amino acids per gram of suppository (this is referred to as 1: 4 molar preparation). Histological observations made at 1 h after the treatment are presented in Table I. The changes in mucosa

TABLE I. Histological Findings in the Rectal Mucosa after the Administration of Several Kinds of Suppositories containing DCNa and L- or D-Amino Acids to Rats

Observation	H-15[a]	DCNa	Arg·HCl		Lys·HCl		L-Lys·Acet.	Met		Phe		L-Leu	L-His	L-Gly	L-Asp	L-Glu	L-Glu·NH_2
			L	D	L	D		L	D	L	D						
Mucosa																	
DEC	±	##	+	##	+	##	+	+	+	+	##	+	+	##	+	+	##
ND	−	+	+	##	+	+	+	±	+	±	##	+	+	+	+	+	+
HY	−	##	+	+	+	##	±	−	+	±	##	+	+	+	+	±	+
HE	−	##	±	+	±	+	+	−	+	−	+	±	±	+	−	−	−
ICI	−	+	±	+	±	+	+	±	+	−	+	±	+	±	±	±	±
Submucosa																	
Edema	−	##	+	+	±	##	+	−	+	±	+	+	+	+	−	+	−
HE	−	+	±	+	−	+	±	−	+	−	+	±	±	+	±	±	±
ICI	−	+	+	##	+	##	±	±	+	±	##	+	±	±	±	±	±
n	3		4	4		4	4	6	4	6	4	4	4	4	4	4	4

DEC: desquamation of epithelial cells, ND: necrosis of degradation, HY: hyperemia, HE: hemorrhage, ICI; inflammatory cell infiltration. ++: severe irritation, +; moderate irritation, ±: slight irritation, −: no irritation.

a) Suppository base alone: Witepsol H-15.
The dose of suppository was 50 mg/animal.

and submucosa are classified into four degrees (卄, +, ±, and −) according to the method of Itoh[6] with some modifications. Representative microphotographs after the administration on the following suppositories are shown in Fig. 1: Witepsol H-15 alone (Fig. 1A), the preparation containing DCNa in Witepsol H-15 (Fig. 1B) and those containing DCNa and L- or D-Met in Witepsol H-15 (Fig. 1C and D). The administration of the base alone (Witepsol H-15 group in Table I and Fig. 1A) induced only slight desquamation of epithelial cells at the luminal border (①→), but no other changes were observed in the mucosal and submucosal layer. This means that the rectal membrane was hardly affected by the base alone. On the other hand, DCNa seriously damaged the rectal tissue. The main histological changes induced in the mucosa and submucosa were desquamation of epithelial cells (②→), hyperemia of capillaries (③→), hemorrhage (④→), inflammatory cell infiltration and edema (Fig. 1B). The severity of these histological changes induced by DCNa was dramatically reduced by the addition of L-form amino acids. Among them, L-Met and L-Phe were the most effective. Fig. 1C, a photograph of a case given L-Met, shows almost normal histological findings except for a slight desquamation of epithelial cells (⑤→). It has been reported that L-Met or some other amino acids can significantly decrease gastric mucosal damage induced by the oral

