Effect of cimetidine and ranitidine on drug induced damage to gastric epithelial cell monolayers *in vitro*

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SUMMARY The effect of the H₂ blockers cimetidine and ranitidine on drug induced damage to gastric cell monolayers has been evaluated in conditions independent of systemic factors and their anti-acid properties. Monolayers of mucous cells from a human cell line MKN 28, obtained from a human gastric adenocarcinoma, have been studied. Cell damage has been assessed qualitatively by trypan blue dye exclusion test and quantitatively by ⁵¹Cr release assay. Cimetidine and ranitidine significantly protected cultured cells against damage induced by sodium taurocholate decreasing taurocholate induced ⁵¹Cr release by 36% (p<0.001) and 28% (p<0.01), respectively. Cimetidine was also protective in concentrations lower than ranitidine. This protection was not prevented by the prostaglandin synthesis inhibitor indomethacin nor by the sulph-hydryl blocker N-ethylmaleimide. Incubation with cimetidine and ranitidine did not increase the production of PGE₂ by cultured cells nor did it affect the cellular level of sulph-hydryl compounds. Cimetidine and ranitidine did not afford protection against damage induced by indomethacin and ethanol. Cimetidine in a concentration of 10⁻⁴ M increased ethanol induced damage significantly. In conclusion (1) cimetidine and ranitidine protect gastric cells against taurocholate induced damage *in vitro*, independently of their anti-acid effect; (2) this protection is not mediated by prostaglandin E₂ or sulph-hydryl compounds; (3) cimetidine and ranitidine do not protect gastric cells against damage induced by indomethacin and ethanol.

Histamine H₂ receptor blockers cimetidine and ranitidine have been shown to protect gastric mucosa against aspirin induced damage *in vivo*^{1,2} Because aspirin induced gastric mucosal damage is dependent on the amount of acid present in the stomach (the more acid, the more damage), H₂ blocker protection may be dependent on the inhibition of gastric acid secretion. Cimetidine and ranitidine do not protect rat gastric mucosa *in vivo* against ethanol induced damage (which is acid independent unlike aspirin)³; this further questions the ability of these drugs to protect independently of acid inhibition. On the other hand, H₂ antagonists have been reported to be 'cytoprotective' even in non-anti-secretory doses.^{2,4,6}

The mechanism of the protection by cimetidine and ranitidine is unknown. Prostaglandins did not

appear to be involved in the protective effect exerted by ranitidine *in vivo*.² The effect of cimetidine on prostaglandin production by gastric mucosa is controversial.^{7,8} Sulph-hydryl compounds such as cysteamine protect against drug induced damage *in vivo* and *in vitro*.^{9,10} Whether endogenous sulph-hydryls mediate the protective effect of the H₂ blockers has not been determined. Furthermore, whether H₂ blockers are able to protect gastric mucosal cells directly, independently of the influence of systemic factors such as blood flow, hormonal, and vascular factors or bicarbonate secretion has not been reported.

The aim of the present study was to evaluate the effect of H₂ blockers on taurocholate, indomethacin, and ethanol induced damage to gastric epithelial cells *in vitro*. Gastric mucosal cell monolayers were derived from a cell line, MKN 28, obtained from a human gastric adenocarcinoma, which has been shown to be suitable for the study of cytoprotection

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by agents such as these *in vitro*.¹¹ In this experimental model, the influence of vascular, neural, and hormonal factors is excluded. Furthermore, as MKN 28 cells are mucus producing cells, the acid inhibitory property of H₂ blockers is not relevant to their protective effect. The mechanism of this protection has been investigated by comparing two H₂ blockers of differing potency and by evaluating the role of prostaglandins and endogenous sulph-hydryl compounds.

