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Inhibitory effects of L-glutamine on the aspirin-induced gastric lesions in the rat

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The effects of an amino acid, L-glutamine, on aspirin-induced gastric lesions and gastric secretion were studied in either intact or pylorus-ligated rats. L-Glutamine had a pronounced inhibitory effect on gastric lesions induced by aspirin administered by oral, intraperitoneal, or intraduodenal routes to intact or pylorus-ligated rats. By the oral route the inhibition was dose-related. However, L-glutamine given either intraduodenally or intraperitoneally did not show any appreciable effects on gastric lesions induced by orally administered aspirin in pylorus-ligated rats. One mechanism of L-glutamine protection was inhibition of a back diffusion of gastric acid caused by aspirin and this was demonstrated in pylorus-ligated rats. The reduction in H^+ and increase in Na^+ concentrations in the lumen caused by aspirin was returned to normal by increasing doses of L-glutamine. In addition, L-glutamine was considered to inhibit the back diffusion of acid caused by pylorus ligation *per se*, because the amino-acid produced an increment of H^+ and reduction of Na^+ and K^+ in comparison with the control group. The role of pepsin on L-glutamine protection was negligible.

Several reviews dealing with aspirin-induced gastric lesions in man and experimental animals have been published (Roth & Valdes-Dapena, 1963; Salter, 1968; Cooke, 1973). Of the various theories of aetiology, that of a back diffusion of acid, proposed by Davenport (1964), has been widely accepted as a principal aetiologic factor for the lesion formation in response to aspirin administration. However, little attention seems to have been paid to the search for prophylactic agents for aspirin-induced lesions of the gastro intestinal tract, agents without effects on the therapeutic benefits of aspirin, i.e., the analgesic and anti-inflammatory effects. Previously, Takagi & Okabe (1968) reported a favourable effect of L-glutamine on the healing process of stress-induced gastric lesions in the rat in comparison with other amino-acids, although the mechanisms remained unexplained. We now describe the inhibitory effect of L-glutamine on aspirin-induced gastric lesions and relate this to a back diffusion of acid.

METHODS AND MATERIALS

Male Donryu strain rats, 190–215 g, were fasted for 24 h before use, but were allowed free access to water.

Induction of lesions by aspirin in intact rats

Aspirin suspended in 1% carboxymethylcellulose solution (CMC) at 100 mg kg^{-1} was given by gastric intubation to fasted rats. Ten min before aspirin dosing, all animals were given either L-glutamine suspended in 1% CMC solution, or to 1% CMC alone by gastric intubation. The dose of aspirin was selected on the basis of

data published by Brodie & Chase (1967) and Pfeiffer & Lewandowski (1971). Seven h after aspirin dosing, during which time food and water had been withheld, the animals were killed by an overdose of ether. Ten min before death, while the animals were under ether anaesthesia, 1 ml of a 5% solution of pontamine sky blue 6 BX (PSB) dissolved in saline (pH adjusted to 7·2 with 0·5 N HCl) was injected into the femoral vein (Brodie, Tate & Hooke, 1970). After death, stomachs were removed, slightly inflated by injection of 1% formalin solution through the oesophageal junction, and immersed in 1% formalin solution for 10 min for fixation of the inner and outer layers of the gastric wall (Brodie & Hanson, 1960). Subsequently, the stomach was opened along the greater curvature, and the length of lesions in the glandular portion were measured under the dissecting microscope (10 X) with a square grid and the lengths (mm) were summed to give a lesion index for each rat.

Induction of lesions by aspirin in pylorus-ligated rats

Pylorus ligation under ether anaesthesia according to Shay, Komarov & others (1945) was found initially to enhance the production of aspirin-induced gastric lesions in comparison with intact rats (non-ligated). In addition, the ligation facilitated the measurement of gastric secretory conditions at the time of lesion formation. Immediately after the pyloric ligation, either L-glutamine, given over a wide dose range, or 1% CMC solution was administered orally. Ten min later, aspirin at 100 mg kg^{-1} was given by the same route. To exclude any interference between aspirin and L-glutamine in the stomach, these two agents were also given by different routes. For this, L-glutamine (1000 mg kg^{-1}) was given intraduodenally or intraperitoneally at the time of pylorus ligation and then aspirin at 100 mg kg^{-1} was administered by gastric intubation. In addition, aspirin at 100 mg kg^{-1} was given intraduodenally or intraperitoneally at the time of pyloric ligation followed by L-glutamine (1000 mg kg^{-1}) given orally. Seven h after dosing by aspirin, these animals were examined for gastric lesions.