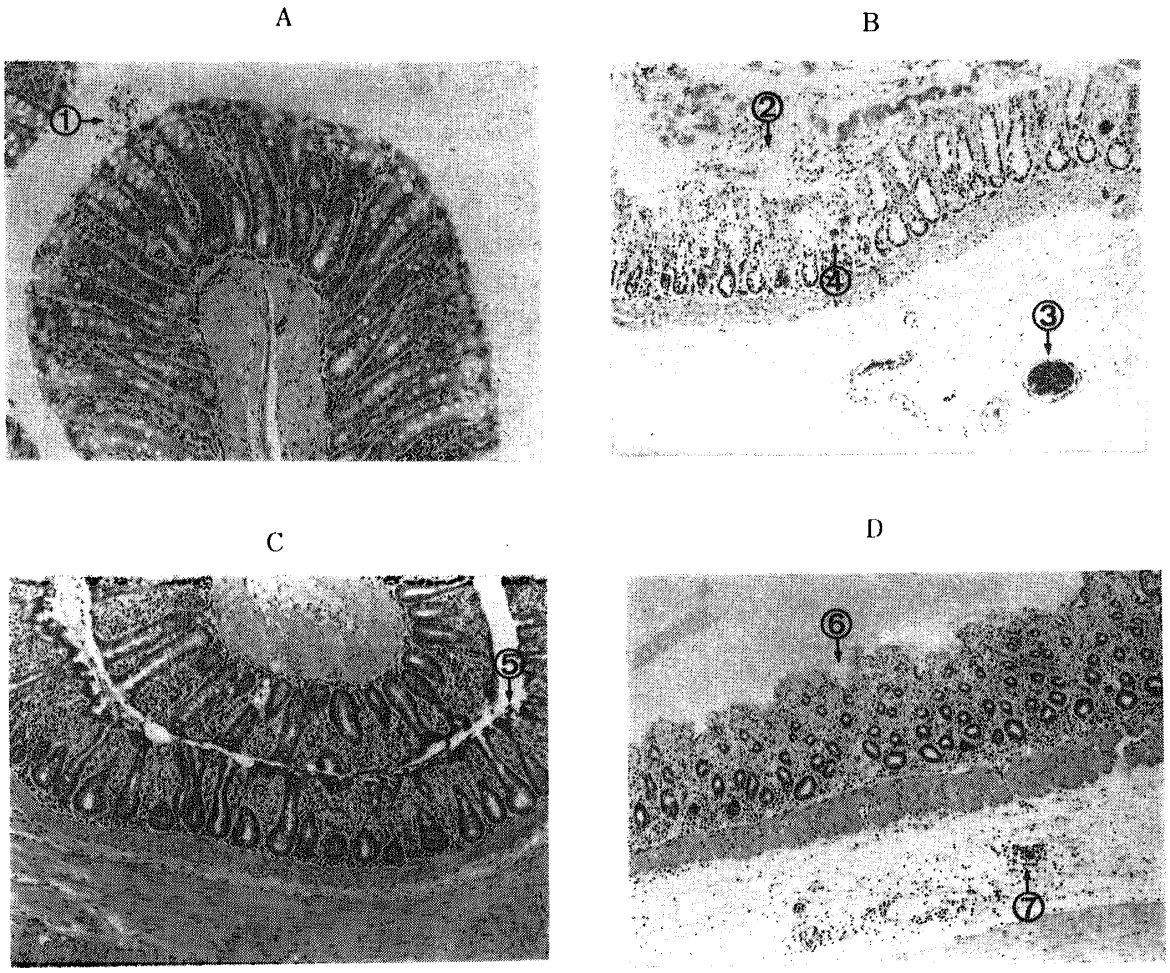

Fig. 1. The Histological Features of the Rectal Mucosa of Rats at 1 h after the Administration of Various Suppositories (×25)

A: suppository base (Witepsol H-15) alone.
B: DCNa alone suppository.
C: DCNa and L-Met suppository (1: 4 molar preparation).
D: DCNa and D-Met suppository (1: 4 molar preparation).
The dose of suppository was 50 mg/animal.

administration of acidic NSAI drugs such as aspirin.[4,7] Another report has claimed that L-Glu has a pronounced protective effect against gastric lesion induced by NSAI drugs.[7] However, it has not been reported hitherto that these amino acids have a protective effect against the damage induced by NSAI drugs on the rectal membrane. The protective effects of D-form Met, Phe, Arg·HCl and Lys·HCl were also compared histologically. It is very interesting that the D-forms were much less effective than the L-forms as shown in Table I and Fig. 1D. The severity of histological changes such as desquamation of epithelial cells (⑥→), hyperemia of capillaries (⑦→) and edema was similar to that with DCNa alone, indicating that the damage was hardly reduced by the coexistence of D-form amino acids in a suppository. The reasons why amino acids had such protective effects on the rectal mucosa and why there were distinct differences between D- and L-forms are not clear at present. It may be presumed that the metabolic disorders induced by DCNa in mucosal cells were mitigated by amino acids contained in the suppository. This will be discussed later from the viewpoint of amino acid contents in the rectal mucosa.

