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Effect of Caffeine on Ibuprofen-induced Gastric Mucosal Damage in Rats

RIKA KOYAMA, HIROFUMI KATAOKA, YOSHIYUKI TANAKA, SEIICHI NAKATSUGI AND MASUMI FURUKAWA

Osaka Research Laboratory, Sawai Pharmaceutical Co. Ltd, 1-8-14 Ikue, Asahi-ku, Osaka 535-0004, Japan

Abstract

During investigations on the effect of caffeine on ibuprofen-induced gastric mucosal lesions in rats, we have found that caffeine (p.o.) inhibits the development of ibuprofen-induced gastric lesions in a dose-dependent manner (ED50 18·4 mg kg^{-1}). To investigate this protective effect of caffeine, we have studied the effect of caffeine on HCl–ethanol-induced gastric mucosal lesions with or without indomethacin pretreatment.

Caffeine inhibited the development of HCl–ethanol-induced gastric lesions with and without indomethacin pretreatment. These results indicate that caffeine did not act as a mild irritant but, on the contrary, had protective effects. We measured the gastric mucosal prostaglandin E_2 (PGE_2) concentrations and gastric mucosal blood flow, as representative protective factors for gastric mucosa. Caffeine did not affect the gastric mucosal PGE_2 concentrations 4 h after administration of ibuprofen. However, topical administration of caffeine resulted in an increase in gastric mucosal blood flow, as measured by laser Doppler flowmetry. We investigated the gastric acid secretion and gastric mucosal myeloperoxidase activity as representative aggressive factors for gastric mucosa. When caffeine was administered intraduodenally in pylorus-ligated rats, gastric acid secretion decreased in a dose-dependent manner, with an ED50 of 44·9 mg kg^{-1}. Caffeine decreased ibuprofen-induced gastric myeloperoxidase activity in a dose-dependent manner, with an ED50 of 9·1 mg kg^{-1}.

These findings indicate that caffeine, at least in rats, may inhibit the development of acute gastric mucosal injury. The mechanisms underlying the protective actions of caffeine are unclear, but may be related in part to an increase in gastric mucosal blood flow and suppression of neutrophil activation.

Caffeine has been known to stimulate gastric acid secretion in man (Cohen & Booth 1975) and rats (Kowalewski 1973; Seegers et al 1979), and to induce gastric lesions after pretreatment with aspirin (acetylsalicylic acid), phenylbutazone or reserpine in rats (Tariq et al 1985). Also, it has been reported that caffeine potentiates the formation of gastric lesions induced by restraint plus water-immersion (25°C) stress in rats (Yano et al 1982). However, it has also been reported that caffeine prevents the formation of gastric lesions induced by aspirin or restraint plus water-immersion stress in rats (Okabe 1981). It remains unclear whether caffeine truly induces gastric mucosal damage.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and aspirin are analgesics frequently prescribed as over-the-counter drugs. Side effects of these drugs include the production of erosions and ulcers in the stomach (Beck et al 1990; Scarpignato 1995). NSAID and caffeine mixtures have been prescribed as over-the-counter drugs. It has therefore been suggested that NSAIDs in combination with caffeine may induce the formation of gastric ulcers to a greater extent than NSAIDs alone because of the potentiating effect of caffeine.

This study was designed to determine whether caffeine indeed potentiates ibuprofen-induced gastric mucosal damage in rats.

Correspondence: M. Furukawa, Osaka Research Laboratory, Sawai Pharmaceutical Co. Ltd, 1-8-14 Ikue, Asahi-ku, Osaka 535-0004, Japan. E-Mail: m-furu@ja2.so-net.ne.jp

Materials and Methods

Animals

Male Donryu rats (Charles River, Tokyo, Japan) weighing 120–180 g (300–400 g for the gastric

mucosal blood flow study) were used. The animals were housed in a light-controlled room under a 12-h light/dark cycle at $23 \pm 2^{\circ}C$ and $55 \pm 10\%$ relative humidity. Animals were deprived of food for 18 h before the experiments, but were allowed free access to water.

Drugs

Caffeine anhydrous, aspirin and hexadecyltrimethylammoniumbromide (HTAB) were from Wako Pure Chemical Industries Ltd (Osaka, Japan). *o*-Dianisidine dihydrochloride, ibuprofen and indomethacin were from Sigma Chemical Co. (St Louis, MO).