Gastric secretion

To examine the effect of L-glutamine on gastric secretion, the pyloric ligation preparation was also used with the same time schedule (24 h fasting + 7 h ligation). The gastric juices were collected, centrifuged and titrated for acidity to pH 7·4 with 0·1 N NaOH. The concentrations of Na^+ and K^+ were measured by flame photometry. Pepsin activity was determined by Anson's hemoglobin method (1938).

The level of significance was calculated by using Student's *t*-test.

RESULTS

Effects of L-glutamine on aspirin-induced lesions in intact rats

As a result of aspirin administration, relatively small mucosal lesions were occasionally observed in the glandular stomach. L-Glutamine (1000 mg kg^{-1}) largely prevented the lesions (74·5%; $P < 0·005$). (The lesions index for controls given 1% CMC 0·5 ml dl^{-1} by intubation was 14·9 $\pm$ 3·2 mm mean $\pm$ s.e. n = 16. That for L-glutamine-treated rats was 3·8 $\pm$ 0·7 mm, n = 16.)

Effects of L-glutamine on aspirin-induced lesions in pylorus-ligated rats

In pylorus-ligated rats, aspirin consistently induced severe haemorrhagic erosions,

and superficial mucosal defects without any appreciable haemorrhage. The difference of severity of gastric lesions between the intact and pylorus-ligated rats was highly significant ($P < 0.001$). The forestomachs of the rats, dosed either with aspirin or 1% CMC solution, were seldom influenced by this technique.

L-Glutamine given orally had an inhibitory effect on the formation of aspirin-induced lesions (by oral route) (Table 1). The inhibition in the lesion index was 93·6, 67·1, 72·6 or 49·0% at doses of 1000, 500, 250 or 125 mg kg^{-1} of L-glutamine,

Table 1. *Effects of* L-*glutamine on gastric lesions induced with aspirin at 100 mg kg^{-1} given orally, in pylorus-ligated rats (7 h ligation after 24 h fasting).*

Treatment	Dose (mg kg^{-1})	No. of rats	Average body weight (g)	Lesion index (mm) m ± s.e.	Inhibition %
Control (1% CMC)		10	188	54·7 ± 3·4	
L-Glutamine	62·5	10	189	57·6 ± 5·6	—5·0
	125	10	179	27·9 ± 4·7	49·0***
	250	12	182	15·0 ± 3·4	72·6***
	500	12	186	18·0 ± 3·1	67·1***
	1000	12	189	3·5 ± 1·5	93·6***

*** Significantly different from control, $P < 0.001$.

respectively compared with the controls. All the values were highly significant ($P < 0.001$). The stomachs of 3 of 12 animals dosed with L-glutamine at 1000 mg kg^{-1} were clear of ulcers and mucosal defects. L-Glutamine at 62·5 mg kg^{-1} did not inhibit the aspirin-induced lesions. Gastric lesions induced by aspirin given orally were not significantly prevented by intraduodenal or intraperitoneal injection of 1000 mg kg^{-1} of L-glutamine (Table 2). Aspirin given intraduodenally or intraperitoneally induced minor but consistent lesions in the glandular portion of stomachs of rats with pyloric ligation. L-Glutamine at 1000 mg kg^{-1} also markedly inhibited the lesion formation by parenterally given aspirin.

Table 2. *Effects of* L-*glutamine on the aspirin-induced gastric lesions in pylorus-ligated rats (7 h ligation after 24 h fasting).*

	Treatment	No. of rats	Average body weight (g)	Lesion index (mm) m ± s.e.	Inhibition %	*P* value
Aspirin (oral)	Control (i.d.)	12	186	63·2 ± 5·5		
	L-Glutamine (i.d.)	12	183	49·7 ± 7·0	21·4	NS
	Control (i.p.)	14	185	61·9 ± 9·4		
	L-Glutamine (i.p.)	14	188	59·6 ± 6·3	3·7	NS
Aspirin (i.d.)	Control (oral)	15	185	12·5 ± 1·9		
	L-Glutamine (oral)	15	187	1·7 ± 0·4	86·4	< 0·001
Aspirin (i.p.)	Control (oral)	11	192	12·5 ± 3·0		
	L-Glutamine (oral)	12	189	0·8 ± 0·2	94·0	< 0·001

Aspirin and L-glutamine were given at 100 and 1000 mg kg^{-1}.
i.d. Intraduodenally, i.p. Intraperitoneally. NS Non significant at $P = 0.05$.

Table 3. *Effects of aspirin, aspirin + L-glutamine, and L-glutamine on gastric secretion in pylorus-ligated rats (7 h ligation after 24 h fasting).*

Group	Treatment	Dose (mg kg^{-1})	No. of rats	Body weight (g)	Gastric contents					
					Volume (ml)	H^+	Na^+ (m mol	K^+	$H^+ + Na^+ + K^+$	Pepsin (mg ml^{-1})
A	Control (1% CMC X 2)		10	150	11·9 ± 0·4	110·5 ± 2·9	49·4 ± 4·0	4·91 ± 0·36	164·0 ± 2·0	26·8 ± 0·6
B	Aspirin (100 mg kg^{-1}) + 1% CMC		10	147	10·3 ± 0·4	66·7 ± 3·6 (−43·8)	91·8 ± 4·9 (+ 42·4)	6·44 ± 0·63 (+ 1·53)	165·0 ± 1·7 (+ 1·0)	26·6 ± 0·9
C	Aspirin (100 mg kg^{-1}) + L-glutamine	62·5	10	149	10·9 ± 0·3	73·2 ± 4·4 (− 37·3)	88·3 ± 4·5 (+ 38·9)	6·25 ± 0·39 (+ 1·34)	167·8 ± 3·6 (+ 3·8)	29·7 ± 0·8
D		125	10	150	10·5 ± 0·3	92·3 + 3·4 (− 18·2)	68·8 ± 2·4 (+ 19·2)	6·42 ± 0·34 (+ 1·51)	167· ± 2·2 (+ 3·3)	27·5 ± ·04
E		250	10	155	10·5 ± 0·4	100·7 ± 3·6 (− 9·8)	66·1 ± 2·9 (+ 16·7)	6·17 ± 0·51 (+ 1·26)	173·0 ± 1·6 (+ 9·0)	25·7 ± 9·9
F		500	10	160	10·8 ± 0·4	117·2 ± 3·9 (+ 6·7)	50·8 ± 3·2 (+ 1·4)	6·20 ± 0·62 (+ 1·29)	174·2 ± 1·7 (+ 10·2)	23·6 ± 0·7
G		1000	10	154	10·3 ± 0·3	129·6 ± 2·5 (+ 19·1)	42·8 + 4·0 (− 6·6)	5·41 ± 0·54 (+ 0·50)	177·8 + 4·60 (+ 13·8)	24·5 ± 0·7
H	L-Glutamine + 1% CMC	1000	10	152	12·1 ± 0·4	137·4 ± 3·6 (+ 26·9)	37·2 ± 2·7 (− 12·2)	3·69 ± 0·56 (− 1·22)	177·5 + 5·3 (+ 13·5)	23·6 ± 0·9
	P value				A:B < 0·05 A:D < 0·05 A:G < 0·01	A:B < 0·001 A:C < 0·001 A:D < 0·001 A:E < 0·05 A:G < 0·001 A:H < 0·001 C:D < 0·01 E:F < 0·001 F:G < 0·01	A:B < 0·001 A:C < 001 A:D < 0·001 A:E < 0·01 C:D < 0·05 A:H < 0·05	A:B < 0·05 A:C < 0·01 A:D < 0·01 A:H < 0·05	A:E < 0·01 A:F < 0·01 A:G < 0·01 A:H < 0·05 B:E < 0·01 B:F < 0·01 B:G < 0·01 D:E < 0·05	A:C < 0·01 A:F < 0·01 A:G < 0·05 A:H < 0·01

All figures represent mean ± s.e. The values in the parentheses represent the net difference from control.

Effects of aspirin, aspirin + L-glutamine, and L-glutamine on gastric secretion.

The collected gastric juice from rats dosed with L-glutamine (doses over 250 mg kg^{-1}) with aspirin was relatively clear while that from the group given aspirin alone was almost invariably coloured brown due to the presence of blood.

As indicated in Table 3, aspirin (100 mg kg^{-1}) produced a significant reduction of volume (−1·6 ml) and H^+ (−43·8 m mol) and increment of Na^+ (+42·2 m mol) and K^+ (+ 1·53 m mol) in gastric juices. Pepsin activity was not changed with aspirin at all. With L-glutamine given, simultaneously with aspirin, the volume was either reduced, or not significantly different from control values. The reduced H^+ concentration in response to aspirin was returned to the normal level or increased as the dose of L-glutamine increased ($P < 0·05$). On the other hand, the increments of Na^+ and K^+ induced by aspirin gradually declined to the control level after L-glutamine; Na^+ was reduced in a dose-dependent manner.

The total concentration of H^+, Na^+ and K^+ in the aspirin-treated group equalled that of the control, with values of 164·0 ± 2·0 and 165·0 ± 1·7 m mol, respectively. However, the total concentration of the cations in gastric juice of rats given aspirin + L-glutamine slightly but gradually increased; the increment was significant at over 250 mg kg^{-1} of L-glutamine ($P < 0·01$). Pepsin activity was significantly increased at 62·5 mg kg^{-1} ($P<0·01$) but slightly reduced at more than 250 mg kg^{-1} of L-glutamine given simultaneously with aspirin.

