Gastric Mucus Effusion Elicited by Oral Copper Compounds: Potential Anti-Ulcer Activity

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Summary. In rats, both Cu(I) and Cu(II) show an irritancy profile not shared with Cu° or Zn(II) or Ni(II). The gastric response to Cu(II), i.e. copius fluid and mucus secretion, can protect the stomach from the acute ulcerative effects of aspirin or physical stress administered subsequently.

Aqueous solutions of $CuCl_2$ and Cu(II) salicylate administered orally to rats induce a marked gastric swelling with copious effusion of mucus[3]. This reflects a protective response by the gastric mucosa to certain intragastric irritants. By contrast, other irritants (notably aspirin and many anti-inflammatory acidic drugs induce ulcerations in the gastric mucosa[4,5] with considerable parietal cell injury[6]. However both classes of gastric irritants (protective or ulcerogenic) induce a rather similar irritant response with local oedema when injected into the rear footpads of rats[3,5], i.e. in a non-acidic environment without parietal cells.

Experimental. Outbred Wistar rats of both sexes (180–220 g) were starved for 24 h but permitted water ad libitum before oral dosing with compounds dissolved or dispersed in saline or water[6]. They were sacrificed 2 h later and both the gastric swelling and the amount of visible mucus lining the stomach were each scored on a scale of 0 to 3+[3]. Ulcerative lesions were scored by the lesion index (LI) described previously[6]. Preformed Cu(I) complexes were dissolved in 5 or 10% v/v specially purified thiodiglycol (Pierce Chemical Co, Rockford, Illinois) in saline. Other metal complexes were prepared by mixing metal chlorides in aqueous solution with the complexing agent (ligand) in the proportions 1:1.1 or 1:2.2, adjusting the pH to 6.5 with $NaHCO_3$ and the osmolarity to 0.3 *M* with NaCl, before injection into the rear paws (2 μmoles metal ion/paw) of 200–300 g rats. Ensuing local oedema was measured by increase in paw thickness, determined with a micrometer screw gauge 1,2,6 and 24 h later.

Results. The Table shows that the irritancy of Cu(II) complexes in both the paw (causing local oedema) and in the stomach (eliciting mucus effusion and gastric swelling) is probably due to free Cu(II) ions, being greatest with $CuCl_2$ and least with those complexes containing tightly liganded Cu (i.e. with high stability constants). In these experiments the pH of the (starved) rats' stomachs was ca. 5.0: therefore there was still considerable buffering of the applied Cu(II) by the Cu-ligands. Calculations showed that only histidine and NTA, among the ligands listed in the Table, would appreciably buffer Cu(II) at pH 2.5 (pCu = 4.0 for 20 m*M* Cu-$Hist_2$, = 4.7 for 20 m*M* Cu-NTA).

Cu(II) preparations which were highly irritating outside the stomach ($\leqslant$1 μmole/paw causing much local oedema) nevertheless caused no ulceration when applied i.g., but always elicited a copious mucus effusion into the stomach. This non-ulcerant effect was confirmed by light microscopic observations of formalin-fixed tissues stained with periodic acid-Schiff reagent[7], Alcian blue (pH 2.5)[8] or the haematoxylin and eosin stains. Mucus discharge from the superficial and gastric pit mucous cells was clearly evident after administering $CuCl_2$ and those Cu(II) complexes which elicited a visible mucus effusion. Some disruption of parietal cells was also seen, especially after giving high doses of Cu(II) compounds.

Soluble Cu(I) compounds administered i.g. also caused considerable mucus effusion, gastric swelling and paw oedema – with the exception of Cu(I)-penicillamine. Finely powdered metallic copper, i.e. Cu° (British Drug Houses, chemically precipitated) was inert in both the stomach and paw over the time span of these experiments.

Nickel salts (Cl^-, NO_3^-, $SO_4^=$) were less potent than the corresponding Cu(II) salts in eliciting a protective gastric response. Complexation of Ni(II) with 2 equivalents of 1,10-phenanthroline (log K_1 = 8.6) at pH 2.9 prevented gastric swelling. Unlike Cu(II), Ni(II) did not cause significant local irridation when injected into the paw.

By contrast, zinc salts (Cl^-, $SO_4^=$, acetate) had no evident effects on the stomach but they were rather irritant in the paw, causing considerable acute, though short-lived (< 12 h), edema. The paw irritancy of Zn(II) was blocked by thiols and the zinc $(penicillamine)_2$ complex caused no edema at all when injected into the paw.

The potential anti-ulcer activity of Cu(II) was demonstrated in the two following sets of experiments: 1. Prolonged physical stress, such as exposure to −15 °C for 3 h, induces considerable gastric ulceration in starved rats (av. number of lesions = 4.8, lesion index = 10) with massive haemorrhage and mucosal hyperemia. Animals predosed with $CuCl_2$ (300 μmoles/kg, i.g.) before this prolonged cold exposure suffered no haemorrhage or gastric lesions. Lower doses of $CuCl_2$ ($\leqslant$ 30 μmoles/kg) afforded partial protection from these effects of cold stress but $CuCl_2$ given parenterally was totally ineffective.

