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Research Article

Protective effects of nedocromil sodium and sodium cromoglycate on gastroduodenal ulcers: a comparative study in rats

Mohammad Tariq[1,*], Meshal Al Moutaery[1], Ibrahim Elfaki[1], Mohammad Arshaduddin[1], Haseeb Ahmad Khan[2]

[1] Research Center, Armed Forces Hospital, P.O. Box 7897 (W-912), Riyadh 11159, Saudi Arabia, e-mail: rkh_research@yahoo.com
[2] Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia

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Abstract. Stabilization of mast cells plays a key mechanism to protect gastrointestinal tract from injury. This study presents a comparative evaluation of mast cell stabilizers nedocromil sodium (NDS) and sodium cromoglycate (SCG) in experimental gastric and duodenal ulcers in rats. Wistar rats of either sex were used in this study. Both NDS and SCG, in the doses of 10, 30 and 100 mg/kg were given intraperitoneally for gastric secretion studies and by gavage for antiulcer studies. Acid secretion studies were undertaken in pylorus-ligated rats. Gastric lesions were induced by water immersion restraint stress (WIRS), indomethacin and ethanol whereas duodenal ulcers were produced by cysteamine. The level of glutathione (GSH) and gastric wall mucus were measured in glandular stomach of rats following ethanol-induced gastric lesions.

SCG was more effective than NDS in preventing WIRS- and indomethacin-induced gastric lesions whereas reverse was true in ethanol- and cysteamine-induced ulcers. All the 3 doses of SCG offered almost equal protection against WIRS-induced gastric lesions whereas only medium and high dose of NDS provided significant protection in this model of ulcer. NDS significantly inhibited cysteamine-induced duodenal ulcers whereas SCG failed to do so. Pretreatment with NDS or SCG significantly and dose-dependently protected gastric mucosa against ethanol-induced injury, while the former drug appeared to be more effective. The cytoprotective effects of these two drugs were accompanied by the attenuation of ethanol-induced depletion of gastric wall mucus and GSH. The differential effects of NDS and SCG against various gastric lesions rationalize the possible benefits of a combined therapy (NDS+SCG) for the treatment of complex gastroduodenal ulcers.

Key words: Gastric ulcers; Duodenal ulcers; Mast cells; Nedocromil; Cromoglycate

* *Corresponding author*

Introduction

The acidity and volume of gastric acid secretion play a vital role in the pathogenesis of gastroduodenal ulcers (Nishida et al., 1994). Gastric acid secretion is precisely regulated by endocrine, paracrine and neurocrine signals via gastrin-histamine and cholecystokinin-somatostatin pathways. Targeting these mediators by pharmacological agents has been shown to modify the healing of gastric ulcers (Brimblecombe et al., 1975; Pendley et al., 1992; Tariq et al., 1987; Hung, 1998; Yamamoto et al., 2001). Besides hyperacidity, which is considered as one of the major aggressive factors for mucosal damage, body's immune system response to ulcerogenic stimuli plays a critical role in the pathogenesis of gastric ulcers. Immunoactivation as a result of noxious stimuli is accompanied by a cascade of proinflammatory cytokines, neutrophil proteases and oxygen-derived free radicals (ODFR), which could be more injurious to host tissue than the primary ulcerogenic stimuli itself (Huang et al., 2001; Watanabe et al., 2000). On the other hand, immunoneutralization or depletion of neutrophils has been shown to promote gastric ulcer healing (Huang et al., 2001; Watanabe et al., 2000; Shimizu et al., 2000).

Gastrointestinal mast cells are one of the main proinflammatory cells which are involved in undesirable pathologic effects such as food hypersensitivity, besides providing a natural defense against parasitic and microbial infections. Recently, mast cells have been implicated in the pathogenesis of Helicobacter pylori-infected gastritis (Nakajima et al., 2004) whereas stabilization of mast cells has been suggested to be a key mechanism to protect the gastrointestinal tract from injury (Penissi et al., 2003). Involvement of mast cells in gastropathy is supported by the findings of animal studies showing the induction of gastric mucosal lesions by mast cell degranulators (Ohta et al., 2003; Kawakubo et al., 2005; Tabuchi et al., 1994) and their protection by mast cell stabilizers (Tabuchi et al., 1994; Ali, 1995; Erkasap et al., 2005; Takeuchi et al., 1986).

Nedocromil sodium (NDS) and sodium cromoglycate (SCG) are mast cell stabilizers that are widely used as prophylactic agents in the treatment of bronchial asthma. Pharmacological studies have demonstrated that NDS is a more potent (Verin et al., 1999; Spezia et al., 1993; Lal et al., 1993) or at least equally effective (Kelly et al., 2001; Robuschi et al., 1997; De Benedictis et al., 1995) as the progenitor of SCG. The beneficial effects of SCG in gastrointestinal injury have been reported in experimental (Ali, 1995; Beck et al., 1989; Ogle and Hui, 1995; Goossens et al., 1987) and clinical (Di Gioacchino et al., 1990; Moots et al., 1988; Grace et al., 1987; Caporali et al., 1986) studies, whereas the gastroprotective activity of NDS remains to be explored. In the present investigation, we performed a comparative evaluation of NDS and SCG in experimental gastric (ethanol-, indomethacin-, and stress-induced) and duodenal (cysteamine-induced) ulcers in rats.

Materials and methods

Wistar rats of either sex, weighing 220 ± 20 g, fed on standard chow diet were maintained in a temperature and humidity controlled room at 12 h light/dark cycles. The animals were divided into experimental groups of 6 animals each. The distribution of animals into groups and the treatment allotted to each group were randomized. The protocol of animal study was approved by Research and Ethical Committee of Armed Forces Hospital, Riyadh, Saudi Arabia and the guidelines of animal care were strictly adhered during animal maintenance and experimentation.

The aqueous solution of ulcerogens and drugs (NDS and SCG) were freshly prepared before administration. Both NDS and SCG, in the doses of 10, 30 and 100 mg/kg body weight were given intraperitoneally for gastric secretion studies and by gavage for antiulcer studies. The animals were sacrificed, stomachs removed and opened along the greater curvature. After washing with saline the gastric lesions were quantified by a person unaware of the treatment protocol. The ulcers were scored according to the method of Valcavi et al. (1992). The circular ulcers induced by indomethacin were assessed on the basis of their diameters; deep circular ulcers more than 8 mm diameter = 10; 7–8 mm = 8; 6–7 mm = 7; 5–6 mm = 6; 4–5 mm = 5; 3–4 mm = 4; 2–3 mm = 3; 1–2 mm = 2; and <1 mm = 1. Deep linear ulcers 10 mm or more in length were scored 3. The scores of each single lesion were then summed up for determination of the ulcer index. Patched lesions of the stomach induced by 100 % ethanol were scored according to the method described by Schiantarelli et al. (1984) using the following scale; 0 = normal mucosa; 1 = hyperemic mucosa or up to 3 small patches; 2 = 4-10 small patches; 3 = more than 10 small or up to 3 medium-sized patches; 4 = 4–6 medium-sized patches; 5 = more than 6 medium-sized or up to 3 large patches; 6 = 4–6 large patches; 7 = 7–10 large patches; 8 = more than 10 large patches or extensive necrotic zones. 'Small' was defined as up to 2 mm across (maximum diameter), 'medium-sized' as between 2 and 4 mm across, and 'large' as more than 4 mm across.

Pylorus ligated (Shay) rats

The animals were fasted for 36 h with access to water *ad libitum* before the pylorus was ligated under ether anesthesia, care being taken not to cause bleeding or to occlude blood vessels (Shay et al., 1945). The animals were sacrificed 6 h after pylorus ligation. The stomachs were removed, contents collected, volume measured, centrifuged and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7 for total acid output calculation.

Induction of gastric lesions by water immersion and restraint stress

Rats were placed in a restraint cage and immersed vertically to the level of the xiphoid process in a water bath (23 °C) for 7 h and then sacrificed. The stomach was examined for lesions and scored according to the method described by Valcavi et al. (1992).

Indomethacin-induced gastric lesions

Indomethacin was suspended in 1 % carboxymethylcellulose in water and administered by gavage at the dose of 30 mg/kg body weight (Bhargawa et al., 1973). The animals were sacrificed 6 h after indomethacin administration.

Cysteamine-induced duodenal ulcers

Duodenal ulcers were induced by two doses of cysteamine hydrochloride (400 mg/kg i. g. in 10 % aqueous solution) at an interval of 4 h according to the method described by Szabo (1978). All the animals were sacrificed 24 h after the first dose of cysteamine and the duodenum was excised carefully and opened along the antimesenteric side. The duodenal ulcers were scored using a scale of 0 to 3 where: 0 = no ulcer; 1 = superficial mucosal erosion; 2 = deep ulcer or transmural necrosis, and 3 = perforated or penetrated ulcer (into the pancreas or liver). The sum of the intensity of each lesion was used as the ulcer index.

Gastric lesions induced by ethanol (cytoprotection studies)

The animals were administered 1 ml of 100 % ethanol by gavage (Natale et al., 2001). One hour after the administration of ethanol the animals were sacrificed and examined for lesions in the stomachs. The assays of gastric wall mucus and glutathione (GSH) in the stomach were done as follows.

Determination of gastric wall mucus

Gastric wall mucus was determined according to the modified procedure of Corne et al. (1974). The glandular segment of the stomach was separated from the lumen of the stomachs, weighed and transferred immediately to 10 ml of 0.1 % w/v Alcian blue solution (in 0.16 M sucrose solution buffered with 0.5 ml sodium acetate at pH 5). Tissue was stained for 2 h in Alcian blue, excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose. Dye complexed with the gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride, which was intermittently shaken for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3000 rpm for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated.

Determination of glutathione (GSH)

Gastric mucosal GSH levels were measured according to the method reported by Owen (1980). The glandular part of stomach was homogenized in ice-cold perchloric acid (0.2 M) containing 0.01 % of EDTA. The homogenate was centrifuged at 10,000 g for 10 min. The enzymatic reaction was started by adding 100 µl of clear supernatant in a spectrophotometric cuvette containing 800 µl of 0.3 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 100 µl of 6 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and 10 µl of 50 units/ml GSH reductase

(all the above three reagents were freshly prepared in phosphate buffer at pH 7.5). The absorbance was measured over a period of 4 min at 412 nm at 30 °C. The GSH level was determined by comparing the change of absorbance (ΔA) of test solution with the ΔA of standard GSH.

Statistics

Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Differences with a *P* value less than 0.05 were considered statistically significant.

Results

Effect of NDS and SCG on the gastric secretion in 6 h pylorus-ligated (Shay) rats

In control rats, pylorus-ligation for 6 h resulted in accumulation of 10.5 ± 0.50 ml of gastric secretion and a total acid output of 788 ± 65.2 mEq (Table 1). The volume of gastric secretion was significantly and dose-dependently reduced in the rats treated with 30 mg/kg and 100 mg/kg of NDS or SCG (ANOVA F = 6.721, P < 0.001). These doses of NDS and SCG also decreased total acid output, significantly and dose-dependently (ANOVA F = 3.85, P < 0.01).

Table 1. Effect of nedocromil sodium (NDS) and sodium cromoglycate (SCG) on gastric secretion and acidity in 6 h pylorus ligated (Shay) rats.

Treatment	Dose (mg/kg)	Gastric secretion (ml)	Total acid output (mEq)
Control[a]	0	10.5 ± 0.50	788 ± 65.2
NDS	10	8.5 ± 0.48	748 ± 28.3
NDS	30	6.6 ± 0.65**	480 ± 54.1*
NDS	100	5.3 ± 0.31***	430 ± 38.1*
SCG	10	7.5 ± 1.28*	559 ± 123.6
SCG	30	5.6 ± 0.66***	473 ± 85.9*
SCG	100	5.5 ± 0.80***	430 ± 93.3*

aLigation only. Values are mean ± standard error of means (SEM). *P < 0.05, **P < 0.01 and ***P < 0.01 versus control group using Dunnett's test.

Effect of NDS and SCG on water-immersion-restraint stress (WIRS)-induced gastric lesions

The rats exposed to WIRS showed considerable ulcerogenicity in the form of hemorrhagic mucosal lesions in the stomach. The lesion area in the control (ulcer only) group was 19.50 ± 3.12 mm^2 (Fig. 1). Pre-treatment of rats with 30 mg/kg (lesion area, 11.16 ± 2.03 mm^2) and 100 mg/kg (lesion area, 8.66 ± 2.61 mm^2) of NDS significantly and dose-dependently attenuated gastric lesions whereas all the three doses of SCG were equipotent in inhibiting WIRS-induced ulcers (ANOVA F = 3.429, P < 0.01).

Effect of NDS and SCG on indomethacin-induced gastric mucosal damage

The administration of indomethacin produced gastric lesions mainly in the glandular stomach of all the animals. The lesion area in the control group was found to be 28.5 ± 1.58 mm^2 (Fig. 2). Pretreatment of rats with medium and high doses of NDS and SCG significantly decreased the intensity of indomethacin-induced ulcers; the effects of SCG being dose-dependent and highly significant (ANOVA F = 4.143, P < 0.01). The indomethacin-induced lesions areas in the rats treated with 30 mg/kg and 100 mg/kg of NDS were 16.33 ± 4.67 mm^2 and 16.66 ± 2.66 mm^2 respectively as compared to 11.40 ± 4.31 mm^2 and 7.80 ± 1.24 mm^2 in the rats treated with same doses of SCG respectively. The low

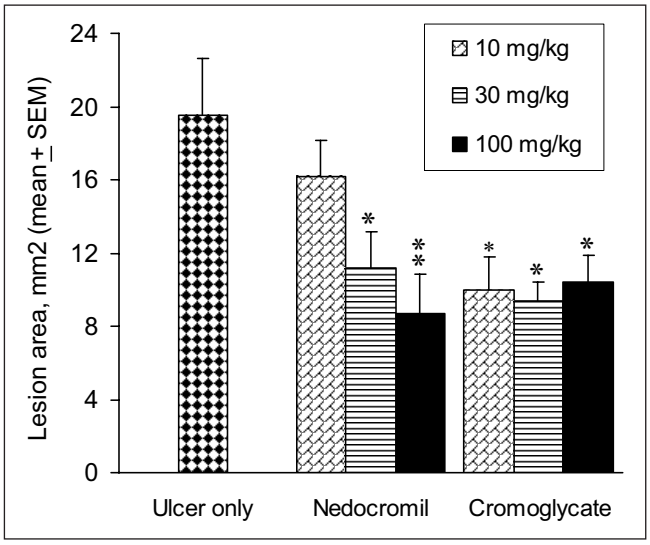

Fig. 1. Effect of nedocromil sodium and sodium cromoglycate on gastric mucosal damage induced by water immersion restraint stress (WIRS) in rats. *P < 0.05 and **P < 0.01 versus ulcer only (WIRS only) group using Dunnett's test.

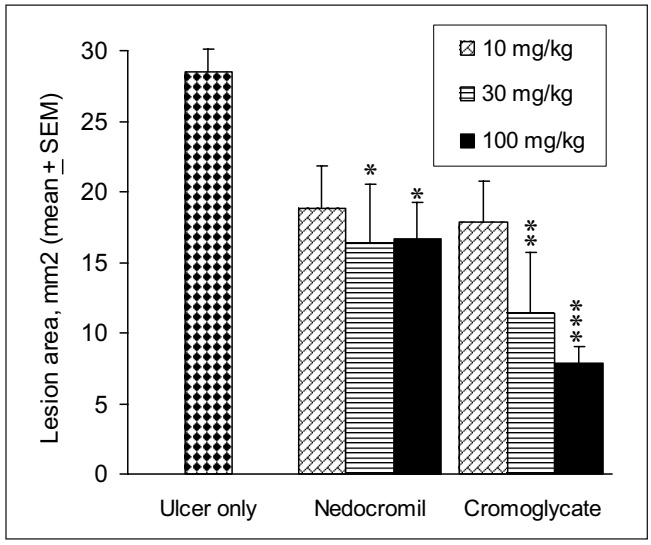

Fig. 2. Effect of nedocromil sodium and sodium cromoglycate on gastric mucosal damage induced by indomethacin in rats. *P < 0.05, **P < 0.01 and ***P < 0.001 versus ulcer only (indomethacin only) group using Dunnett's test.

dose of both the drugs insignificantly reduced indomethacin-induced mucosal damage (Fig. 2).