Methods

MATERIAL

The MKN 28 cell line is derived from a well differentiated human gastric adenocarcinoma consisting of mucus secreting epithelial cells.¹² Culture medium consisted of a 1:1 mixture of Dulbecco's modified Eagle medium (DME) and Coon's modified Ham's F-12 medium (both from GIBCO, Grand Island, NY). Culture medium was supplemented with 15 mM HEPES buffer (Sigma Chemical Co, St Louis, Mo), 1.2 g/l NaHCO₃ (Sigma), heat inactivated (at 56°C for 30 min) 10% fetal bovine serum (Armour Pharmaceutical Co, Kankakee, Ill), and 1% antibiotic antimycotic solution (100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml fungizone) obtained from Irvine Scientific Co, Santa Ana, Cal. Ranitidine was a gift from Glaxo Research Group Ltd. Cimetidine, sodium taurocholate, indomethacin, and N-ethylmaleimide (NEM) were purchased from Sigma. Chromium 51 (sodium chromate 200–900 Ci/g chromium) and trypsin were from ICN Biomedicals, Irvine, Cal (¹²⁵I) prostaglandin E₂ radioimmunoassay kit was obtained from New England Nuclear, Boston, Mass. Reduced glutathione, 5,5-dithiobis-2-nitrobenzoic acid, and sulphosalicylic acid were purchased from Sigma. Sodium taurocholate, N-ethylmaleimide, and ranitidine were dissolved in DME-F12 medium and used immediately after adjusting pH to 7.4. A stock solution of indomethacin 50 mM was obtained by dissolving the drug in Na₂CO₃ (Sigma) 0.2 M; pH was adjusted to 7.4 by adding NaH₂PO₄. Desired concentrations of the drug were obtained by diluting the stock solution with culture medium (pH 7.4). A stock solution of cimetidine 100 mM was prepared by dissolving the drug in dimethyl sulphoxide. The drug solution was aliquoted and stored frozen (–20°C) until the experiment day. Desired concentrations were obtained by diluting the stock solution with DME-F12 medium.

CELL CULTURE

MKN 28 cells were grown as monolayers in DME-F12 medium and confluent monolayers were sub-

cultured every four days by treatment with 0.1% trypsin and 0.03% EDTA (Sigma) in CA⁺⁺- and Mg⁺⁺-free phosphate buffered saline. After trypsin treatment, cells were plated onto 35×10 mm culture dishes (Corning, Corning, NY). Cells were studied when they were at the confluent state, usually from four to five day old cultures. The cultures were maintained in a sterilitic incubator (Forma Scientific, Marietta, Ohio) at 37°C in a humidified atmosphere of 5% CO₂ in air. The medium was changed every other day. MKN 28 cells have been previously identified as mucus producing cells histochemically and electron microscopically.^{11,12}

51 CHROMIUM RELEASE ASSAY

The method for isotope release assay was as described previously.^{13,14} The media were discarded and the monolayers washed three times with 1 ml medium (37°C) to remove floating cells in culture. The cells were then incubated in medium containing 5 µCi/ml of ⁵¹Cr for 60 min in the culture condition. The labelled cells were washed five times with medium (4°C) to remove excess isotope and then 1 ml medium containing test reagents as described under experimental studies was added to monolayers. After incubation for the experimental period in the culture condition, supernatant medium was collected. The monolayers were dissolved in 1N NaOH for 24 hours. Radioactivity of the cells and of the supernatants was counted with an automatic gamma counter (Beckman 7000, Beckman Instruments Inc, Fullerton, Cal). The percentage of ⁵¹Cr release per sample was expressed as:

$$^{51}\text{Cr Release (\%)} = \frac{\text{cpm supernatant}}{\text{cpm supernatant} + \text{cpm cells}} \times 100$$

or as:

Specific ⁵¹Cr Release (%) = ⁵¹Cr release (%) in the presence of the test drug – spontaneous ⁵¹Cr release (%) – that is, ⁵¹Cr release in control monolayers. The medium used in ⁵¹Cr release assay was not supplemented with fetal bovine serum and was antibiotic-antimycotic free.

