Cysteamine protects gastric epithelial cell monolayers against drug induced damage: evidence for direct cellular protection by sulphydryl compounds

M Romano, M Razandi, A Raza, S Szabo, J Ivey

Abstract
The sulphydryl containing drug cysteamine protects gastric mucosa in vivo against acute injury. It is not known whether this protection includes a direct effect on gastric cells. Using gastric epithelial cell monolayers derived from a well differentiated human cell line, we evaluated whether cysteamine protects against taurocholate or indomethacin induced damage in conditions which completely exclude the influence of vascular, hormonal, and neural factors. The effect of cysteamine on prostaglandin production by monolayer cells in vitro was also assessed. Cysteamine decreased damage brought about by sodium taurocholate and indomethacin by 40% (p<0.01) and 50% (p<0.01) respectively. The sulphhydryl blocker iodoacetamide prevented the protective effect of cysteamine. Pretreatment with indomethacin, which inhibited prostaglandin E2 output by 60%, did not prevent protection by cysteamine; incubation with cysteamine decreased prostaglandin E2 production by cultured cells. We conclude that (i) cysteamine directly protected gastric epithelial cells in vitro (ii) this protection occurred with indomethacin, which interferes with cellular metabolism of prostaglandins, and taurocholate, whose damaging action at neutral pH is unrelated to interference with prostanoïd metabolism, (iii) cysteamine protection in vitro is unrelated to endogenous prostaglandins and is probably mediated by endogenous sulphydryl compounds.

Sulphydryl containing drugs, such as cysteamine, have been shown to protect in vivo against acute gastric mucosal injury caused by ethanol,1 indomethacin,2 and endogenous agents released by exposure to cold and restraint stress.3 Gastric vascular endothelium has been reported to be the target of the protective effect afforded by sulphphydryl compounds in vivo.4 It is not known whether sulphhydryl containing drugs protect gastric epithelial cells directly without the mediation of vascular or other systemic factors against damage brought about by sodium taurocholate and indomethacin.

Several agents, such as histamine H2 receptor antagonists and omeprazole,6,7 protect against damage to the cell membranes by taurocholate, but only prostaglandins have been found capable of protecting against agents that alter internal cellular metabolism such as indomethacin.8 The role of endogenous prostaglandins in the protection of gastric mucosa by sulphphydryl containing drugs is unclear.9,10 The effect of cysteamine on prostaglandin E2 (PGE2), production by gastric epithelial cells is unknown.

The aims of the present study were: (i) to evaluate whether the sulphphydryl agent cysteamine protects gastric epithelial cell monolayers against damage brought about by sodium taurocholate and indomethacin in vitro in conditions independent of vascular, neural, and hormonal factors; (ii) to evaluate the effect of cysteamine on PGE2 production by gastric cells using monolayers derived from a well differentiated human gastric cell line, MKN 28.11 The MKN 28 cell line has been shown to be an effective model for the study of drug induced damage to and protection of gastric cells in vitro giving results comparable with those for monolayers of human gastric epithelial cells in primary cultures derived from surgical specimens.12

Methods

MATERIALS

The characteristics of the MKN 28 cell line have been described.12 Culture medium was a 1:1 mixture of Dulbecco's modified Eagle medium (DME) and Ham's nutrient F-12 medium (F-12 medium) (both from Gibco, Grand Island, NY) supplemented with 15 mmol/l N-hydroxyethylpiperazine-N-ethane sulphonic acid (Hepes) buffer (Sigma Chemical, St Louis, MO), 2 g/l NaHCO3 (Sigma), heat inactivated (at 56°C for 30 min) 10% fetal bovine serum (Armour Pharmaceutical, Kankakee, IL), and 1% antibiotic-antimycotic solution, 100 µl/ml penicillin, 100 µg/ml streptomycin, 0-25 µg/ml amphotericin B (Irvine Scientific, Santa Ana, CA). Sodium taurocholate, cysteamine, indomethacin, iodoacetamide, monochloroacetic acid, reduced glutathione, cysteine, and ethylendiamine tetra-acetic acid (EDTA) were purchased from Sigma. Taurocholate, cysteamine, and iodoacetamide were dissolved in DME-F12 medium, pH was adjusted to 7.4, and drugs were used immediately. Indomethacin 50 mmol/l was freshly prepared, obtained by dissolving the drug in 0.2 mol/l Na2CO3, pH was adjusted to 7.4 by adding 5 mol/l NaH2PO4. This solution was diluted in DME-F12 medium to reach the desired concentration and the solution was used immediately. 3H-Chromium (3Cr) (sodium chromate 200–900 Ci/g chromium) and trypsin were from ICN Biomedicals, Irvine, CA.

1I prostaglandin E2 (PGE2) radioimmunoassay

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Cysteamine protection in gastric cell culture

Fig 1A

Fig 1B

Figure 1: Phase contrast photomicrograph of four day old cultures of MKN 28 cells (×200) 10 minutes after the addition of trypan blue 0-4% (A) Control monolayer incubated with medium only. