EFFECTS OF L-GLUTAMINE ON ACETYLSALICYLIC ACID INDUCED GASTRIC LESIONS AND ACID BACK DIFFUSION IN DOGS

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Abstract—Effects of L-glutamine on acetylsalicylic acid (ASA)-induced gastric mucosal lesions were studied in mongrel dogs. It was confirmed that when oral ASA at 1.0 or 2.0 g per dog is given in two divided doses, there is severe and consistent dose-dependent mucosal damage in the glandular portion of the stomach in fasted dogs. However, when L-glutamine 2.0 or 4.0 g per dog in two divided doses is given concomitantly with ASA 2.0 g per dog orally, the gastric irritation is significantly inhibited. Instillation of 20 mM of ASA in 100 mM HCl solution into the Heidenhain pouch of Beagle dogs produced a significant loss of H^+ from the pouch and a gain of Na^+ in the lumen compared with ASA-free controls. When L-glutamine (100 mM) was given concomitantly with ASA (20mM) into the pouch, changes of electrolyte fluxes in response to ASA alone were significantly suppressed. However, 50 mM of L-glutamine had no appreciable effect on acid back diffusion caused by ASA 20 mM. The amino acid itself had little effect on the ionic movement in the pouch. Gross bleeding from the pouch treated with ASA was never observed with the concomitant dosing of ASA and L-glutamine 50 or 100 mM.

We have already reported the marked inhibitory effect of L-glutamine on acetylsalicylic acid (ASA)-induced gastric irritation in normal, cirrhotic or pregnant rats, or in rats with a chronic gastric ulcer (1-4). It was experimentally determined that L-glutamine produces a dose-dependent inhibition of acid back diffusion in response to ASA in the rat stomach (2-5). As dogs are frequently used in the study of gastric problems related to ASA (6-9), our present work was an attempt to determine the effects of L-glutamine in this species. This report contains data on the potent effect of this amino acid in preventing the noxious effect of ASA on the gastric mucosa in normal dogs and on the acid back diffusion in response to ASA in the stomach of Heidenhain pouch Beagle dogs.

MATERIALS AND METHODS

ASA-lesion formation: Thirty-six male mongrel dogs, weighing 8-10 kg, were deprived of food but allowed free access to water from 10:00 a.m. until the end of the experiments. ASA packed in a gelatin capsule was given orally to the dogs in the dose of 0.25, 0.5 or 1.0 g per dog at 6:00 p.m. of the same day. At 10:00 a.m. the next day, ASA in the same dose was given to these same dogs, i.e., the total dose of ASA was 0.5, 1.0 or 2.0 g per dog per day. An overdose of sodium pentobarbital was given i.v. at 6:00 p.m. of the same day after the

dogs had fasted from food for a total of 32 hr. The stomach of each dog was removed, inflated by pouring in one liter of 1% formalin solution for 10 min, then incised along the greater curvature and examined for lesions which had developed in the glandular portion. The length of each lesion was measured, summed and arbitrarily divided into 7 degrees as a lesion index as follows.

Total length of each lesion (mm)	0	1-100,	101-200,	201-300,	301-400,	401-500,	501-600,	600<
Lesion index	0	1	2	3	4	5	6	7

The gastric specimens were put into Bouin solution for histological study. To determine the effect of L-glutamine on ASA-induced gastric lesions, L-glutamine at 0.5, 1.0 or 2.0 g per dog was mixed well with ASA at 1.0 g per dog, packed in a gelatin capsule and then given orally to the dogs, twice at a 16 hr interval. As a control, lactose at 1.0 g per dog was mixed with ASA 1.0 g per dog and also given twice at the same interval.