TRYPAN BLUE DYE EXCLUSION TEST

Trypan blue dye exclusion test was carried out as described elsewhere.^{15,16} Briefly, after incubation with test drugs, monolayers were washed three times with F-12 medium. Washed monolayers were kept in 1 ml F-12 medium until the test was performed. One tenth of a millilitre of trypan blue (0.4%) was then added to monolayers and mixed. Ten minutes later, monolayers were observed in an inverted phase

Fig. 1 Phase contrast photomicrograph of four day old cultures of MKN 28 cells. (a) Control monolayer incubated with medium only. (b) Monolayer incubated with sodium taurocholate 10 mM for 30 min. Arrows indicate holes which represent areas formed from cells which died and were detached. (c) Monolayer treated with indomethacin 5 mM for 60 min. Many cells are dead and stained darkly with trypan blue (arrows) (d) Monolayer treated with ethanol 15% for 30 min showing cell damage. Short arrows indicate cells stained darkly with trypan blue. Long arrow shows a large hole resulting from detached cells. (e) and (f) Monolayers pretreated with cimetidine 10^{-5} M and ranitidine 10^{-5} M, respectively, before the addition of sodium taurocholate 10 mM. The structure of the monolayers is still disorganised but the amount of cell damage is less than in (b) with fewer holes (arrows) caused by detachment of dead cells.

contrast microscope by an observer unaware of treatment.

RADIOIMMUNOASSAY OF PROSTAGLANDIN E_2
Monolayers were washed three times with DME-F12 medium (37°C) and incubated with medium for 30 min for further washing. This medium was discarded and monolayers were incubated with medium containing test reagents or medium only (control) for 60 min. The medium was collected and centrifuged for 5 min at 2000 rpm and 4°C. The supernatant was collected and stored at -70°C in N_2 atmosphere until assayed. The pellets were combined with corresponding monolayers and protein was determined with the dye binding test according to Bradford.¹⁷ PGE_2 was measured directly in duplicate with a highly sensitive ^{125}I radioimmunoassay kit. Standards in the range of 0.2 to 50 pg PGE_2 were treated in the same way as the samples and the curve calculated on semilogarithmic paper after counting in a Beckman 7000 gamma counter. The limit of detection was 0.1 pg PGE_2 in 0.1 ml volume per assay tube. For PGE_2 , cross reactivity with prostaglandin E_1 was 3.7% and with all other prostaglandins less than 0.4%. Cross reactivity between drugs and antisera to PGE_2 was evaluated in each assay experiment and found to be not significant. The medium used in PGE_2 radioimmunoassay was not supplemented with fetal bovine serum and was antibiotic antimycotic free.

DETERMINATION OF TOTAL REDUCED SOLUBLE SULPH-HYDRYLS

Reduced soluble sulph-hydryl content was measured as follows.^{18,19} After incubation for the experimental periods, monolayers were washed three times with 0.02% EDTA in phosphate buffered saline (PBS); then, 1.4 ml 0.2% Triton X-100 and 2.5% sulphosalicylic acid in EDTA/PBS buffer was added to monolayers. Cells were scraped off with a rubber policeman and sonicated. Solutions were cleared by centrifugation at 11000 g for five minutes. A 1 ml aliquot of the acid soluble supernatant medium was then added to 2.0 ml 0.3 M Na_2HPO_4 buffer. Spectrophotometric determinations were performed with a Gilford Stasar II spectrophotometer (Gilford Instruments Laboratories Inc, Oberlin, Ohio) at 412 nm immediately after the addition of 0.25 ml of 5,5-dithiobis-2-nitrobenzoic acid (40 mg/dl in 1% sodium citrate).²⁰ With each assay a standard curve was generated with known amounts of reduced glutathione (10-100 nmol/ml). Results are expressed as nanomoles of soluble reduced sulph-hydryls/10⁶ cells. The medium used in sulph-hydryl assay experiments was not supplemented with fetal bovine serum and was antibiotic antimycotic free.

Experimental studies

DRUG INDUCED DAMAGE TO CULTURED GASTRIC MUCOSAL CELLS

Prelabelled cells were incubated with medium containing sodium taurocholate 5-15 mM, or indomethacin 2.5-10 mM, or ethanol 5-20%, or medium only, control. Incubation was 30 min with sodium taurocholate or ethanol and 60 min with indomethacin.

EFFECT OF CIMETIDINE AND RANITIDINE ON VIABILITY OF CULTURED CELLS AND ON DRUG INDUCED DAMAGE

To assess whether H_2 blockers produce any damage to cultured gastric mucosal cells, prelabelled cells were incubated with cimetidine or ranitidine 10^{-8} to 10^{-4} M or medium only (control), for 60 min. In order to evaluate whether cimetidine and ranitidine protect gastric cells against drug induced damage, prelabelled cells were preincubated with cimetidine or ranitidine 10^{-8} to 10^{-4} M or medium only (control) for 60 min and then with sodium taurocholate 10 mM (30 min), or indomethacin 5 mM (60 min), or ethanol 15% (30 min).