L-Glutamine at 1000 mg kg^{-1} plus 1% CMC did not exert any changes on volume but significantly increased H^+ ($P < 0·001$) and reduced Na^+ and K^+ ($P < 0·05$) compared with control values. The total concentration of above cations was significantly ($P < 0·05$) increased in comparison with that of controls; the extent of the increase (13·5%) was almost the same as that observed with the L-glutamine (1000 mg kg^{-1}) + aspirin-treated group (13·8%). Pepsin activity was significantly reduced by L-glutamine in comparison with the control group ($P < 0·01$).

DISCUSSION

L-Glutamine, given orally, had a pronounced inhibitory effect on gastric lesions induced by aspirin given orally in intact and pylorus-ligated rats. In particular, the inhibition was most marked in pylorus-ligated rats in which almost complete inhibition (93·6%) appeared at 1000 mg kg^{-1} of L-glutamine; even at 125 mg kg^{-1} 49% inhibition was observed. One possible interpretation is that L-glutamine might inactivate aspirin in the stomach before it irritates the gastric mucosa. However, gastric lesions induced by intraduodenally or intraperitoneally administered aspirin, even though less severe than those obtained after oral administration, were also markedly inhibited by L-glutamine given orally. This finding excludes any physical or chemical interference between L-glutamine and aspirin in the stomach. In contrast, L-glutamine given intraduodenally or intraperitoneally did not exert any appreciable inhibition on lesions induced by aspirin orally given. Thus L-glutamine should be given orally and be present in the stomach at the time of lesion formation by aspirin. Examination of the fate (absorption and metabolism) of both aspirin and L-glutamine in the pylorus-ligated rats may afford some clue to the mechanisms involved. The most plausible interpretation on the effect of L-glutamine is the prevention of a back diffusion of acid. Brodie & Chase (1967) have reported that aspirin reduced the volume to some extent and acidity to a greater extent in pylorus-ligated rats. In addition, Overholt, Brodie &

Chase (1969) described the occurrence of a back diffusion of acid through aspirin damaged mucosa in vagotomized rats. Our present data agree with the above authors, even though we used the innervated rat stomach. The present study also seems to provide the evidence for the above views because a back diffusion of acid was confirmed with aspirin dosing. The net loss of H^+ concentration was well matched by the increased concentration of Na^+ and K^+ in the lumen, there being no difference in the total concentration of these cations between the control and aspirin-treated group. As might be expected, L-glutamine strongly inhibited the back diffusion induced by aspirin, in a dose-related manner. Slight but gradual increase of the total concentration of those cations was found as the dose of L-glutamine was increased. The same phenomenon was observed with L-glutamine alone. L-Glutamine might either stimulate the gastric secretion of acid or influence the titration of acid in samples of gastric juice. However, it seems unlikely that L-glutamine is a secretagogue because Elwin & Uvnas (1966) have reported that amino-acids with four carbon atoms do not evoke an appreciable increase in acid secretion. It was clear from an unpublished *in vitro* study that an acid solution containing L-glutamine did not require an excess sodium hydroxide on titration in comparison with acid solution alone. However, there is a possibility that L-glutamine was partly metabolized to L-glutamic acid in the acidic gastric juices during the experimental period and an additional but small amount of the sodium hydroxide was required at the time of titration. L-Glutamine at 1000 mg kg^{-1} increased H^+ concentration and reduced Na^+ and K^+ concentrations compared with the control values. These facts suggest that L-glutamine might prevent the back diffusion of acid elicited by the pyloric ligation procedure *per se*. Overholt & others (1969) have stated that a back diffusion of acid probably occurs normally in significant quantities. Pepsin activity changed to a lesser extent by the administration of aspirin, aspirin + L-glutamine and L-glutamine alone. Therefore, participation of pepsin in either the aetiology of aspirin-induced lesions or the L-glutamine protection was assumed to be negligible.

It has been confirmed that antacids prevent aspirin-induced gastric lesions in man and animals (Salter, 1968; Brodie & Chase, 1967; Pfeiffer & Lewandowski, 1971). We have found the strong inhibition of aspirin lesions produced by the method described in this paper with the administration of antacids (unpublished data). The findings reported by Matsumoto & Grossman (1959), Scott, Porter & others (1961) and Wood, Harvey-Smith & Dixon (1962) emphasize the difficulty of avoiding gastric irritation in man by addition of antacids to aspirin, even when large amounts are used. In addition, it has been demonstrated that a high percentage of alkali incorporated into aspirin preparations make them inappropriate for long-term use (Salter, 1968).

Accordingly, L-glutamine may have a part to play in the clinical use of aspirin.

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