Table II. Histological Findings in the Rectal Mucosa after the Administration of Suppositories containing DCNa and Various Concentrations of L-Amino Acids to Rats

Observation	L-Met		L-Phe	
	1:1	1:10	1:1	1:10
Mucosa				
DEC	+	++	++	++
ND	++	++	++	+
HY	+	+	+	+
HE	+	+	±	+
ICI	+	+	+	+
Submucosa				
Edema	++	++	++	+
HE	+	+	+	+
ICI	+	+	+	+
n	4	4	4	4

DEC, ND, HY, HE, ICI, ++, +, ±, and − are the same as in Table I. 1:1 and 1:10 are 1:1 molar and 1:10 molar preparations, respectively.
The dose of suppository was 50 mg/animal.

In order to determine the optimal content of amino acid to be added to the DCNa suppositories, the relative concentrations in the preparations were changed variously and the histological changes were observed at 1 h after administration to rats. Suppositories were prepared so as to contain 0.156 mmol (50 mg) of DCNa and 0.157 mmol (1:1 molar preparation) or 1.57 mmol (1:10 molar preparation) of amino acids per gram of suppository. The dose of DCNa was the same as in the case of the experiment mentioned above. The results are presented in Table II as histological findings and Fig. 2 shows the microphotographs. Table II shows that the damage to rectal mucosa induced by 1:1 and 1:10 molar preparations was more serious than that by 1:4 moler preparations for all amino acids studied. Fig. 2 shows microphotographs at 1 h after the administration of 1:1 and 1:10 molar preparations of DCNa and L-Met. The histological changes in the cases of both 1:1 molar preparation and 1:10 molar preparation suggested enhanced damage to the rectal membrane. The weaker protective or reverse effects of 1:1 and 1:10 molar preparations could not be explained in terms of the results of dissolution tests because the dissolution rates of DCNa were not influenced by the presence of amino acids.

In order to clarify the reasons for the difference between L- and D-forms in protective

effect against the rectal membrane damage observed in the experiments mentioned above, the contents of amino acids in the rectal tissue were measured in rats at 1 h after the treatment. The amounts of neutral and basic amino acids are shown in Figs. 3 and 4, respectively. When suppositories containing DCNa alone were administered, the contents of all amino

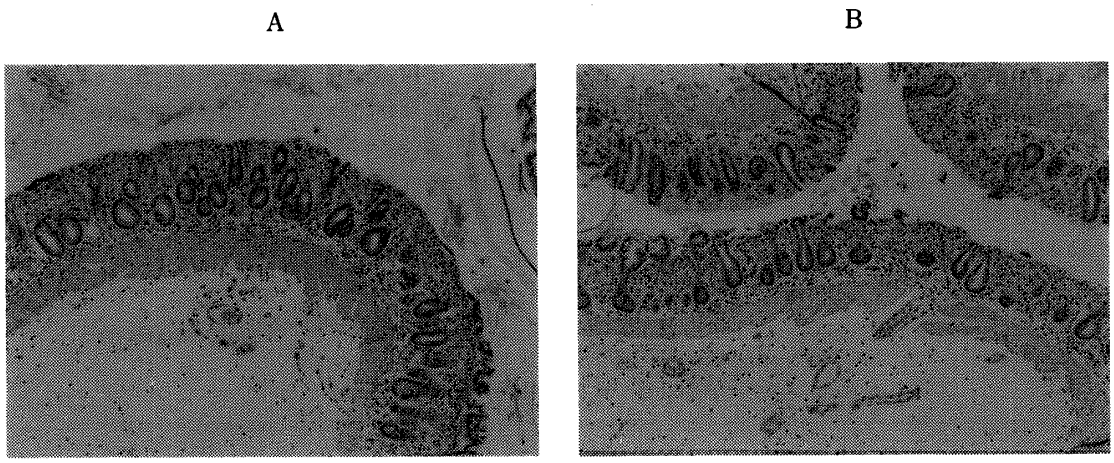

Fig. 2. The Histological Features of the Rectal Mucosa of Rats at 1 h after the Administration of Suppositories containing DCNa and L-Met (×25)

A: 1:1 molar preparation.
B: 1:10 molar preparation.
The dose of suppository was 50 mg/animal.

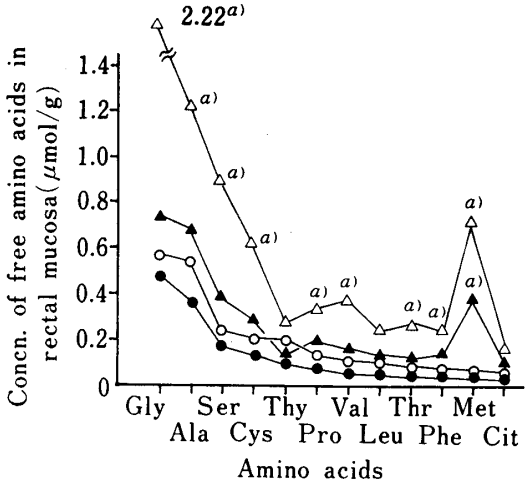

Fig. 3. Aminogram of Neutral Amino Acids in the Rectal Tissue of Rats at 1 h after the Administration of Various Suppositories

—○—: control untreated.
—●—: DCNa alone suppository.
—△—: DCNa and L-Met suppository (1:4 molar preparation).
—▲—: DCNa and D-Met suppository (1:4 molar preparation).
The dose of suppository was 50 mg/animal.
Each point represents the mean of four animals.
a) $p<0.05$ significant difference from control.

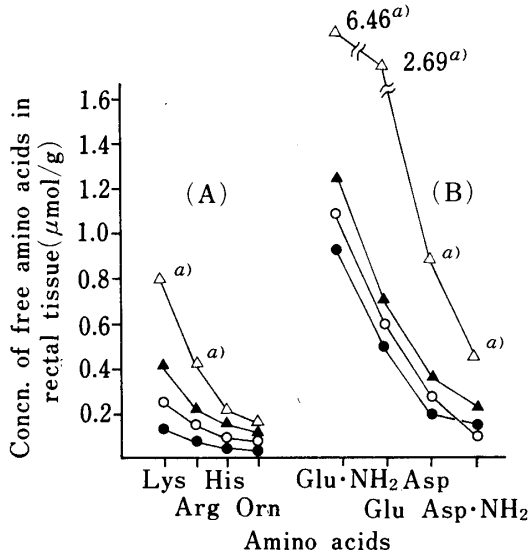

Fig. 4. Aminograms of Basic (A) and Acidic (B) Amino Acids in the Rectal Tissue of Rats at 1 h after the Administration of Various Suppositories

—○—: control untreated.
—●—: DCNa alone suppository.
—△—: DCNa and L-Met suppository (1:4 molar preparation).
—▲—: DCNa and D-Met suppository (1:4 molar preparation).
The dose of suppository was 50 mg/animal.
Each point represents the mean of four animals.
a) $p<0.05$ significant difference from control.

acids in the rectal tissue were found to decrease (*cf.* Fig. 3 and 4). This meant that considerable amounts of free amino acids were lost as a result of the atrophy of mucous cells which was previously ascertained by an electron microscopic study.[8] However, on coadministration of both forms of methionine, the content of all amino acids tended to increase (*cf.* Fig. 3). Furthermore, acidic amino acids were greatly influenced by the coadministration of either form of methionine. It is very interesting that the increase in many kinds of amino acids in rectal tissue induced by L-Met was markedly larger than that by D-Met. Rainsford *et al.*[9] reported that the concurrent administration of L-form amino acids reduced the degree of damage induced by the oral administration of NSAI drugs on the gastric mucosa, and the protective action has been explained in terms of the ability of L-amino acids to mitigate the metabolic disorder induced by NSAI drugs in mucosal cells. The same explanation may be applicable in the present case. Furthermore, the present findings that D-Met did not show the protective effect in spite of its good uptake into rectal epithelial cells is in good accordance with the well known fact that L-forms of amino acids are utilized exclusively in mammalian metabolism. Thus, the protective effect of L-form amino acids against the barrier damage may be considered to be a result of a normalizing effect on the metabolic function in mucosal cells. However, further work is necessary to elucidate the precise mechanism (s) involved.