Effect of NDS and SCG on cysteamine-induced duodenal ulcers

Administration of cysteamine produced elongated lesions extending longitudinally down the duodenum. The lesion area in the rats of the control group was 3.5 ± 0.50 mm^2 (Fig. 3). All the 3 doses of NDS significantly inhibited (ulcer inhibition, 52.37–61.91 %) the formation of cysteamine-induced duodenal ulcers (ANOVA F = 4.531, P < 0.01). On the other hand, all the 3 doses of SCG appeared to be ineffective against duodenal ulcers (ulcer inhibition, 9.71–14.28 %).

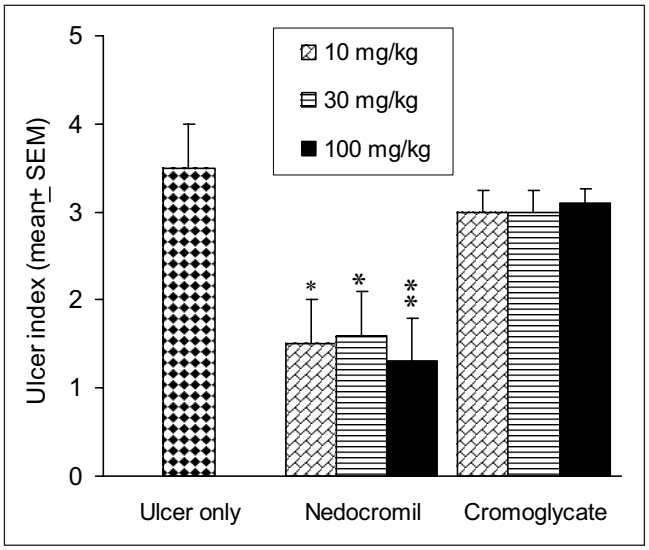

Fig. 3. Effect of nedocromil sodium and sodium cromoglycate on duodenal ulcers induced by cysteamine in rats. *P < 0.05 and **P < 0.01 versus ulcer only (cysteamine only) group using Dunnett's test.

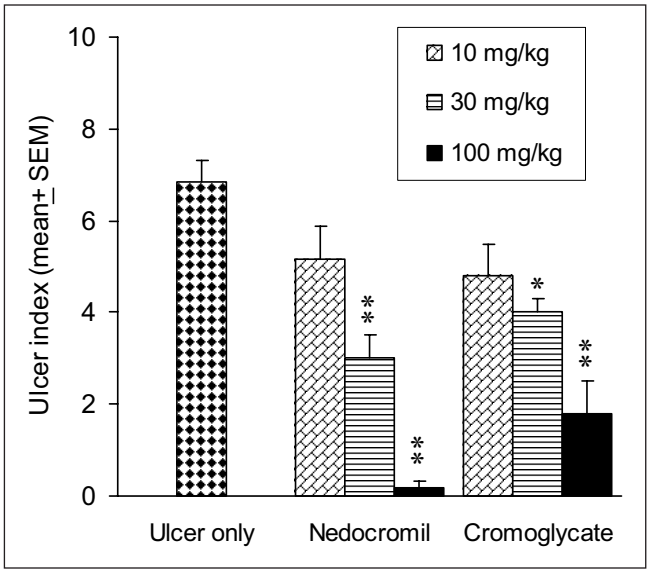

Fig. 4. Effect of nedocromil sodium and sodium cromoglycate on gastric mucosal damage induced by ethanol (EtOH) in rats. *P < 0.01 and **P < 0.001 versus ulcer only (EtOH only) group using Dunnett's test.

Effect of NDS and SCG on ethanol-induced gastric lesions

The treatment of rats with ethanol produced extensive gastric lesions in the glandular mucosa of stomach in all the control animals. These lesions were characterized by multiple hemorrhagic red bands (patches) of different sizes along the axis of glandular stomach. The ulcer index was found to be 6.83 ± 0.47 in control animals (Fig. 4). Pretreatment of rats with medium and high doses of NDS or SCG significantly and dose-dependently reduced the formation of ethanol-induced gastric lesions (ANOVA F = 17.174, P < 0.001). NDS appeared to be more potent than SCG in the doses of 30 mg/kg (ulcer inhibition, 56.09 % versus 41.46 %) and 100 mg/kg (ulcer inhibition, 97.57 % versus 73.05 %) for the inhibition of ethanol-induced gastric lesions (Fig. 4).

Effect of NDS and SCG on ethanol-induced changes in gastric wall mucus

The treatment of rats with ethanol significantly decreased the Alcian blue binding capacity of gastric wall mucus (568 ± 8.25 μg Alcian blue/g of tissue) as compared to control rats (676 ± 39.7 μg/g). Pretreatment of animals with NDS or SCG dose-dependently reversed the adverse effect of ethanol on gastric wall mucus, however only the high dose (100 mg/kg) of SCG exerted significant effect (ANOVA F = 4.35, P < 0.01) (Table 2).

Effect of NDS and SCG on ethanol-induced depletion of gastric mucosal GSH

Administration of ethanol significantly decreased the level of GSH in the gastric mucosa of rats (ANOVA F = 3.249, P < 0.01). Concomitant treatment with NDS or SCG dose-dependently attenuated the ethanol-induced depletion of gastric mucosal GSH, whereas the high dose of NDS significantly restored GSH levels (Table 2).

Table 2. Effect of nedocromil sodium (NDS) and sodium cromoglycate (SCG) on ethanol-induced changes in gastric Alcian-blue binding capacity and gastric NP-SH levels in rats.

Treatment	Dose (mg/kg) of NDS or SCG	Alcian-blue binding (Mean ± SEM)	GSH (μmol/g) (Mean ± SEM)
Control[a]	0	676 ± 39.7	4.43 ± 0.18
Ulcer (EtOH)	0	568 ± 8.25#	3.32 ± 0.22##
EtOH + NDS	10	574 ± 34.9	3.46 ± 0.21
EtOH + NDS	30	615 ± 20.9	3.97 ± 0.16
EtOH + NDS	100	618 ± 29.5	4.14 ± 0.12*
EtOH + SCG	10	658 ± 13.9	3.55 ± 0.13
EtOH + SCG	30	664 ± 21.6	3.87 ± 0.16
EtOH + SCG	100	740 ± 34.1*	4.06 ± 0.36

#P < 0.05 and ##P < .0.01 versus control; *P < 0.05 versus ulcer (EtOH only) group using Dunnett's test.

Discussion

The results of this study showed differential effects of NDS and SCG on various types of experimental ulcers. SCG was more effective than NDS in preventing WIRS- and indomethacin-induced gastric lesions whereas reverse was true in ethanol- and cysteamine-induced ulcers. The specific response of these drugs may be attributed to the involvement of different mechanisms in the pathogenesis of various types of ulcers as well as the variations in the mode of drug action. However, both the drugs significantly and dose-dependently reduced the acidity and volume of gastric secretions (Table 1). Increased gastric acidity is considered to be an important contributing factor in the pathogenesis of gastric ulcers (Goa and Monk, 1987). Drugs with the ability to reduce acid secretion have been shown to attenuate ulcerogen-induced gastric mucosal damage (Takeuchi et al., 2003; Patel et al., 2001). Histamine plays an important role in regulation of gastric acid secretion and is considered to be a final common mediator which stimulates the parietal cell in response to other secratagogues (Black et al., 1972). Histamine is present in the gastrointestinal tract of nearly all vertebrates in concentrations ranging from 1 μg to over 100 μg per gram of tissue (Vugman and Rocha, 1996; Lorenz et al., 1973). The highest concentrations are those in the stomach particularly in the acid secreting fundus and body of the stomach. Endogenous histamine is closely related to the early development of ethanol-induced acute gastric mucosal injury (Shindo et al., 1997). Administration of SCG has been shown to block hypersecretory response to both histamine and gastrin in iodacetamide-induced gastritis in mice (Piqueras et al., 2003). SCG also inhibits gastric acid secretion in response to ulcerogens, pentagastrin and C48/80 in rats (Tabuchi et al., 1994; Nicol et al., 1981).

