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Rectal Delivery of Antiinflammatory Drugs. III.[1] Effect of Basic Amino Acid Salts of Diclofenac on the Rectal Absorption of Ampicillin Sodium[2]

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The effects of basic amino acid and sodium salts of diclofenac on the rectal absorption process of ampicillin sodium were studied. All diclofenac salts enhanced the permeation of ampicillin sodium through the membrane in rabbits, rats and dogs. The absorption of ampicillin sodium sigmoidally increased with increase of the concentration of diclofenac salts and the maximum plasma level was attained at 1.5% incorporation for all salts. The permeation of ampicillin sodium increased in the following order; arginine salt<histidine salt<lysine salt≪sodium salt. This order did not change with the animal species studied. From the results of repeated administration experiments, it was found that the leakiness of the barrier induced by diclofenac sodium recovered to the normal condition within about 20 h after the administration, but in the case of diclofenac lysine, the membrane returned to the normal state within only about 6.5 h.

Keywords——diclofenac; lysine; arginine; histidine; ampicillin; suppository; rectal absorption

The use of rectal suppositories has been recommended for drugs having irritative effects on the gastrointestinal tract following oral administration.[3] In spite of many reports[4] concerning the local irritation of the rectal membrane, nonsteroidal antiinflammatory (NSAI) drugs have been clinically used as suppositories.

In the previous papers,[1,5] it was demonstrated that the basic amino acid salts of diclofenac (DC) were rapidly absorbed from the rectum at a rate comparable or superior to that obtained by oral administration of the sodium salt of DC (DCNa), and these salts significantly reduced the local irritation in comparison to DC or DCNa.

Extensive efforts toward reducing the undesirable side effects of NSAI drugs are currently being made, especially for oral delivery preparations. For example, the concomitant administration of some amino acids, including basic ones, successfully reduced the gastric lesions induced by the NSAI drugs.[6] Thus the basic amino acid salts of acidic NSAI drugs are considered to be potential candidates for clinical application as suppositories without marked side effect on the rectal mucosa.

The present study was performed to confirm the effects of basic amino acid salts of DC on the rectal absorption of water-soluble materials. As a water-soluble material, ampicillin sodium (ABPCNa) was used because of its poor absorptivity from the rectum. The results were compared with the histological findings reported in the previous paper.[1]

Experimental

Materials and Procedure of Suppository Preparation——These were the same as those reported previously.[1] The suppositories were prepared by the hot-melt method using a lipophilic base, Witepsol H-15 (Dynamit Nobel A.G., West Germany). They were formulated to contain 10% ABPCNa and various concentrations of a DC salt. The concentration of DC salts was expressed in percent equivalent to DCNa.

***In Vivo* Studies**——Adult male rabbits weighing 2.3—2.5 kg, adult male beagle dogs weighing 10—12 kg, and adult male Wistar rats weighing 230—250 g were used. Animals were kept fasting for 24 h before the experiments, but water was given freely. Administration of suppositories, blood sample collection, and the

analytical method were as reported previously.[1] The dose of ABPCNa was fixed at 15 mg/kg for all experiments.

To study the duration of barrier leakiness to ABPCNa induced by DC salts, suppositories containing ABPCNa and 1.5% DCNa or 1.5% lysine salt (DC-Lys) in terms of DCNa were rectally administered to rabbits. Then, suppositories containing ABPCNa alone were administered at 1-h intervals after the initial administration. As a control experiment, suppositories containing ABPCNa alone were administered at 1-h intervals.

Results and Discussion

To compare the effects of basic amino acid salts of DC and that of DCNa on the rectal absorption of ABPCNa, the values of area under the plasma concentration of ABPC-time curve during 0—120 min (AUC_{0-120}) were plotted against the concentration of DC salts in terms of DCNa in the suppository (Fig. 1).

The permeation of ABPCNa sigmoidally increased with increase of the concentration of NSAI drugs and reached plateau levels at 1.5% incorporation in all cases studied. The AUC_{0-120} of ABPC in the presence of DCNa was larger than that in the presence of various amino acid salts of DC. From the findings reported by others,[7] it is reasonable to speculate that, in this case, the diclofenac acid anion may be responsible for making the barrier leaky. In the present experiment, it was first found that formation of amino acid salts of diclofenac acid decreased the membrane damage, although the absorption of diclofenac acid was scarcely affected, as seen in Fig. 3A and B. Consequently, the injurious effect of the carboxylic anion appears to be influenced significantly by the coexisting counter cation. The plateau levels decreased in the order of sodium>L-lysine>L-histidine>L-arginine. This order is the same as that obtained from the results of histological changes reported in the previous paper.[1] Thus, it may be concluded that the enhanced rectal absorption of ABPCNa is attributable

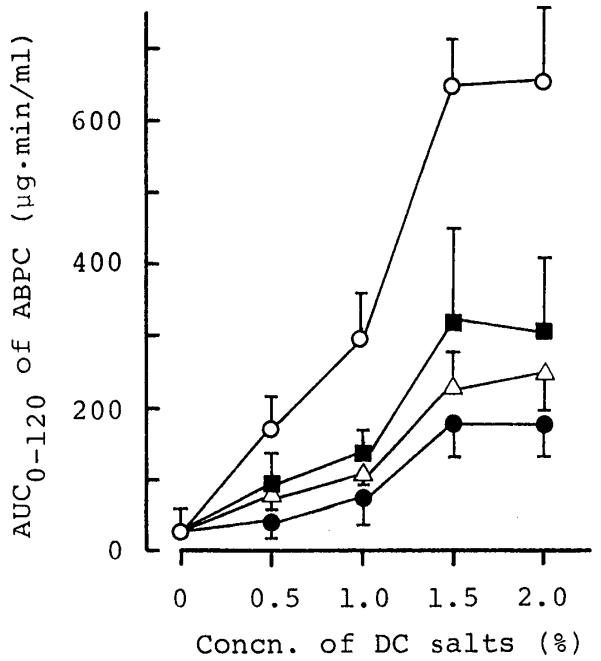

Fig. 1. AUC during 0—120 Min for ABPC following Administration of Suppositories containing 10% ABPCNa and Various Concentrations of DC Salts in Terms of DCNa in Rabbits

—○— ; DCNa, —■—; DC-Lys, —△— ; DC-His, —●— ; DC-Arg.
Dose of ABPCNa; 15 mg/kg.
Each point represents the mean±S.D. of six animals.

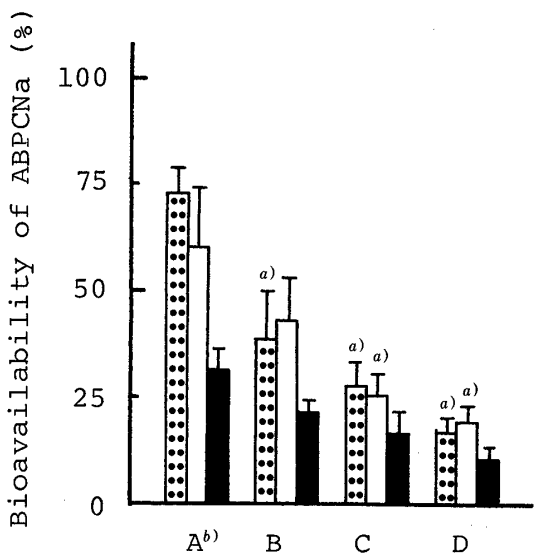

Fig. 2. Comparison of Bioavailability of ABPCNa following Administration of Suppositories containing 10% ABPCNa and Four Kinds of DC Salts (1.5% in Terms of DCNa) in Rabbits, Rats and Dogs

▦ ; rabbits, □ ; rats, ■ ; dogs.
A; DCNa, B; DC-Lys, C; DC-His, D; DC-Arg.
Dose of ABPCNa; 15 mg/kg.
Each histogram represents the mean±S.D. of five or six animals.
a) $p<0.05$ Significant difference from the result for DCNa.
b) $p<0.05$ Significant difference between rabbits or rats and dogs.

to an irritative or inflammatory effect of DC present at a rather high concentration in the rectal fluid. For all experiments, the peak plasma levels of ABPC were observed early, at 10—15 min after the administration, suggesting a rapid change in the mucosal barrier. A good linear correlation was obtained between the peak plasma level, C_{max} and the values of AUC_{0-120} (Eq. 1); $C_{max}=0.0265\ AUC_{0-120}+1.566$ ($n=16$, $r=0.958$, $s=2.066$) Eq. 1 where n is the number of experiments, r is the correlation coefficient, and s is the standard deviation.

From these results, it may be considered that the change in the rectal barrier making it leaky to ABPCNa or other water-soluble materials is induced at an early stage after the administration, and the degree of leakiness depends upon the concentration of DC anion and the kind of counter ion.

In another experiment, the enhanced absorption of ABPCNa induced by DC salts was examined in three species of animal. The concurrent administration of DC salts was fixed at 1.5% equivalent to DCNa, which was the miniumum concentration required to attain the maximum absorption of ABPCNa in rabbits. Fig. 2 shows the relative bioavailability with respect to intravenous administration of ABPCNa at the same dose plotted against the AUC_{0-120} in each animal species. It should be noted that ABPCNa permeates through the rabbit rectum without DC salts to give a bioavailability of about 3%. Still, in general, the promoting efficacy of DC salts in dogs was less than in rats and rabbits.[1] This result is supported by the results of Itoh[8] that the histological changes in rectal mucosa induced by phlogistic materials such as citric acid were qualitatively similar in the three animal species, but a 4-fold greater dose was required in dogs to induce the same degree of irritation as in rabbits and rats, suggesting that the mucosal barrier in dogs is rather strong.