Caffeine, ibuprofen, indomethacin and aspirin were suspended in 0·5% sodium carboxymethylcellulose (CMC-Na) for oral or intraduodenal administration. Caffeine was dissolved in saline for intraluminal, intraperitoneal or subcutaneous administration. Control rats received a similar volume of 0·5% CMC-Na or saline.

NSAID-induced gastric lesions

Rats were administered NSAIDs orally, and 4 h later were killed with ether and their stomachs removed. After formalin treatment, each stomach specimen was examined for lesions. Caffeine was given orally concomitantly with NSAIDs.

HCl–ethanol-induced gastric lesions

HCl–ethanol (60% ethanol in 150 mM HCl) was administered orally to rats at a volume of 1 mL per rat (Mizui & Doteuchi 1983). The animals were killed 1 h later with ether and their stomachs removed. After formalin treatment, each stomach specimen was examined for lesions. Caffeine was administered 0·5 h before the administration of HCl–ethanol.

Indomethacin was used to block endogenous formation of prostaglandin through inhibition of prostaglandin cyclooxygenase. Indomethacin was suspended in 0·5% CMC-Na, and was administered orally 1 h before the administration of 20% ethanol (mild irritant) or caffeine. This was followed 15 min later by the administration of HCl–ethanol solution.

Determination of gastric mucosal damage

The length (mm) of the gastric lesion was measured under a dissecting microscope ($\times 10$) with a square grid ($\times 10$), and the sum of the lengths was regarded as the lesion index.

Measurement of gastric mucosal PGE_2

Gastric mucosa was prepared using the method described by Kobayashi et al (1985). The stomachs were quickly excised, opened along the greater curvature and washed with phosphate-buffered saline (PBS) containing 10^{-4} M indomethacin. The gastric corpus was cut into approximately 5×5 mm pieces, placed between two glass slides, and frozen with hexane in a dry ice/acetone bath. The frozen gastric mucosa was pulled away from the slides and homogenized in acetone and centrifuged, and the supernatant was stored at $-30^{\circ}C$. The PGE_2 in the supernatants was purified on Isolute Bond Elute C_{18} columns according to the method of Powell (1980), and its concentrations were measured using an enzyme-linked immunoassay kit (Cayman Chemical Company, Ann Arbor, MI). Drugs were administered 4 h before the rats were killed. Control animals were given 0·5% CMC-Na alone.

Measurement of gastric mucosal blood flow (GMBF)

Animals were anaesthetized with urethane (i.p.). Measurement of GMBF was performed in the chambered stomach according to Matsumoto et al (1992). The abdomen was incised and the stomach was exposed, mounted on a chamber and perfused at a flow rate of 0·7 mL min^{-1} with saline preheated to $37^{\circ}C$, and kept in a reservoir. GMBF was measured by laser Doppler flowmetry (BPM2; Vasamedics Inc., St Paule, MN) after placing the probe (P-433-5, 1·4 mm diam.; Vasamedics Inc.) softly on the surface of the corpus mucosa using a balancer. At least 30 min after the parameter stabilized, the perfusion was discontinued, the luminal solution was removed, and the mucosa was exposed for 15 min to 5 mL kg^{-1} saline. After saline treatment, the mucosa was rinsed with saline, and the perfusion was resumed at least 15 min before the parameter stabilized. Then, after the luminal solution was removed, caffeine was applied topically to the stomach for 120 min.

Measurement of gastric secretion

Effect of caffeine on gastric secretion was estimated following pyloric ligation (Shay et al 1945). The animals were administered caffeine, intraduodenally, immediately after pylorus ligation and killed 4 h later. The stomachs were removed after ligating the oesophagogastric junction and the gastric contents were collected. The gastric contents were centrifuged for 10 min at 3000 rev min^{-1} and the volume of the supernatant measured.

Acidity was determined by titration of the gastric contents against 0·1 M NaOH to pH 7·0 in a potentiometric automatic titrator (AT-400 Kyoto Electronics, Kyoto, Japan). Acid output was expressed as μEq h^{-1}.