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Paw irritancy (oedema) and gastric irritancy (mucus effusion, gastric swelling) of Cu, Zn and Ni ions in rats: Correlation with free Cu(II)[a] applied in stomach after fasting or in extragastric tissue

Compound	pCu at		Paw irritancy[b]	Gastric irritancy[c]	
	pH 4.5	pH 7.4		Mucus effusion	Swelling
$CuCl_2$	1.7	1.7	+++	3.0	3.0
$Cu(imidazole)_2Cl_2$	2.3	4.7	+++	3.0	3.0
Cu(II)-carnosine	2.0	7.5	+++	2.7	3.0
Cu(II)-salicylate$_2$	2.6	5.4	++	1.0	0.4
Cu(II)-histidine$_2$	8.2	12.6	+	1.3	1.3
Cu(II)-TRIEN	6.8	14.7	0	0.3	0.5
Cu(II)-Gly · His · Gly	3.7	10.3	0	0.4	0.2
Cu(II)-Gly$_2$ · His · Gly$_2$	2.7	12.5	0	0.2	0.1
Cu(II)-NTA	7.0	9.9	0	0	0
Cu(I)Cl[d]			++++	1.0	2.5
$Cu(thiourea)_3Cl$[d]			++++	3.0	3.0
$Cu(CH_3CN)_4ClO_4$[d]			++	3.0	3.0
Cu(I)-thiomalate, Na$_2$			++	1.0	1.0
Cu(I)-penicillamine			0	0	0.3
10% thiodiglycol			0	0	0
Cu° (metal powder)			0	0	0
$ZnCl_2$			+++	0.3	0
$NiCl_2$			+	2.3	2.3

TRIEN, triethylenetretramine; NTA, nitrilotriacetic acid.

[a] pCu(II) = negative logarithm$_{10}$ of the Cu(II) concentration (cf. pH), calculated from pK_a's and stability constants[10,11] of the Cu-ligands, for total Cu = 20 m*M* using SPECON 18, a variant of the programme COMICS[12].

[b] Paw irritancy scored by increase in paw thickness: <1 mm = 0; 1–2 mm = +; 2–3 mm = ++; 3–4 mm = +++; >4 mm = ++++, 2 h after injecting 2 µmoles metal ion in isotonic saline; pH metal complexes adjusted to 6–6.5.

[c] 2 h after 100 µmoles/kg p.o. in starved rats.

[d] Cuprous compounds stabilized in aqueous solution with 10% (v/v) thiodiglycol.

2. Aspirin (100 mg/kg) causes considerable gastric lesions – even in unstressed rats within 2 h[5,9]. Pretreating a group of animals with oral $CuCl_2$ (50 µmoles/kg) 60 min before administering this dose of aspirin (= 550 µmoles/kg) induced sufficient effusion of mucus and watery exudate to wholly protect the mucosa from the deleterious effects of the aspirin. [Lesion index with no pretreatment = 17.2; with $CuCl_2$ pretreatment = 0]. Removing the available i.g. Cu(II) by subsequent administration of TRIEN or NTA (55 µmoles/kg) or L-histidine (110 µmoles/kg) 10 min before giving aspirin did not block the anti-ulcerant effect (i.e. lesion index was still zero). This showed that it was probably mucus released in response to Cu(II) that afforded protection from the aspirin since a) Cu-TRIEN and Cu-NTA are not protective irritants in the stomachs of fasted rats (Table), and b) this quantity of Cu(II) could 'neutralize' only 18% of the aspirin applied. (However, even Cu(II)-aspirin$_2$ is ulcerogenic[3], being decomposed by the normal gastric acidity to liberate free aspirin).

Cupric chelates have been reported to have anti-ulcer activity in the Shay rat ulcer model[13,14]. Our findings suggest that it is probably uncomplexed Cu(II), rather than the chelated Cu in the stomach, that has the ulcer-protectant activity.

Effect of Sulfated and Non-Sulfated Gastrin and Octapeptide-Cholecystokinin on Cat Gall Bladder in vitro[1]

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Summary. This study demonstrates that for the isolated cat gall bladder a smaller molar dose of the sulfated form of OP-CCK and gastrin is required to produce contraction as compared to the respective non-sulfated forms. For OP the D_{50} for the sulfated form versus the non-sulfated form was 1.94. For gastrin it was 1.10.

Structure-activity relationships of several gastrointestinal hormones and their analogues have been studied on a variety of tissues. Of particular interest has been the importance of sulfation of the tyrosine residue in the C-terminal 7th position in cholecystokinin (CCK) and its analogues, and in the 6th position in gastrin. The present

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