EFFECT OF INDOMETHACIN ON PGE_2 PRODUCTION BY CULTURED CELLS

Cells were incubated with indomethacin 10^{-8} to 10^{-4} M or medium only (control) for 60 min. Cell free supernatant was then collected and assayed for PGE_2 .

EFFECT OF INDOMETHACIN ON CIMETIDINE AND RANITIDINE INDUCED PROTECTION

After preincubation with indomethacin 10^{-4} M or medium only (control) for 60 min, prelabelled cells were incubated with cimetidine or ranitidine 10^{-5} M for 60 min and then with sodium taurocholate 10 mM for 30 min.

Fig. 2 Sodium taurocholate (NaT), indomethacin (Ind), and ethanol (ETOH) damage cultured cells in a dose dependent manner, as assessed quantitatively by ^{51}Cr release assay. Mean (SE) of seven cultures (taurocholate) and six cultures (indomethacin and ethanol).

EFFECT OF N-ETHYLMALEIMIDE ON TOTAL REDUCED SOLUBLE SULPH-HYDRYL

Cells were incubated with N-ethylmaleimide 0.01–0.5 mM or medium only (control) for 30 min. Cells were then harvested and sulph-hydril content measured.

EFFECT OF N-ETHYLMALEIMIDE ON CIMETIDINE AND RANITIDINE INDUCED PROTECTION

After 30 min incubation with ranitidine 10^{-5} M, the sulph-hydril blocker agent N-ethylmaleimide was added at a final concentration of 0.05 mM. After 30 min, monolayers were washed and incubated with sodium taurocholate 10 mM for 30 min.

EFFECT OF CIMETIDINE AND RANITIDINE ON PGE_2 AND SULPH-HYDRYL PRODUCTION BY CULTURED CELLS

Monolayers were incubated with cimetidine or ranitidine 10^{-7} to 10^{-4} M or medium only (control) for 60 min. Cell free supernatant was then collected and assayed for PGE_2 . Cells were harvested and sulph-hydril content measured colorimetrically.

STATISTICAL ANALYSIS

Data are expressed as mean standard error of mean (SE). Significance of differences was assessed by one-way analysis of variance and, when the F value was significant, by Duncan's new Multiple Range Test.²¹ Differences were considered significant if $p < 0.05$. Dose dependency was assessed by regression analysis.

Fig. 3 Cimetidine (Cim), at all dose levels 10^{-8} to 10^{-4} M and ranitidine (Ran) at dose levels 10^{-5} and 10^{-4} only, significantly reduced 10 mM sodium taurocholate (NaT) induced damage to cultured gastric mucosal cells. Cimetidine and ranitidine 10^{-5} M decreased taurocholate-induced specific ^{51}Cr release by 37% and 27%, respectively. Mean (SE) of nine cultures.

The data for ^{51}Cr release related to the effect of H_2 blockers on taurocholate induced damage have been analysed before normalisation *versus* taurocholate induced specific ^{51}Cr release.

Results

CELL CULTURE AND DRUG INDUCED MORPHOLOGICAL CHANGES

Figure 1a shows a four day old culture of MKN 28 cells. These cells have been shown to be mucus producing cells by means of histochemical staining (PAS positive, negative reaction for succinic dehydrogenase and for pepsinogen granules) and electron microscopy.^{11,12} Figures 1b to 1d shows that incubation with sodium taurocholate 10 mM (Fig. 1b), indomethacin 5 mM (Fig. 1c), or ethanol 15% (Fig. 1d), respectively, disrupted the structure of the monolayer causing death of a large percentage of cells. Pretreatment with cimetidine (Fig. 1e) and ranitidine (Fig. 1f), reduced the damage induced by sodium taurocholate but not by indomethacin or ethanol.