Acid back diffusion: The methods used in this study were much the same as those employed by Davenport (8) or Chvasta and Cooke (9). Studies were done on 5 unanesthetized Beagle dogs (3 males and 2 females) over a 4 month period. Each dog had a separate denervated (Heidenhain) pouch of the oxyntic glandular area of the stomach which drained via a stainless steel cannula with a 3 way stopcock. Water was provided for 18 hr prior to the test. Studies were done twice a week only and never on consecutive days. Prior to testing, each dog was given atropine sulfate (0.015 mg/kg) i.m. to ensure basal secretion. At the beginning of each experiment, the pouch was rinsed thoroughly with a solution of 100 mM HCl and 54 mM NaCl and drained with a syringe. Forty ml of a solution of 100 mM HCl and 54 mM NaCl with phenol red (40 mg/liter) were injected into the pouch using a calibrated syringe. The contents were mixed for 30 sec and a 5 ml sample (initial sample) was removed for analysis. The contents of the pouch were mixed at 10 min intervals using a syringe connected to the 3 way stopcock. At the end of 30 min, the pouch was emptied as completely as possible by gentle aspiration with a syringe, and the volume of fluid recovered (final sample) was measured. The residual volume, the volume of fluid remaining in the pouch after recovery of the injected solution, was calculated by dilution of the phenol red. The pouch was then rinsed twice with 154 mM NaCl and twice with the solution without phenol red which was to be used in the next period.

Each experiment consisted of 5 consecutive 30 min observation periods. Period 1 was the control; in period 2, ASA, ASA+L-glutamine, or L-glutamine alone was instilled, and in periods 3 to 5 the same solution as used in period 1 was tested. During period 2, the drug was instilled in an acid solution consisting of 100 mM HCl, 54 mM NaCl and phenol red 40 mg/liter (pH 1.1). The residual volume of the pouch was determined for each test period and for the 5 dogs (mean $\pm$ S.E.) was 2.95 ± 0.22 ml, 2.50 ± 0.19 ml, 2.15 ± 0.20 ml, or 2.17 ± 0.20 ml; the number of experiments was over 40.

The initial and final samples were analyzed for titratable acidity, Na^+ , K^+ and phenol red concentrations. The acidity was measured by titrating a 1 ml sample of the gastric con-

tents with 0.1 N NaOH to pH 7.0 using an automatic buret (Radiometer). The concentrations of Na^+ and K^+ were measured by a flame photometer (Hitachi). Phenol red concentration was measured by the method of Hunt and Knox (10). Osmolarity was measured using an Advanced Wide-Range Osmometer (Advanced Instruments). The ionic fluxes were calculated as the difference between the product of the final volume and concentration, and the product of the initial volume and concentration. Positive values indicate that the net flux was from mucosa to lumen while negative values indicate that the net flux was from lumen to mucosa.

Test solutions: The solution of ASA (20 mM), ASA (20 mM)+L-glutamine (50 or 100 mM) or L-glutamine (100 mM) alone was prepared by mixing each substance with 200 ml of distilled water for 1 hr in a 250 ml of volumetric flask. The solution was made acid (pH 1.1) by adding conc. HCl solution (approx. 2.1 ml) to form 100 mM HCl solution, 54 mM of NaCl solution and 10 mg of phenol red and a final volume of 250 ml was accomplished using distilled water. The solutions were prepared daily and mixed thoroughly before instillation in the pouch.

Statistics: Student's *t*-test was employed to determine the statistical significance of both lesion index and difference of ionic flux.

RESULTS

Effects of L-glutamine on gastric lesions induced with ASA

ASA, when given at 0.5 g per dog per day in two divided doses, induced no detectable injuries to the gastric mucosa in 2 fasted dogs. However, the dosing of 1 g of ASA per dog per day resulted in an apparent development of mucosal erosions either punctate or elongated, mainly in the oxyntic glandular area including the so-called "magenstrasse" and partly in the antrum. ASA at 2 g per dog per day induced much more severe and consistent irritation of gastric mucosa than that seen at 1 g per dog per day (Table 1). Histologically, the damage proved to be superficial erosions and there was never penetration beyond the muscularis mucosa. L-glutamine, given together with ASA at 2 g per dog per day, produced a definite inhibition of ASA-induced gastric lesions in dogs, i.e., the inhibition rate was 25.5%, 43.1% or 62.7% at the dose of 1.0, 2.0 or 4.0 g per dog per day, respectively. The protective