All the three doses of SCG offered almost equal protection against WIRS-induced gastric ulcers whereas only medium and high dose of NDS provided significant protection in this model of ulcer (Fig. 1). Protective effect of SCG against stress-induced gastric ulceration in rats has been reported earlier (Ogle and Hui, 1995). Stress is known to trigger hyperacidity (Nishida et al., 1993; 1994) and impaired gastric motility (Watanabe et al., 2000). Both NDS and SCG significantly reduced gastric acidity (Table 1). SCG also possess inhibitory effects on gastric motor activity (Takeuchi et al., 1986). SCG appeared to be more protective than NDS against indomethacin-induced gastric lesions (Fig. 2). Gastropathy associated with nonsteroidal anti-inflammatory drugs (NSAIDs) is accompanied by increased gastric acidity, imbalance of arachidonic acid metabolites (Lippman, 1974; Kasuya et al., 1979) and elevated oxidative stress (Otamiri and Tagesson, 1989; Vaananen et al., 1991). The gastroprotective effect of SCG against indomethacin-induced ulcers may be attributed to its inhibitory effects on arachidonic acid metabolism (Okayama et al., 1992), neutrophil infiltration (Furuta et al., 1998) and ODFR generation (Etienne et al., 1988).

In the duodenal ulcer model, NDS significantly inhibited cysteamine-induced duodenal ulcers whereas SCG failed to do so (Fig. 3). The pathogenesis of cysteamine-induced duodenal ulcers is not fully understood. Cysteamine ulcers are considered to be associated with the hypersecretion of gastrin and hydrochloric acid and decreased mucosal resistance (Szabo et al., 1977). Gastrin inhibitors appeared to be as potent as proton-pump inhibitors in prevention of cysteamine-induced duodenal ulcers (Pendley et al., 1995; Makovec et al., 1999). Recently, an important role of chloride transporters including Na^+-K^+-$2Cl^-$ (Mc Daniel et al., 2005), Cl^--HCO_3^- (Simpson et al., 2005), H^+-Cl^- (Tanigaki et al., 2002) and ClC^{-2}-Cl^- (Sherry et al., 2001) channels in gastric secretion and ulceration. Modulation of these channels has been shown to alleviate hypersecretion and gastroduodenal ulceration (Akiba et al., 2005; Cheon et al., 2001; Hagen et al., 2000). NDS is capable of inhibiting Cl^- flux (Alton and Norris, 1996) which may lead to the modulation of Cl^- symporters and antiporters to normalize gastric secretion and acidity.

Pretreatment with NDS or SCG significantly and dose-dependently protected gastric mucosa against ethanol-induced injury, where the former drug appeared to be more effective (Fig. 4). The cytoprotective effects of these two drugs were accompanied by the attenuation of ethanol-induced depletion of gastric wall mucus and GSH (Table 2). The gastric mucus coat plays an important role in protecting gastric mucosa against ulcerogens and facilitates the repair of the damaged gastric epithelium (Wallace and Whittle, 1986). The mucus gel adhering to the gastric mucosal surface protects the underlying epithelium against acid (Bell et al., 1985; Slomiany et al., 1985), pepsin (Bell et al., 1985) and necrotizing agents, like ethanol (Allen et al., 1987). Earlier studies have shown that restoration of GSH improves the formation of gastrointestinal mucus which protects gastric mucosa against necrotizing agents (Guardia et al., 1994; Salim, 1992). It has been reported that neutrophils are the major inflammatory cells type infiltrating the injured mucosa following exposure to ethanol (Laine and Weinstein, 1998). Neutrophil adherence to the vascular endothelium is one of the early events in the process of ulcergen-induced mucosal injury (Kitahora and Guth, 1987; Lee et al., 1992). The activated neutrophils injure the microvasculature via release of ODFR and proteases (Elsbach and Weiss, 1988; Hernandez et al., 1987). Thus, the protective effect of NDS and SCG may be attributed to their inhibiting effect on neutrophil infiltration and oxidative stress (Ali, 1995; Furuta et al., 1998; Etienne et al., 1988; Braga et al., 1997).

In conclusion, both NDS and SCG offer significant protection against experimental gastric ulcers. NDS is more potent in preventing ethanol- and cysteamine-induced lesions whereas SCG is more effective against stress- and indomethacin-induced ulcers. It is therefore anticipated that a combined therapy (NCS+SCG) may be more beneficial for the treatment of complex gastroduodenal ulcers. However, the clinical relevance of these findings is yet to be established.

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