Measurement of myeloperoxidase activity

Myeloperoxidase tissue activity was assayed using *o*-dianisidine as substrate (Krawisz et al 1984). Gastric mucosa (200–400 mg) was prepared using the method of Kobayashi et al (1985) and homogenized in 1 mL 50 mM phosphate buffer, pH 6·0 containing 0·5% HTAB on ice. The homogenate was sonicated for 10 s and freeze–thawed twice. The homogenate was then centrifuged at 40 000 *g* for 15 min at 4°C. The supernatant was assayed for myeloperoxidase activity spectrophotometrically. A 0·1-mL sample of the supernatant was combined with 2·9 mL 50 mM phosphate buffer, pH 6·0, containing 0·167 mg mL^{-1} *o*-dianisidine dihydrochloride and 0·0005% hydrogen peroxide. The change in absorbance at 460 nm was measured. One unit of myeloperoxidase activity was defined as the amount of myeloperoxidase degrading 1 μmol peroxide min^{-1} at 25°C. Rats were killed 3 h after the drug treatment.

Measurement of gastric motility

Gastric motility was measured in conscious rats using a miniature balloon as described by Yano et al (1978). Under ether anaesthesia, the balloon and the support catheter were placed in the stomach through an incision on the forestomach. The animals were kept in Bollman cages and after complete recovery from anaesthesia the gastric motility was monitored on a Nihon Koden recorder using a pressure transducer (Nihon Koden, Tokyo, Japan) and a polygraph (Nihon Koden, Tokyo, Japan). After basal motility had stabilized, the animals were administered caffeine subcutaneously at a dose of 10–100 mg kg^{-1}. Gastric motility was measured for 2 h thereafter. To quantify gastric motility, the amplitude of each contraction (clear spike) was measured over a 10-min period, determining the mean for this period in each rat from these values and by calculating the mean ± s.e. for each time period from four to five different rats per group.

Statistical analysis

Results were expressed as mean ± s.e. Statistical significance was evaluated by Student's *t*-test or Dunnett's test. Differences were considered significant at $P < 0.05$ and $P < 0.01$.

Results

Effect of caffeine on NSAID-induced gastric lesions

Oral administration of 100 mg kg^{-1} ibuprofen produced haemorrhagic gastric lesions (lesion index 17·7 ± 3·0 mm). When rats were administered caffeine orally at 10, 30, or 100 mg kg^{-1} in combination with ibuprofen, the development of mucosal injury was inhibited in a dose-dependent manner, with an ED50 of 18·4 mg kg^{-1} (Table 1).

Caffeine (50 mg kg^{-1}) significantly inhibited indomethacin-induced gastric lesions and aspirin-induced gastric lesions (Table 2).

Effect of caffeine on HCl–ethanol-induced gastric lesions

Oral administration of HCl–ethanol (60% ethanol in 150 mM HCl) to rats caused haemorrhagic gastric lesions (lesion index 75·0 ± 10·9 mm). Oral administration of caffeine at 10–100 mg kg^{-1}, 30 min before HCl–ethanol administration, reduced lesions in a dose-dependent manner, with an ED50 of 56·4 mg kg^{-1} (Table 3). Caffeine at the

Table 1. Effect of caffeine on ibuprofen-induced gastric mucosal lesions in rats.

Treatment		Lesion index (mm)
Control (ibuprofen alone)		17·7 ± 3·0
Ibuprofen plus caffeine	10 mg kg^{-1}	16·8 ± 3·7
	30 mg kg^{-1}	5·5 ± 1·9*
	100 mg kg^{-1}	1·4 ± 0·5**

Caffeine (p.o.) was given concomitantly with ibuprofen (100 mg kg^{-1}, p.o.). Animals were killed 4 h after drug treatment. The values are mean ± s.e. of 12 rats. *$P < 0.05$, **$P < 0.01$, significantly different from the control group.

Table 2. Effect of caffeine on various NSAID-induced gastric mucosal lesions in rats.

Treatment	Lesion index (mm)
Ibuprofen (100 mg kg^{-1}, control)	15·8 ± 3·2
Ibuprofen (100 mg kg^{-1}) plus caffeine (50 mg kg^{-1})	0·4 ± 0·1**
Indomethacin (20 mg kg^{-1}, control)	21·7 ± 3·9
Indomethacin (20 mg kg^{-1}) plus caffeine (50 mg kg^{-1})	5·2 ± 2·4**
Aspirin (50 mg kg^{-1}, control)	32·4 ± 8·7
Aspirin (50 mg kg^{-1}) plus caffeine (50 mg kg^{-1})	8·7 ± 5·0*

Caffeine (50 mg kg^{-1}, p.o.) was given concomitantly with NSAID. Animals were killed 4 h after drug treatment. The values are mean ± s.e. of six to ten rats. *$P < 0.05$, **$P < 0.01$, significantly different from the corresponding control group.

Table 3. Effect of caffeine on HCl–ethanol-induced gastric mucosal lesions in rats.

Treatment		Lesion index (mm)
HCl–ethanol (control)		75·0 ± 10·9
Caffeine pretreatment	10 mg kg^{-1}	85·5 ± 10·5
	30 mg kg^{-1}	51·8 ± 9·5
	100 mg kg^{-1}	23·3 ± 6·8**

Rats were given 1mL 60% ethanol in 150mM HCl orally 30 min after caffeine treatment (p.o.). Animals were killed 90 min after caffeine treatment. The values are mean ± s.e. of 11–12 rats. $^{**}P < 0{\cdot}01$, significantly different from the control group.

dose of 100 mg kg^{-1} intraperitoneally also prevented significantly the development of mucosal injury (Table 4).

The effects of endogenous prostaglandins on HCl–ethanol-induced gastric lesions were examined (Table 5). The administration of caffeine (100 mg kg^{-1}) and 20% ethanol almost completely inhibited HCl-ethanol induced gastric lesions. Oral pretreatment with indomethacin 1 h before the administration of caffeine or 20% ethanol inhibited the inhibitory activity of 20% ethanol, but the inhibitory activity of caffeine remained.

Table 4. Effect of caffeine given orally or intraperitoneally, on HCl-ethanol-induced gastric mucosal lesions in rats.

Treatment	Lesion index (mm)
Oral administration	
HCl–ethanol (control)	106·8 ± 19·6
Caffeine plus HCl–ethanol	5·0 ± 1·4**
Intraperitoneal administration	
HCl–ethanol (control)	97·3 ± 4·9
Caffeine plus HCl–ethanol	27·1 ± 6·3**

Rats were given 1 mL 60% ethanol in 150 mM HCl orally 30 min after caffeine treatment (100 mg kg^{-1}, p.o. or i.p.). Animals were killed 90 min after caffeine treatment. The values are mean ± s.e. of six rats. $^{**}P < 0{\cdot}01$, significantly different from the corresponding control group.

Table 5. Effect of caffeine on gastric mucosal lesions induced by HCl–ethanol with or without indomethacin pretreatment in rats.

Treatment	Lesion index (mm)
Without indomethacin pretreatment	
HCl–ethanol (control)	987.2 ± 20.4
Caffeine plus HCl–ethanol	4.9 ± 1.9**
20% Ethanol plus HCl–ethanol	9.7 ± 3.6*
With indomethacin pretreatment	
HCl–ethanol (control)	164.5 ± 21.1
Caffeine plus HCl–ethanol	43.7 ± 12.6**
20% Ethanol plus HCl–ethanol	147.8 ± 18.4

Animals were given 1 mL 60% ethanol in 150 mM HCl orally 15 min after caffeine (100 mg kg^{-1}) or 20% ethanol treatment (p.o.). Animals were also given oral indomethacin (5 mg kg^{-1}) 60 min before drug treatment. Animals were killed 75 min after drug treatment. The values are mean ± s.e. of six rats. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, significantly different from the corresponding control group.

Effect of caffeine on the gastric mucosal concentration of PGE_2

Gastric mucosal PGE_2 concentration was 408·7 ng g^{-1} wet tissue in normal rats. Gastric mucosal PGE_2 concentration was significantly reduced following the administration of ibuprofen alone (38·7 ± 1·6 ng (g tissue)$^{-1}$, $P < 0{\cdot}01$, n = 9) or caffeine alone (234·9 ± 31·7 ng (g tissue)$^{-1}$, $P < 0{\cdot}05$, n = 9), the inhibition being 90·5% and 42·5%, respectively. Concomitant administration of ibuprofen and caffeine markedly decreased the mucosal PGE_2 concentration (35·7 ± 2·6 ng (g tissue)$^{-1}$, $P < 0{\cdot}01$, n = 9) to the same degree as observed in animals following the administration of ibuprofen alone.

Effect of caffeine on GMBF

Exposure of the mucosa to saline as vehicle had no effect on GMBF. However, the GMBF increased immediately after exposure to caffeine at the dose of 30 and 100 mg kg^{-1}, reaching maximal levels of about 130 and 160% of basal values, at 10 and 15 min, respectively. These levels reverted to the saline control level at about 50 and 90 min, respectively, after exposure (Figure 1).

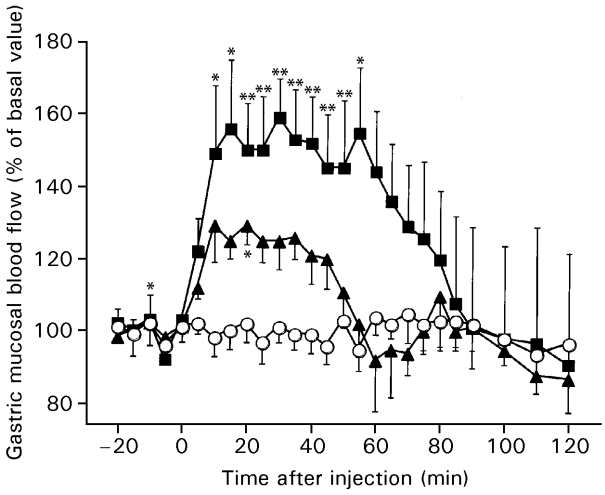

Figure 1. Effect of mucosal application of caffeine on gastric mucosal blood flow in anaesthetized rat stomachs. The mucosa was superfused with saline (○) and then exposed to caffeine (▲ 50 mg kg^{-1}, ■ 100 mg kg^{-1}). Each point represents the mean ± s.e. of values determined every 1 min in five rats. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ compared with the saline group.

Table 6. Effect of caffeine on gastric secretion in pylorus-ligated rats.

Treatment	Dose ($mg\,kg^{-1}$)	Volume (mL)	Acid output ($\mu Eq\,h^{-1}$)	Acid output inhibition (%)
Vehicle	—	7·6 ± 0·9	248·2 ± 33·2	—
Caffeine	10	7·6 ± 0·5	240·8 ± 22·7	3·0
	30	6·1 ± 0·7	161·8 ± 27·0	34·8
	100	3·2 ± 0·2**	49·8 ± 6·3**	79·9

Caffeine was given intraperitoneally just after pylorus ligation. Animals were killed 4 h after ligation. Each value represents the mean ± s.e. of nine to ten rats. $^{**}P < 0{\cdot}01$, significantly different from the vehicle group.

Effect of caffeine on basal gastric secretion

Four hours after pylorus ligation, the volume of the gastric contents and acid output in the control group was about 7·6 mL and 248·2 $\mu Eq\,h^{-1}$, respectively. When caffeine was administered orally at a dose of 10–100 $mg\,kg^{-1}$, the volume and acid output significantly decreased, in a dose-dependent manner. The ED50 of caffeine for acid output was 44·9 $mg\,kg^{-1}$ (Table 6).

Time-course of changes in gastric mucosal lesions and gastric mucosal myeloperoxidase activity in ibuprofen-treated rats

Gastric mucosal lesions developed following the administration of ibuprofen, reaching the maximum aggregate length 4 h after administration (Figure 2A). Myeloperoxidase activity increased significantly after oral administration of ibuprofen (100 $mg\,kg^{-1}$) and reached the maximal levels 3 h after administration (Figure 2B). Therefore, the effect of caffeine on myeloperoxidase activity was evaluated 3 h after the administration of ibuprofen.

Effect of caffeine on myeloperoxidase activity

Myeloperoxidase activity in the vehicle-treated rat stomach was 0·08 unit $(g\,tissue)^{-1}$. Oral administration of ibuprofen (100 $mg\,kg^{-1}$) increased myeloperoxidase activity, to values of 0·30 unit $(g\,tissue)^{-1}$. Administration of caffeine (10, 30 or 100 $mg\,kg^{-1}$) suppressed the increase in myeloperoxidase activity induced by ibuprofen in a dose-dependent manner, with an ED50 of 9·1 $mg\,kg^{-1}$ (Figure 3).