EFFECT OF TAUROCHOLATE, INDOMETHACIN, AND ETHANOL ON VIABILITY OF CULTURED CELLS

Sodium taurocholate, indomethacin, and ethanol damaged monolayers in a dose dependent manner as assessed quantitatively by ^{51}Cr release assay ($r = 0.97$, $p < 0.01$ with taurocholate, $r = 0.97$, $p < 0.01$ with indomethacin, $r = 0.90$, $p < 0.05$ with ethanol) (Fig. 2).

Fig. 4 Cimetidine (Cim) and ranitidine (Ran) did not prevent cell damage induced by indomethacin 5 mM (Ind). Mean (SE) of nine cultures.

Fig. 5 Cimetidine (Cim) and ranitidine (Ran) did not exert a protective effect against 15% ethanol (ETOH) induced damage. Cimetidine, but not ranitidine, showed a tendency to increase ethanol induced specific ⁵¹Cr release; this was significant at the concentration of 10⁻⁴ M. Mean (SE) of nine cultures.

EFFECT OF CIMETIDINE AND RANITIDINE ON DRUG INDUCED DAMAGE

Cimetidine and ranitidine over the range of concentrations used did not produce any significant change in ⁵¹Cr release by cultured cells as compared with control. Cimetidine 10⁻⁸ to 10⁻⁴ M decreased significantly the ⁵¹Cr release induced by sodium taurocholate 10 mM. Cimetidine 10⁻⁵ M decreased taurocholate induced specific ⁵¹Cr release by 37% ($p < 0.001$) (Fig. 3). Ranitidine 10⁻⁵ M and 10⁻⁴ M caused significant protection ($p < 0.01$) decreasing taurocholate induced damage by 27%. Ranitidine did not afford significant protection in the lower concentrations 10⁻⁸ to 10⁻⁶ M (Fig. 3). Cimetidine and ranitidine did not reduce indomethacin and ethanol induced damage (Figs 4 and 5, respectively). Cimetidine, but not ranitidine, showed a tendency to increase ethanol induced specific ⁵¹Cr release; this was significant at the concentration of 10⁻⁴ M (Fig. 5).

EFFECT OF INDOMETHACIN AND N-ETHYLMALIMIDE ON CIMETIDINE AND RANITIDINE INDUCED PROTECTION

Indomethacin 10⁻⁸ to 10⁻⁴ M decreased PGE₂ production by cultured cells in a dose dependent manner (Fig. 6). The concentration of indomethacin 10⁻⁴ M reduced PGE₂ production by cultured cells by 60% (Fig. 6). Pretreatment with the prostaglandin synthesis inhibitor indomethacin in the concentration of 10⁻⁴ M did not prevent the protection afforded by cimetidine and ranitidine (Fig. 7).

Fig. 6 Indomethacin (Ind) decreased PGE₂ production by cultured cells in a dose dependent manner. Indomethacin 10⁻⁴ M inhibited PGE₂ synthesis by approximately 60%. Mean (SE) of five cultures.

N-ethylmaleimide in a concentration of 0.05 mM reduced the sulph-hydryl content of cultured cells by 45% (Fig. 8). Higher concentrations of N-ethylmaleimide (0.1 and 0.5 mM) reduced the sulph-hydryl content of cultured cells to a much greater extent but these concentrations themselves signifi-

Fig. 7 Prostaglandin synthesis inhibitor indomethacin (Ind) did not prevent the protective effect exerted by cimetidine (Cim) and ranitidine (Ran) against taurocholate (NaT) induced damage. Mean (SE) of eight cultures.

Fig. 8 *N*-ethylmaleimide (NEM) significantly and in a dose dependent manner decreased levels of total reduced soluble sulph-hydryls. Mean (SE) of eight cultures.

cantly damaged cells and increased taurocholate induced damage. The sulph-hydryl blocker agent *N*-ethylmaleimide did not prevent the protection afforded by cimetidine and ranitidine against damage brought about by sodium taurocholate (Fig. 9). Indomethacin and *N*-ethylmaleimide at the concentrations used did not cause any cell damage nor did they increase taurocholate induced damage (Figs 7 and 9).

Fig. 9 Sulph-hydryl blocker agent *N*-ethylmaleimide (NEM) did not prevent the protective effect of cimetidine (Cim) and ranitidine (Ran) against taurocholate (NaT) induced damage. Mean (SE) of six cultures.