Experiments were also performed in order to study how long it took for the barrier leakiness induced by DC salts to return to the normal state. The degree of damage of the rectal mucous membrane was thought to be correlated with the absorbability of ABPCNa administered.

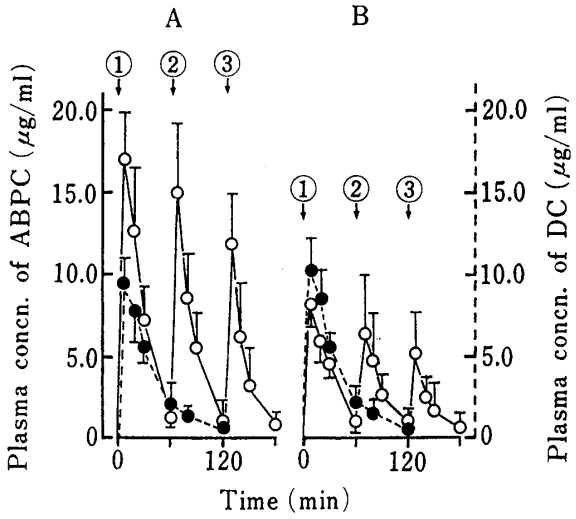

Fig. 3. The Time Course of Plasma Concentration following Repeated Administration of Suppositories Containing ABPCNa with DC Salts and ABPCNa Alone in Rabbits

—○—; ABPC, --●-- ; DC.
A ; After the suppository containing 10% ABPCNa and 1.5% DCNa had been given at first administration (①), the second and third suppositories containing 10% ABPCNa alone were administered at intervals of 60 min (②,③).
B; After the suppository containing 10% ABPCNa and DC-Lys of 1.5% in terms of DCNa had been given at first administration (①), the second and the third suppositories containing 10 % ABPCNa alone were administered at intervals of 60 min (①,②).
Each point represents the mean±S.D. of eight animals.

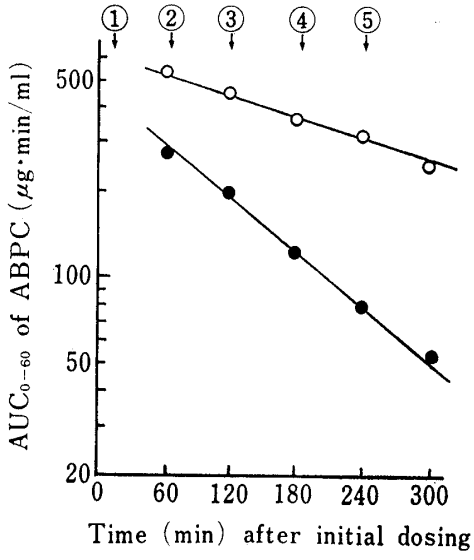

Fig. 4. Relationship between the Values of AUC_{0-60} of ABPC and Time after Initial Dosing in Repeated Administration Experiments in Rabbits

After the suppository containing 10% ABPCNa and 1.5 % DCNa (—○—) or DC-Lys of 1.5% in terms of DCNa (—●—) had been given at first administration (①), suppositories containing 10% ABPCNa alone were administered four times at intervals of 60 min (②—⑤). Each point represents the mean of eight animals.

The value of area under the plasma concentration of ABPC-time curve during 0—60 min (AUC_{0-60}) after each administration in rabbits was used as a parameter of the absorbability of ABPCNa.

The AUC_{0-60} value after administration of ABPCNa alone was determined as an index of the normal membrane state and was compared with AUC_{0-60} of ABPCNa after treatment with DCNa or DC–Lys. When AUC_{0-60} of ABPC after DC salts treatment returned to the level before treatment, the mucous membrane was judged to be restored completely to the normal condition. The changes of plasma concentration and AUC_{0-60} of ABPC under various experimental conditions are shown in Figs. 3 and 4. A linear relationship was apparently obtained between AUC_{0-60} of ABPC and time after initial dosing. Extrapolation of the plot for DCNa or DC–Lys in Fig. 4 to the AUC_{0-60} of the normal state (AUC_{0-60}=25.5 μg·min/ml) gives the period required for the leaky barrier to return to the normal state. The damage by DCNa required about 20 h for recovery ,while that by DC–Lys required only about 6.5 h.

From these results, it may be concluded that the barrier leakiness at the rectal mucosa induced by the irritative effect of DCNa is greater than that of DC–Lys. The time required for recovery of the barrier to the normal state is closely correlated to the irritative effect of the salts, so is considered to be an important factor relating to the safety of rectal delivery preparations of NSAI drugs, because of possible absorption of unfavorable materials present in the rectal fluid. A short duration of leakiness would reduced any such unfavorable absorption. From the result that the bioavailability of basic amino acid salts of DC was comparable to that of DCNa, it is also concluded that the formation of a leaky barrier is not necessary for the absorption of DC.

References and Notes

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