Effect of caffeine on gastric motility

Gastric motility increased immediately following the administration of caffeine (30 $mg\,kg^{-1}$, s.c.), reaching the maximum level of 150% of the basal level approximately 20 min later, and reverting to the basal level approximately 60 min later. Gastric motility also increased 10 min after ibuprofen administration (100 $mg\,kg^{-1}$, s.c.), reaching the maximal level of 150% of the basal level

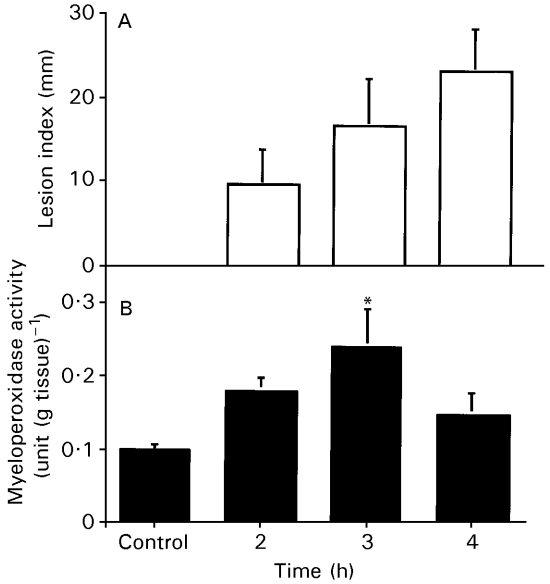

Figure 2. Time-course of changes in gastric mucosal lesions (A) and gastric mucosal myeloperoxidase activity (B) in ibuprofen-treated rats. Animals were killed 2, 3 or 4 h after oral administration of ibuprofen (100 $mg\,kg^{-1}$). Each column represents the mean ± s.e. of six (A) or four (B) rats. $^{*}P < 0{\cdot}05$ compared with the control group.

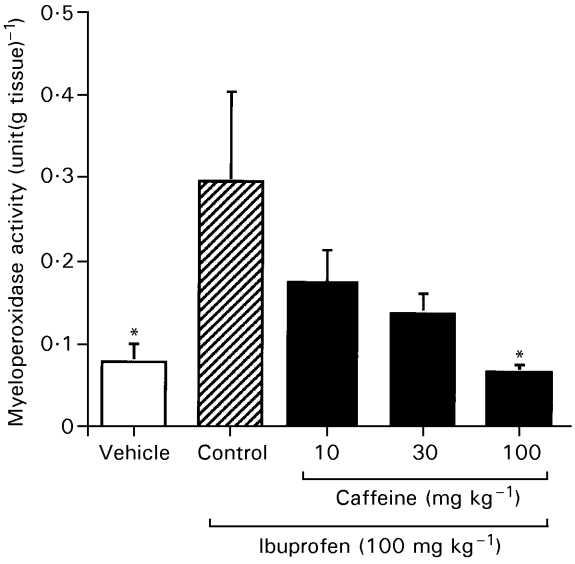

Figure 3. Effect of caffeine on gastric mucosal myeloperoxidase activity in ibuprofen-treated rats. Animals were killed 3 h after drug treatment (p.o.). Each column represents the mean ± s.e. of five rats. $^{*}P < 0{\cdot}05$ compared with the control group.

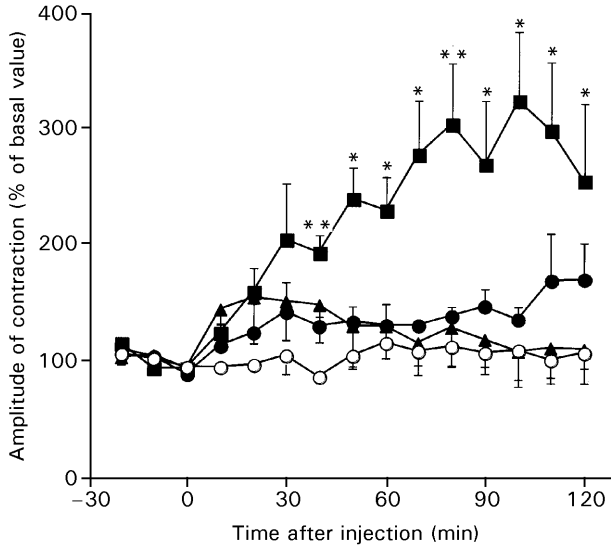

Figure 4. Effect of ibuprofen, caffeine and ibuprofen plus caffeine on gastric motility in rats. Animals were given the drugs subcutaneously (○ saline, ● ibuprofen 100 mg kg^{-1}, ▲ caffeine 30 mg kg^{-1}, ■ ibuprofen + caffeine 100 + 30 mg kg^{-1}). Each value represents the mean ± s.e. of values determined every 10 min from four to five rats per group. *$P < 0.05$, **$P < 0.01$ compared with the saline group.