Fig. 10 Cimetidine (Cim) and ranitidine (Ran) did not increase PGE₂ production by cultured cells (A) nor did they affect total reduced soluble sulph-hydryl content of cells (B). Mean (SE) of six cultures.

EFFECT OF CIMETIDINE AND RANITIDINE ON PGE₂ AND SULPH-HYDRYL PRODUCTION BY CULTURED CELLS

MKN 28 cells produced PGE₂ in the amount of 458 (37) pg/mg protein/h. Incubation with cimetidine and ranitidine did not increase PGE₂ production by cultured cells (Fig. 10). The cellular content of reduced sulph-hydryl compounds was 22.7 (1) nmol/10⁶ cells. Cimetidine and ranitidine did not affect the sulph-hydryl content of monolayers (Fig. 10).

Discussion

This study shows that the histamine H₂ receptor blocking agents cimetidine and ranitidine are able to protect gastric mucosal cells grown in tissue culture against drug induced damage in conditions independent of their acid inhibitory effect as well as of systemic factors. This protection occurs against

sodium taurocholate, but not against indomethacin or ethanol induced damage. Endogenous prostaglandin and sulph-hydryl compounds do not appear to play a major role in the protective effect exerted by cimetidine and ranitidine.

The H₂ blockers cimetidine and ranitidine have been shown to protect against gastric mucosal damage induced by aspirin *in vivo*.^{1,2,22,23} Cimetidine and ranitidine do not, however, protect rat gastric mucosa against ethanol induced damage *in vivo*.^{3,24,25} As aspirin induced gastric mucosal damage is dependent on the pH of gastric content,²⁶ whereas ethanol induced damage is not,^{27,28} H₂ blocker mediated protection of gastric mucosa *in vivo* may merely be the result of inhibition of gastric acid secretion and, therefore, not true cytoprotection. On the other side, H₂ antagonists have been shown to be protective in non-anti-secretory doses.^{2,4,6} Also, Hogan *et al* have recently shown that cimetidine decreases the damaging effect of acute aspirin administration on the human gastric mucosa in conditions independent of suppression of gastric acid secretion.²² Using an experimental model of mucus producing cells and carrying out experiments at pH 7.4 we found cimetidine and ranitidine significantly protected gastric cells against taurocholate induced damage. These findings are in agreement with a report from Utley *et al* who found protection by cimetidine against taurocholate induced damage to rat gastric mucosa.²⁹ Also, Muller *et al* reported that ranitidine prevents the taurocholate induced drop in gastric mucosal potential difference in man.³⁰ Cimetidine and ranitidine 10⁻⁵ M decreased taurocholate induced ⁵¹Cr release by 37% (p<0.001) and 28% (p<0.01), respectively. Cimetidine exerted a significant protective effect also in lower concentrations (10⁻⁸ to 10⁻⁶ M), whereas ranitidine protected cells only in higher concentrations (10⁻⁵ and 10⁻⁴ M). Both cimetidine and ranitidine failed to prevent the damaging effect of indomethacin and ethanol. This is in agreement with other *in vitro* studies which showed no protection by cimetidine against damage induced by indomethacin to rat gastric mucosal cells or isolated rat gastric glands.^{31,32} Tarnawski *et al* have reported that cimetidine and ranitidine increase the amount of damage induced by ethanol to rat gastric mucosa *in vivo*.³ Our findings are in partial agreement with this study in as much as we found a significant increase in ethanol induced cell damage after pretreatment with cimetidine 10⁻⁴ M, but not with ranitidine. It can be postulated that the cimetidine induced increase in cell damage brought about by ethanol is caused by an inhibitory effect exerted by cimetidine on gastric alcohol dehydrogenase, the enzymatic system responsible for the oxidation of alcohol.³³

No agents to date *in vitro* are able to offer complete

100% protection against damaging drugs. Prostaglandins, considered to be the ultimate cytoprotective agent, in *in vitro* tissue culture studies reduced taurocholate induced damage by 30%.¹⁵ Failure by H₂ blockers and prostaglandins to protect gastric mucosal cells *in vitro* completely is consistent with the significant percentage of mucosal cell damage *in vivo* after damaging agents such as ethanol in spite of prostaglandin administration when the tissues are examined microscopically as in the current study.³⁴⁻³⁷

