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Gastric and Duodenal Anti-ulcer Activity of SKF 38393, a Dopamine D_1-Receptor Agonist in Rats

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Abstract

The effect of SKF 38393 (1-phenyl-7,8-diol-2,3,4,5-tetrahydro-1H-3-benzazepine), a specific dopamine D_1-receptor agonist, was studied on pylorus-ligation and water immersion plus restraint stress-induced gastric ulcers, and cysteamine-induced duodenal ulcers in rats.

Repeated administration of SKF 38393 (5 and 10 mg kg^{-1}, p.o.) for six days was found to be effective in the prevention of gastric ulceration induced by water immersion plus restraint stress in rats. In 19-h pylorus-ligated rats, repeated treatment with SKF 38393 showed a significant reduction in the number and severity of ulcers. SKF 38393 did not alter the total gastric-mucosal carbohydrates : protein ratio; however, the gastric content volume and the free and total acidity were significantly reduced. In cysteamine-induced duodenal ulcers, the treatment with SKF 38393 for 6 days prevented the duodenal lesions.

Our data suggests the involvement of dopamine D_1 receptors in the anti-ulcer activity of SKF 38393, which could be largely attributed to its anti-secretory effect. Its anti-ulcer activity against water immersion plus restraint, also points towards a central mode of action, but its failure to alter the carbohydrate : protein ratio rules out any protective effect through the strengthening of the gastric mucosal barrier.

In recent years dopamine has been regarded as not only an important central neurotransmitter, but also an enteric neurotransmitter located in the nerve terminals of the myenteric plexus as well as in the mammalian gastric mucosa (Eaker et al 1987). It has been implicated in the genesis of experimentally-induced gastric and duodenal ulcers (Szabo 1979; Hernandez et al 1984; Sikiric et al 1985). Clinically, a high incidence of duodenal ulcers has been observed in patients with Parkinson's disease (associated with dopamine deficiency) and a low occurrence is seen in schizophrenics (associated with dopamine excess or hyperactivity) (Strang 1965; Hinterhuber & Hochenegg 1975). Ulcerogenic as well as anti-ulcer activity has been reported with different dopamine agonists and antagonists (Szabo 1979; Parmar et al 1984; Sikiric et al 1986; Glavin & Dugani 1987). It appears that dopamine D_1 and D_2 receptors have opposing effects on gastric and duodenal ulceration, gastrointestinal motility, gastric mucosal blood flow and gastric acid secretion (Parmar et al 1986; Glavin & Dugani 1987; Glavin & Szabo 1990; Nagahata et al 1992; Desai & Parmar 1994). While dopamine D_2-receptor antagonists such as sulpiride (Lam et al 1979; Desai & Parmar 1994) and domperidone (Weihrauch & Ewe 1981; Parmar et al 1986) have been reported to produce an anti-ulcer effect, dopamine antagonists such as pimozide (Pihan & Szabo 1984), butaclamol and spiperone (Chakravorty 1992) which possess more dopamine D_1 activity have been reported to produce ulcerogenic activity in various models of gastric and duodenal ulcers. The dopamine D_2 agonist, bromocriptine, has been reported to produce a pro-ulcerogenic effect when administered as a single dose treatment (Parmar et al 1984). It was suggested by Glavin (1989) that the gut dopamine receptors may be of the D_1 subtype and the anti-ulcer effect of SKF 38393 involves the reduction of gastric acid output. Glavin (1989) however, could not rule out the possibility of the involvement of other gastroprotective mechanisms such as the effect of the gastric mucosal barrier. In the present investigation we have studied the effect of the selective dopamine D_1-receptor agonist, SKF 38393 (Setler et al 1978), using pylorus ligation, water immersion plus restraint stress and cysteamine hydrochloride-induced duodenal lesion models in rat.

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Materials and Methods

Wistar albino rats of either sex, 200–220 g, and fed on standard rat chow diet were divided into groups of 8–10 animals. The distribution of animals in groups, the sequence of trials and the treatment allotted to each group were randomized. The animals were killed using anaesthetic ether after the completion of experiments, the stomachs were removed, opened along the greater curvature, washed with saline and examined with a 6.4× binocular magnifier. Lesions were assessed by two observers unaware of the experimental protocol.