TABLE 1. Effect of L-glutamine on ASA-induced gastric lesions in dogs

Treatment	Dose (g per dog per day)	No. of dogs	ASA lesions Lesion index (mean $\pm$ S.E.)	% inhibition	P value
ASA	0.5	2	0		
ASA	1.0	2	1.5		
ASA-lactose	2.0 $\pm$ 2.0	8	5.1 $\pm$ 0.5		
ASA + L-glutamine	2.0 $\pm$ 1.0	8	3.8 $\pm$ 0.9	25.5	N.S.
ASA + L-glutamine	2.0 $\pm$ 2.0	8	2.9 $\pm$ 0.5	43.1	<0.01
ASA + L-glutamine	2.0 + 4.0	8	1.9 $\pm$ 0.4	62.7	<0.001

ASA, ASA+L-glutamine, or ASA+lactose was given orally in two divided doses.
(Student's *t*-test)

FIG. 1. Gross appearance of gastric lesions induced by oral acetylsalicylic acid (ASA: 2 g/dog) (A) and ASA (2 g/dog) plus L-glutamine (2 g/dog) (B). Note that ASA-induced lesions were almost completely inhibited by L-glutamine given concomitantly.

effect of L-glutamine against ASA lesions is illustrated in Fig. 1.

Effects of L-glutamine on electrolyte fluxes induced by ASA

Details of the net ionic fluxes are given in Table 2. Gastric instillation of 20 mM of ASA at a pH of 1.1 produced a significant loss of H^+ from the lumen and gain of Na^+ into the lumen of Heidenhain pouch of dogs at periods 2 or 3 compared with the control period 1. The net K^+ flux into the lumen significantly increased at period 2. However, the changes of H^+ and K^+ fluxes in response to ASA tended to recover to the control level (period 1) by period 5, whereas the increased flux of Na^+ recovered almost completely by period 5. Gross bleeding from the pouch was observed in all studies with ASA; this commenced during period 3 and continued in periods 4 and 5. ASA 20 mM, given together with 50 mM of L-glutamine, also produced a significant loss of H^+ and Na^+ at periods 2 and 3, the degree of which was almost the same as seen with ASA alone. When 100 mM of L-glutamine with 20 mM of ASA was instilled into the pouch, the substantial electrolyte changes between period 1 and periods 2 or 3 were considerably less compared to those of ASA alone. Actually in cases of ASA alone, the mean $\pm$ S.E. of net difference of H^+ and Na^+ fluxes between periods 1 and 3 was -428.2 ± 5.1 and 208.0 ± 60.5 , respectively. In contrast, in cases of ASA + L-glutamine 100 mM, the net difference of H^+ and Na^+ was -13.1 ± 31.3 and 23.1 ± 38.2 , respectively. These values between ASA alone and ASA + L-glutamine 100 mM were significantly different (H^+ : $P < 0.001$ and Na^+ : $P < 0.05$). L-glutamine at 100 mM produced little alteration of ionic fluxes in the pouch compared with control period 1 except the significant reduction of Na^+ flux in period 2. It should be noted that ASA-induced bleeding from the pouch was almost completely prevented as the result of concomitant administration of ASA and L-glutamine at either 50 or 100 mM.

DISCUSSION

These studies provide further data that oral ASA produces gastric mucosal damage in fasted dogs (6, 7). The incidence of ASA-induced gastric lesions reported herein was 100% and the severity of lesions was quite consistent in each animal. As in our previous work

TABLE 2. Effects of L-glutamine (Gln) on acid back diffusion induced by acetylsalicylic acid (ASA) in Heidenhain pouch dogs

Periods	ASA 20 mM [5/5] (311.1)						ASA 20 mM + Gln 50 mM [5/4] (310.2)						Gln 100 mM [5/5] (325.6)						Gln 100 mM [5/5] (300.6)					
	H ⁺	Na ⁺	K ⁺	H ⁺	Na ⁺	K ⁺	H ⁺	Na ⁺	K ⁺	H ⁺	Na ⁺	K ⁺	H ⁺	Na ⁺	K ⁺	H ⁺	Na ⁺	K ⁺	H ⁺	Na ⁺	K ⁺	H ⁺	Na ⁺	K ⁺
	Net flux, $\mu\text{Eq}/30\text{ min (mean } \pm \text{S.E.)}$																							
1	89.0 ± 35.2	221.2 ± 52.3	0	17.9 ± 39.0	278.7 ± 51.1	6.0 ± 3.8	-23.2 ± 9.0	213.9 ± 16.3	2.9 ± 2.9	22.9 ± 22.7	258.6 ± 44.9	2.1 ± 1.3	22.9 ± 22.7	258.6 ± 44.9	2.1 ± 1.3	22.9 ± 22.7	258.6 ± 44.9	2.1 ± 1.3	22.9 ± 22.7	258.6 ± 44.9	2.1 ± 1.3	22.9 ± 22.7	258.6 ± 44.9	2.1 ± 1.3
2	-622.8 ± 69.8	405.2 ± 14.2	14.2 ± 3.3	-499.1 ± 58.0	469.3 ± 50.6	19.6 ± 5.5	-247.6 ± 28.0	282.0 ± 29.3	4.5 ± 2.2	7.3 ± 20.7	122.6 ± 19.4	0	7.3 ± 20.7	122.6 ± 19.4	0	7.3 ± 20.7	122.6 ± 19.4	0	7.3 ± 20.7	122.6 ± 19.4	0	7.3 ± 20.7	122.6 ± 19.4	0
3	-339.0 ± 62.6	429.3 ± 49.1	18.1 ± 7.2	-303.7 ± 38.0	487.7 ± 44.8	17.1 ± 7.0	-154.3 ± 24.1	237.0 ± 38.7	3.0 ± 1.4	23.3 ± 22.8	233.6 ± 54.6	1.3 ± 1.3	23.3 ± 22.8	233.6 ± 54.6	1.3 ± 1.3	23.3 ± 22.8	233.6 ± 54.6	1.3 ± 1.3	23.3 ± 22.8	233.6 ± 54.6	1.3 ± 1.3	23.3 ± 22.8	233.6 ± 54.6	1.3 ± 1.3
4	-273.2 ± 68.5	265.0 ± 70.1	6.6 ± 3.5	-252.3 ± 75.6	404.1 ± 65.5	11.4 ± 4.3	-85.5 ± 39.7	338.9 ± 27.8	3.0 ± 1.4	-34.0 ± 23.6	236.6 ± 51.2	2.2 ± 2.2	-34.0 ± 23.6	236.6 ± 51.2	2.2 ± 2.2	-34.0 ± 23.6	236.6 ± 51.2	2.2 ± 2.2	-34.0 ± 23.6	236.6 ± 51.2	2.2 ± 2.2	-34.0 ± 23.6	236.6 ± 51.2	2.2 ± 2.2
5	-200.4 ± 46.5	210.4 ± 60.4	8.6 ± 3.1	-151.5 ± 101.9	392.4 ± 56.5	9.7 ± 4.2	-147.3 ± 63.3	252.0 ± 53.1	5.7 ± 3.4	-47.3 ± 37.3	324.6 ± 54.2	0.7 ± 0.7	-47.3 ± 37.3	324.6 ± 54.2	0.7 ± 0.7	-47.3 ± 37.3	324.6 ± 54.2	0.7 ± 0.7	-47.3 ± 37.3	324.6 ± 54.2	0.7 ± 0.7	-47.3 ± 37.3	324.6 ± 54.2	0.7 ± 0.7
(1:2)	<0.001	<0.01	<0.01	<0.001	<0.05	N.S.	<0.001	N.S.	N.S.	<0.001	N.S.	<0.05	<0.001	N.S.	N.S.	<0.001	N.S.	N.S.	<0.001	N.S.	<0.05	N.S.	<0.05	N.S.
(1:3)	<0.001	<0.01	N.S.	<0.001	<0.05	N.S.	<0.001	N.S.	N.S.	<0.001	N.S.	<0.05	<0.001	N.S.	N.S.	<0.001	N.S.	N.S.	<0.001	N.S.	<0.05	N.S.	<0.05	N.S.

ASA, ASA + Gln, or Gln alone in HCl 100 mM, NaCl 54 mM, phenol red 40 mg/l solution was instilled during period 2. [] indicates the number of experiments/the number of dogs. () indicates osmolarity of the solution (mosmoles/kg). (Student's *t*-test)

done in rats, L-glutamine, given together with ASA, produced a marked dose-dependent protection of the gastric mucosa against the irritative effects of ASA. The dose of L-glutamine which inhibited ASA gastric lesions by about 40–60% was equivalent or only double that of ASA.

Many substances, including ASA, are known to break down the gastric mucosal barrier and permit back diffusion of acid through the disrupted mucosa, resulting in gastric mucosal damage (11). To our knowledge, only one substance, carbenoxolone Na, was found to inhibit acid back diffusion induced by human bile through the Heidenhain pouch of dogs, even though the drug did not affect the flux of Na^+ (12). In contrast to the data observed in dogs, carbenoxolone Na was not reported to exert any appreciable effect on acid back diffusion induced by intragastric instillation of taurocholic acid in man (13).

We have already reported data that L-glutamine inhibits the back diffusion of H^+ and the flux of Na^+ induced by ASA in rats, as well as ASA-induced lesions (5). The inhibitory effect of 100 mM of L-glutamine on acid back diffusion induced by 20 mM of ASA was demonstrated herein by using a standard method of Heidenhain pouch in dogs. Unlike the effect of carbenoxolone Na, L-glutamine also significantly inhibited the increment of Na^- ions in the test solution in response to ASA. Although the osmolarity of the solution which contained 100 mM L-glutamine and 20 mM ASA was slightly higher (14.5 mosmoles/kg in net difference) than that of ASA alone, the difference would hardly contribute to the effect of L-glutamine on acid back diffusion observed in the present study. The addition of certain amounts of mannitol to the 20 mM ASA solution increased the osmolarity of the solution to approx. 325.6 mosmoles/kg, but had no notable influence on acid back diffusion. Reduction of the dose of L-glutamine, i.e., 50 mM, exerted no inhibition on H^+ and Na^+ fluxes due to ASA. L-glutamine itself had little influence on ionic fluxes in Heidenhain pouch dogs. An explanation is now being sought for the significant reduction of Na^+ flux in period 2 in response to L-glutamine.

Davenport (14) and Chvasta and Cooke (9) found that when a Heidenhain pouch of dogs was irrigated with 20 mM of ASA in a 100 mM HCl solution, the recovered fluid frequently contained blood, indicating a severe mucosal damage by the agent. We also found blood in the fluids recovered in periods 3, 4 and 5 after the ASA treatment. L-glutamine given concomitantly with ASA at the dose level of 50 or 100 mM solution completely prevented this bleeding from the pouch. Therefore, this amino acid may to some extent inhibit ASA-induced gastric bleeding by a mechanism other than prevention of acid back diffusion, since the dose of 50 mM of L-glutamine did not result in any significant inhibition of acid back diffusion in response to ASA in acid solution.

In rats, the absorption of ASA from either the gastric mucosa or gastroduodenal mucosa and the resulting pharmacologic effects, such as the analgesic, antipyretic or anti-inflammatory effects, remained unchanged in the presence of L-glutamine (15). We found in preliminary work that the absorption of ASA from Heidenhain pouch of dogs was not affected by the presence of L-glutamine in a dose which inhibited the back diffusion of acid and gastric bleeding in response to ASA. Thus, the therapeutic effect of ASA is apparently

not influenced by a concomitant dosing of L-glutamine. Data from clinical studies on the concomitant administration of ASA and L-glutamine to patients with rheumatic diseases will be reported in a succeeding paper.

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