The mechanism of the protective effect exerted by cimetidine and ranitidine is unknown. In particular, there is controversy as to the role of endogenous prostaglandin for this protection to occur. The present study shows that pretreatment with the prostaglandin synthesis inhibitor indomethacin does not prevent the protective effect of cimetidine and ranitidine. Furthermore, incubation with cimetidine and ranitidine does not increase the amount of PGE₂ produced by cultured cells. These findings are in agreement with those of Hiraishi *et al* who found no effect of cimetidine on PGE₂ and 6-keto PGF_{1α} production by rat gastric mucosal cells in tissue culture³¹ and with those of Konturek *et al* who showed that ranitidine, in a cytoprotective concentration, does not prevent aspirin induced inhibition of PGE₂ and prostacyclin production by rat gastric mucosa.³ Because indomethacin does not inhibit prostaglandin production by 100%, a role for prostaglandins in this protection cannot be excluded completely.

Sulph-hydryl containing drugs protect gastric mucosa against exogenous injury.⁴ We have recently shown that the sulph-hydryl agent cysteamine protects gastric mucosal cells in tissue culture against taurocholate and indomethacin induced damage¹⁰ and that endogenous sulph-hydryls might be involved in the protection of gastric cells by acetaminophen *in vitro*.³⁸ We have therefore evaluated whether the protective effect of cimetidine and ranitidine was related to endogenous sulph-hydryls. The sulph-hydryl blocker N-ethylmaleimide was not able to prevent the protection afforded by cimetidine and ranitidine against taurocholate induced damage nor did incubation with cimetidine and ranitidine affect the cellular level of endogenous sulph-hydryls. Therefore, it seems unlikely that endogenous sulph-hydryl compound is involved in the protection exerted by H₂ blockers.

Because these experiments have been carried out *in vitro* in conditions independent of vascular, hormonal, and neural factors, protection against taurocholate induced cell damage must include the direct effect of the H₂ blockers on cell membrane or metabolism. Ranitidine and cimetidine affect the barrier function of the external membrane as indicated by preventing trypan blue from penetrating the

cell and preventing ^{51}Cr efflux; this suggests that the cell membrane is the site of the protective action of the H_2 blockers. This is consistent with the physiological properties of taurocholic acid which has a pK_a of 1.4. Therefore, at pH 7.4 it is entirely in the dissociated state with no lipid solubility or ability to penetrate cell membranes. On the other hand, ethanol and indomethacin are both more capable of penetrating the cell at neutral pH. In a recent study,³⁹ Tarnawski *et al* presented ultrastructural evidence that indomethacin and ethanol produce cell injury by damaging cytoskeletal elements (actin filaments, intermediate filaments, and microtubules) within isolated gastric epithelial cells *in vitro*. The inability of H_2 blockers to protect against damage induced by these agents suggests that while they can protect against the external detergent action of taurocholate to the cell membrane, they are unable to protect against internal cell damage by indomethacin and ethanol. Prostaglandins, on the other hand, do have this protective ability *in vitro*, in tissue culture¹⁵ and isolated gland preparations.³⁹ The protective effect of the H_2 blockers does not seem to be directly mediated by H_2 receptors on the cell membrane; ranitidine is a more potent H_2 receptor blocker yet it is a less potent protector of the mucosal cell than cimetidine.

In conclusion, (1) cimetidine and ranitidine protected gastric mucosal cells against taurocholate induced damage in conditions independent of their acid inhibitory effect and of systemic factors; (2) cimetidine was effective in lower concentrations than ranitidine, suggesting that protection was not directly related to ability to block H_2 receptors on the cell membrane; (3) cimetidine and ranitidine did not protect against indomethacin and ethanol induced damage; (4) cimetidine and ranitidine did not stimulate production of endogenous PGE_2 or sulph-hydril compounds; thus the protective effects of cimetidine and ranitidine against taurocholate induced damage seem unlikely to be the result of prostaglandin or sulph-hydril compounds but (5) are probably because of a direct action at the level of the external cell membrane.

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