Influence of Amino Acids on Rectal Absorption of Diclofenac Sodium

Rainsford *et al.*[10] reported that the concurrent oral administration of aspirin with amino acids reduced its therapeutic efficacy because the amino acids inhibited aspirin absorption though the adjuvant provided moderate protection against the membrane damage induced by aspirin. In the above-mentioned experiments, amino acids incorporated into a DCNa suppository were also proved to damage to the rectal mucosa by NSAI drug. However, if the bioavailability of DCNa is lowered by their addition, the practical significance of this finding would be much less. Thus, the absorption of DCNa through the rectum was measured after joint administration with amino acids in rabbits. The rectal absorption of DCNa after the administration of 1 : 4 molar preparations is presented in Table III, in which drug absorption is shown by the values of the area under the plasma concentration-time curve during 0—120 min (AUC_{0-120}). The control value was obtained by the administration of a suppository with DCNa alone. These results indicate that absorption of DCNa was not influenced by the concurrent administration of amino acids. In another experiment on rabbits, the relative

TABLE III. Influence of Amino Acids on Rectal Absorption of DCNa in Rabbits

Amino acid	AUC_{0-120} of DCNa	
	L-Form	D-Form
Arg·HCl	337±59	288±69
Lys·HCl	330±59	309±36
Met	317±35	330±37
Phe	317±26	316±37
Leu	344±80	303±98
Gly	298±19	
Ile	354±60	
Asp	294±58	
Glu	343±28	
Glu·NH_2	312±62	
Control[a]	347±64	

Each suppository was the 1: 4 molar preparation.
Each value represents the mean±S.D. of six animals.
The dose of DCNa was 2.3 mg/kg.
a) DCNa alone suppository.

concentrations of amino acids such as L-Met and L-Phe in a suppository were variously changed and the absorbed amounts of DCNa were determined. The absorption of DCNa was not influenced by the changes in the concentrations of amino acids, as can be seen from Fig. 5. This indicated that DCNa could apparently be absorbed independent of the condition of the rectal mucosa, because DCNa possesses a high affinity for lipid, favoring its permeation through the rectal mucosa, as shown in the previous paper.[11]

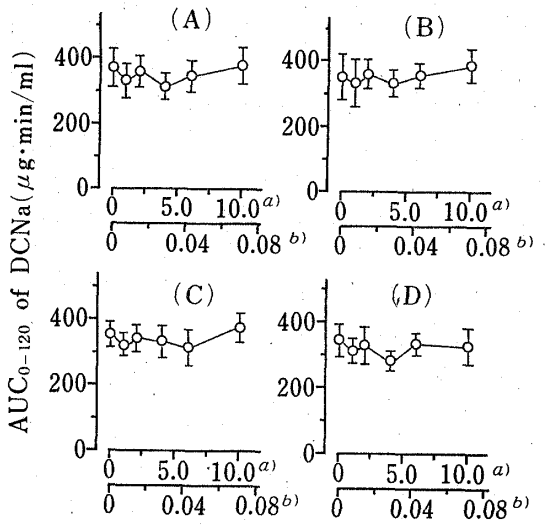

Fig. 5. AUC_{0-120} of DCNa after the Administration of Suppositories containing 5% DCNa and Various Concentrations of L-Amino Acids in Rabbits

A: DCNa and L-Met.
B: DCNa and L-Phe.
C: DCNa and L-Arg. HCl.
D: DCNa and L-Glu.
The dose of DCNa was 2.3 mg/kg.
Each point represents the means ± S.D. of six animals.
a) Molar ratio of amino acid against DCNa
b) Dose of amino acid (mmol/kg)