approximately 50 min later, and remaining elevated for 2 h. Following concomitant administration of ibuprofen and caffeine (100 + 30 mg kg^{-1}, s.c.), gastric motility increased gradually reaching the maximal level of 300% of the basal level, and decreased thereafter (Figure 4).

Discussion

The irritant effects of caffeine on the gastric mucosa (Cohen & Booth 1975; Seegers et al 1979; Tariq et al 1985) and the clinically inducing damage of NSAIDs in the gastrointestinal mucosa of experimental animals (Beck et al 1990; Wagner et al 1995) and man (Scarpignato 1995) has been widely reported. It has therefore been suggested that concomitant administration of NSAIDs and caffeine is associated with a higher risk of development of gastric ulcer than administration of NSAIDs alone. However, the results of this study show that caffeine prevented the formation of ibuprofen-induced gastric lesions in a dose-dependent manner; caffeine also prevented indomethacin- or aspirin-induced gastric lesions. These results demonstrate that caffeine inhibits the development of gastric mucosal injury.

We examined the effect of caffeine on HCl–ethanol induced gastric mucosal injury, which is known to be unrelated to acid output and is frequently used for the screening of gastric protective drugs (Mizui & Doteuchi 1983). Caffeine inhibited the development of such lesions in a dose-dependent manner. Therefore, we investigated the effect of caffeine as a mild irritant (such as 20% ethanol, 0·10–0·35 M HCl) (Robert et al 1983) to the gastric mucosa. When endogenous prostaglandin synthesis was blocked by indomethacin pretreatment, the administration of caffeine inhibited HCl–ethanol-induced gastric lesions, but injury induced by 20% ethanol, a mild irritant, was not inhibited. Oral and intraperitoneal administration of caffeine inhibited the development of gastric mucosal lesions. These results suggest that caffeine has gastric protective activity and does not act as a mild irritant to the gastric mucosa.

To clarify the mechanism of action of caffeine, we examined its effect on gastric mucosal protective factors such as mucosal prostaglandin levels and GMBF which are representative of gastric mucosal protective mechanisms (Miller 1983). Robert et al (1979) reported that the administration of PGE_2 prevented the formation of gastric lesions induced by absolute ethanol in rats. It is known that NSAIDs decrease gastric mucosal PGE_2 concentration (Kobayashi et al 1985; Wagner et al 1995). Therefore, we examined the effect of caffeine on gastric mucosal PGE_2 concentrations. Caffeine did not affect the gastric mucosal PGE_2 concentration decreased by ibuprofen; on the contrary, caffeine administration itself decreased the gastric mucosal PGE_2 concentration. It was previously considered that endogenous gastric mucosal prostaglandins play an important role in the maintenance of mucosal integrity. However, Ligumsky et al (1982) and Redfern et al (1987) reported that there was no correlation between the degree of decrease in prostaglandin concentration and mucosal damage. Thus, it may be assumed that gastric mucosal protection by caffeine does not involve gastric mucosal PGE_2 concentrations.

It is assumed that GMBF is one of the most important factors for the supply of oxygen and energy, translocating H^+ back-diffused into the gastric mucosa (Guth 1980). In this study, exposure of the mucosa to caffeine induced an increase in GMBF. Therefore, GMBF may be one of the gastric mucosal factors of caffeine conferring protection against injury. Beubler & Lembeck (1976) reported that caffeine increased mesenteric blood flow by causing an elevation in cAMP content in mesenteric arteries. Our results revealed that caffeine at the dose of 50–100 mg kg^{-1} (p.o.), which causes elevation in cAMP content, increased GMBF (Sawynok & Yaksh 1993). Therefore, caffeine might increase the microcirculation in the gastric mucosa, an action secondary to its effect on cAMP.

Whittle & Lopez Belmonte (1993) have shown that nitric oxide (NO) plays a role in maintaining gastric mucosal integrity, mainly through regulation of the gastric mucosal microcirculation. We investigated the role of NO in gastric mucosal protection by caffeine. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME; $10\,mg\,kg^{-1}$, i.v.), an NO synthase inhibitor, before oral administration of HCl–ethanol did not influence caffeine-induced gastric protection (data not shown). This suggests that the protective effect of caffeine may not be mediated by endogenous NO.

Reports by Cohen & Booth (1975), Seegers et al (1979), and Kowalewski (1973) indicate that caffeine stimulates gastric acid secretion in man and animals. This stimulation of acid secretion is thought to increase cAMP content in the gastric mucosa through inhibition of phosphodiesterase. Thus, we examined the effect of caffeine on gastric acid secretion as an aggressive factor. However, our results indicate that caffeine induced a decrease in gastric juice volume and acid output in a dose-dependent manner in pylorus-ligated rats. Yano et al (1982) and Ozturkcan et al (1974) have reported that higher doses of caffeine inhibited acid output in pylorus-ligated rats. Yano et al (1982) suggested that the inhibition of gastric secretion might result from the release of adrenal catecholamines mediated by caffeine (Snider & Waldeck 1974). The decrease of acid output by caffeine observed in our study could be due to an increase in adrenal catecholamine release rather than an increase in cAMP content. However, it is not clear how caffeine decreases acid-output in pylorus-ligated rats.

The mechanism of NSAID-induced gastric mucosal lesions is still unclear. Recently it has been suggested that neutrophil activation (Alican et al 1995; Yoshida et al 1995) and gastric motility (Ueki et al 1988) are important factors in the pathogenesis of indomethacin-induced gastric mucosal lesions in rats. Several investigators have shown that myeloperoxidase activity can be used as a marker of tissue neutrophil infiltration (Alican et al 1995; Yoshida et al 1995; Takeuchi et al 1997). Thus, we examined the effect of caffeine on gastric mucosal myeloperoxidase activity in ibuprofen-treated rats. Oral administration of ibuprofen caused an increase in myeloperoxidase activity in a time-dependent manner, with maximal levels observed 3 h after administration. However, caffeine inhibited the myeloperoxidase activity in a dose-dependent manner. Takeuchi et al (1997) reported that the gastric infiltration of leucocytes induced by indomethacin is not a secondary effect but a cause of tissue damage. Our results indicate that accumulation of neutrophils preceded maximum gastric mucosal injury induced by ibuprofen. These findings indicate that neutrophil infiltration of the gastric mucosa may be involved in the mucosal lesions induced by NSAIDs and that caffeine inhibited this neutrophil infiltration. However, the mechanism of inhibition of neutrophil infiltration by caffeine is not clear.

We examined the effect of caffeine on gastric motility. Ibuprofen increased gastric motility 1·5-fold compared with the saline group. Ibuprofen in combination with caffeine significantly increased gastric motility to about three times that in the saline group. This increase in gastric motility may indicate that caffeine stimulates parasympathetic nerve supply through its action on the central nervous system. Our results show that caffeine decreases the extent of mucosal injury induced by ibuprofen, but increases gastric motility. Takeuchi et al (1991) reported that both superoxide dismutase and dimethylsulphoxide decreased the extent of mucosal injury induced by indomethacin, but did not inhibit the increase in gastric motility induced by indomethacin. Therefore, gastric motility may not necessarily be an important factor in the induction of gastric mucosal lesions by NSAIDs. The present results indicate that the increase in GMBF may be due to an increase in gastric mucosal cAMP content induced by caffeine. It was previously considered that an increase in the cAMP content caused an increase in acid output and decrease of gastric motility, but our present data show a decrease in acid output and an increase in gastric motility. At present, we cannot account for this discrepancy. Further experiments are necessary to elucidate the mechanisms underlying the protective actions of caffeine.

In conclusion, the results of the present study show that caffeine inhibits gastric mucosal injury induced by ibuprofen. The mechanism underlying the protective actions of caffeine are unclear, but may be related in part to an increase in GMBF and suppression of neutrophil activation.

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