SKF 38393 (1-phenyl-7,8-diol-2,3,4,5-tetrahydro-1H-3-benzazepine) obtained from SmithKline Beecham Pharmaceuticals, USA was administered orally as an aqueous suspension prepared in 1% carmellose in doses of 5 and 10 mg kg^{-1}, given once daily for six consecutive days before the induction of ulceration.

Experimental gastric ulcers

The animals were starved for 36 h with free access to water before subjecting them to one of the following procedures.

Pylorus ligated rats. The pylorus was ligated under light ether anaesthesia with care being taken not to cause bleeding or to occlude blood vessels. Nineteen hours after ligation, the animals were killed by an overdose of ether. The stomachs were removed, their contents collected, measured, centrifuged and subjected to the analysis for total acidity by titrating against 0·01 M NaOH to pH 8·0 using phenolphthalein as indicator. Peptic activity was determined using haemoglobin as substrate according to the modified method of Debnath et al (1974). Two aliquots of gastric juice were also taken and 95% ethyl alcohol was added to these aliquots in 1 : 10 ratio. After 10 min they were centrifuged at 5000 rev min^{-1} for 10 min and the supernatants were removed. The precipitate of one of the tubes was used to estimate sialic acid (Warren 1959). The precipitate of the other tube was utilized to estimate fucose (Dische & Schettles 1948), hexosamine (Dische & Boronfreund 1950), total hexoses (Winzler 1958) and protein (Lowry et al 1951). The ratio between the total carbohydrate and protein was then determined and this was used as an index of dissolved mucosubstances in the gastric juice.

Each stomach was examined for lesions in the stomach portion which were measured and expressed in terms of the ulcer index calculated as the total ulcerated area divided by the total mucosal area.

Ulcers induced by restraint plus water immersion. The rats were immobilized in a restrainer and then immersed to the level of the xiphoid process in a waterbath at 20° ± 0.5°C. After 16 h the animals were killed by an overdose of ether. The stomachs were removed and examined for the severity of intraluminal bleeding according to the following scale: 0, no blood detectable; 1, thin blood follows the rugae; 2, thick blood follows the rugae; 3, thick blood follows the rugae with blood clot in certain areas; and 4, extensive covering of the whole gastric mucosal surface with thick blood. The blood was wiped off, and the intensity of lesions was scored by measuring their maximum continuous length.

Experimental duodenal ulcers

Cysteamine-induced duodenal ulcers in rats. The method described by Szabo (1978) was followed. Wistar albino rats of either sex, 200–220 g, were used. Food and water were freely available throughout the study. Duodenal ulcers were induced by the administration of cysteamine hydrochloride (400 mg kg^{-1}, p.o.) in 10% aqueous solution at intervals of 4 h. SKF 38393 was administered orally, 30 min before each dose of cysteamine. Animals were killed 24 h after the first dose of cysteamine and duodenum were excised carefully and opened along the antimesenteric side. The duodenal ulcers were scored for intensity using a scale of 0 to 3 where 0 = no ulcer, 1 = superficial mucosal lesion, 2 = deep ulcer or transmural necrosis, and 3 = perforated or penetrated ulcer (into the pancreas or liver). The ulcer index is the sum of the arithmetic mean of the intensity in a group and the ratio of positive/total multiplied by 2.

Statistical analysis

Statistical analysis of the severity of gastric and duodenal lesions and other parameters was by Student's *t*-test. The data for the incidence of intraluminal bleeding was analysed statistically using Fischer's exact test.

Results

Pylorus-ligated rats

Pylorus ligation for 19 h resulted in the accumulation of gastric secretory volume and increase in the total acid output and pepsin activity of the gastric juice. Circular lesions, usually localized in the rumenal as well as the glandular mucosa of the stomach, were observed in all the control animals. Linear lesions and petechiae were frequently seen. Pretreatment with SKF 38393 before pylorus ligation significantly reduced the ulcer index, volume of gastric acid secretion, free acidity and total acid output. The pepsin activity however, remained unchanged (Table 1). SKF 38393 treatment did not alter the total carbohydrate content of the gastric juice. The protein content as well as the total carbohydrate : protein ratio remained unchanged after pretreatment with either dose of SKF 38393 (Table 2).

Lesions induced by restraint plus water immersion

The animals subjected to restraint plus water immersion for 16 h showed the presence of considerable ulcerogenecity in the form of haemorrhagic mucosal lesions in the stomach. The area of involvement in all the animals was confined to the glandular segment only. There was also evidence of intraluminal bleeding in these animals. SKF 38393 produced a significant decrease in the area of ulceration which is represented as total gastric lesion length per rat (Table 3). A slight but statistically insignificant ($P > 0·05$) decrease in the incidence as well as intensity of the intraluminal bleeding was observed after both the doses of SKF 38393 used.

Cysteamine-induced duodenal ulcers

Administration of cysteamine hydrochloride caused mortality in 25% of rats in 24 h. The rats which died had perforated ulcers. Cysteamine produced duodenal ulcers in all the control rats. Usually two ulcers were produced close to the pylorus, the larger one on the anterior and the smaller one on the posterior wall of the duodenum. They were elongated

Table 1. Volume of gastric secretion acidity, pepsin activity and intensity of gastric lesions in pylorus-ligated rats treated orally with SKF 38393 for six days (n = 8).

Treatment (mg kg^{-1} day^{-1})	Volume of gastric contents (mL/100 g)	Total acid output (μEq/19 h)	Pepsin activity (μg mL^{-1})	Ulcer index
Control	11·23 ± 0·78	1134·23 ± 130·67	12·30 ± 0·28	0·196 ± 0·021
5	9·55 ± 0·43	742·03* ± 85·34	13·70 ± 1·36	0·072*** ± 0·006
10	8·07** ± 0·32	544·73** ± 63·08	11·92 ± 0·56	0·059*** ± 0·014

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

Table 2. Carbohydrates and protein contents of gastric secretions in pylorus-ligated rats treated orally with SKF 38393 for six days (n = 8).

Treatment (mg kg^{-1} day^{-1})	Carbohydrates (μg mL^{-1})				Total carbohydrates (μg mL^{-1})	Proteins (μg mL^{-1})	Carbohydrate /protein ratio
	Total hexoses	Hexosamine	Fucose	Sialic acid			
Control	204·4 ± 20·1	98·6 ± 8·1	50·0 ± 3·2	26·9 ± 5·9	379·90 ± 30·3	240·44 ± 20·2	1·58 ± 0·06
5	171·8 ± 29·2	112·0 ± 6·5	58·4 ± 3·6	43·2 ± 7·6	385·40 ± 26·3	230·78 ± 10·7	1·67 ± 0·12
10	190·4 ± 12·8	97·4 ± 10·7	53·6 ± 2·9	35·1 ± 4·2	376·50 ± 38·0	221·47 ± 12·8	1·70 ± 0·09

Table 3. Water-immersion plus restraint-stress ulcers in rats treated orally with SKF 38393 for six days (n = 8).

Dose (mg kg^{-1} day^{-1})	Intraluminal bleeding				Gastric lesions			
	Incidence		Score	% of control	Incidence		Length (mm/rat)	% of control
	Number	%			Number	%		
Control	8/8	100	2·60 ± 0·33	100	8/8	100	48·40 ± 3·03	100
5	6/8	75	2·01 ± 0·14	77	8/8	100	31·16** ± 2·93	64
10	5/8	62	1·91 ± 0·21	73	8/8	100	25·34*** ± 3·03	52

**$P < 0·01$, ***$P < 0·001$.

Table 4. Cysteamine-induced duodenal ulcers in rats treated orally with SKF 38393 for six days (n = 8).

Dose (mg kg^{-1} day^{-1})	Ulcer incidence		Ulcer score		Mortality		Mean ulcer area (mm^2)	Ulcer index
	Number	%	Score	% of control	Number	%		
Control	8/8	100	1·56 ± 0·37	100	2/8	25	27·42 ± 2·36	0·356
5	8/8	100	0·91 ± 0·31	60	0/8	00	14·62*** ± 1·37	0·241
10	8/8	100	0·78 ± 0·10	51	0/8	00	11·13*** ± 1·01	0·203

***$P < 0·001$.

extending longitudinally down the duodenum. Treatment with SKF 38393 produced non-significant decrease in the ulcer score ($P > 0·05$). A decrease in lethality was observed after pretreatment with SKF 38393. The mean ulcer area decreased markedly following the treatment with either dose of SKF 38393 (Table 4).

Discussion

In the present study, six days treatment with SKF 38393 was found to produce significant anti-ulcer activity in the models studied. There was a significant decrease in the gastric acid output in the pylorus-ligation model, indicating the involvement of an antisecretory effect of SKF 38393. These results are consistent with those reported by Glavin (1989). It has been postulated that the cysteamine duodenal ulcer is associated with gastric-acid hypersecretion (Ishii et al 1976). Consequently, the efficacy of SKF 38393 in inhibiting cysteamine ulcer can be largely explained by its potent anti-secretory activity, although its effect on peptide activity remained insignificant. This observation supports earlier studies indicating that gastric acid output was significantly

reduced by dopamine and dopamine receptor agonists (Glavin & Dugani 1987; Glavin 1989). In addition to the gastric acid secretion, reflex or neurogenic effects have also been proposed to play an important role in the formation of gastric ulcers in the pylorus-ligation model (Anichkov et al 1971). A possible central effect of SKF 38393, interfering with the vagal outflow cannot be ruled out, since vagal overactivity appears to contribute substantially to stress ulcer formation (Ogle et al 1985).

Experimental stress-induced gastric ulcerations have been suggested to result from autonomic nervous system hyperactivity and parasympathetic hyperactivity leading to focal vascular stasis of gastric mucosa and altered gastric mucosal microcirculation (Athey & Iams 1981). Apart from these peripheral events, the CNS has also been implicated in stress-induced gastric pathology and the available neurochemical data indicate the involvement of complex transmitter mechanisms in its regulation (Henke 1982). The dopaminergic system in particular has been postulated to play an inhibitory role in this phenomenon. Both centrally and peripherally administered dopamine and dopamine receptor agonists have been reported to attenuate stress ulcerogenesis, whereas opposite effects were observed with drugs which reduce dopaminergic activity (Sikiric et al 1986). The protective effect of SKF 38393 against cold-restraint stress lesions was reported to be blocked largely by indomethacin, meclofenamate, *N*-ethylmaleimide and doperidone (Glavin 1989). It was suggested from these findings that SKF 38393 may exert its anti-lesion and anti-secretory effects through prostaglandins (Pace-Asciak 1972; Glavin 1989).

The essential criterion which determines the status of the mucosal defense barrier against the offensive assault of acid-pepsin is the quality and quantity of gastric mucus secretion (Sanyal et al 1983). Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions; improving the buffering of acid gastric juice and by acting as an effective barrier to back diffusion of hydrogen ions. Increased protein content of the gastric juice has been suggested to represent exfoliation and shedding of gastric mucosal cells induced by the ulcerogenic agent. The total carbohydrate : protein ratio has been accepted as a reliable index of mucosal resistance (Sanyal et al 1983). Since SKF 38393 (5 and 10 mg kg^{-1}, p.o.) did not alter the total carbohydrates or the proteins, or the carbohydrate : protein ratio of the gastric juice, it can be postulated that cytoprotection is not involved in the mechanism of action of the dopamine D_1-receptor agonist SKF 38393.

In conclusion, our data suggest that dopamine D_1-receptor stimulation has a protective effect against gastric and duodenal ulcers. The mechanism of anti-ulcer activity appears to be by inhibition of gastric acid secretion and not by cytoprotection.

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