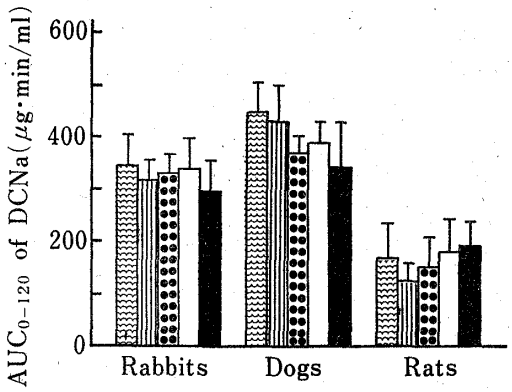

Fig. 6. Species Difference in the Absorption of DCNa after the Administration of Suppositories

▦: DCNa alone suppository.
▤: DCNa and L-Met suppository (1: 4 molar preparation).
▥: DCNa and L-Phe suppository (1: 4 molar preparation).
□: DCNa and L-Arg·HCl suppository (1: 4 molar preparation).
■: DCNa and L-Glu suppsository (1: 4 molar preparation).
The dose of DCNa was 2.3 mg/kg.
Each point represents the mean ± S.D. of six animals.

There have been no reports on the systematic investigation of species differences in rectal drug absorption. In some preliminary experiments, we found that the rectal absorption of several drugs in rabbits was much easier and greater than that in dogs or human. Thus, it is neceassary to investigate the absorbability of DCNa when it is given rectally alone or with amino acids in various animal species. The results in the case of rabbits were identical with those described above. An additional experiment was carried out by using dogs and rats. The results obtained when the suppositories of 1: 4 molar preparation were rectally administered to dogs and rats are presented as a histogram of AUC_{0-120} in Fig. 6 and their time courses of plasma concentration of DCNa are presented in Fig. 7. The coexistence of various kinds of amino acids hardly affected the rectal absorption of DCNa or its elimination pattern in dogs and rat, as in the case of rabbits.

From these results, it may be concluded that the concurrent incorporation of amino acids, especially L-Met or L-Phe, into the rectal preparation of DCNa is very favorable for clinical use without undesirable side effects. The details of the mechanism (s) operating in the protective action of amino acids against rectal membrane irritation by DCNa remain to be clarified at present, and further investigations are in progress in our laboratory.

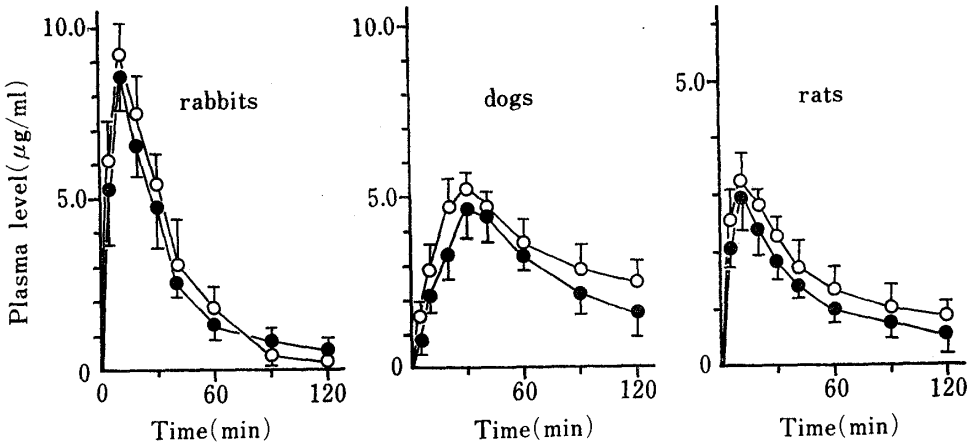

Fig. 7. Time Courses of Plasma Level of DCNa after the Administration of Various Suppositories to Several Species

—○—: DCNa alone suppository.
—●—: DCNa and L-Met suppository (1: 4 molar preparation).
The dose of DCNa was 2.3 mg/kg.
Each point represents the means ± S.D. of six animals.

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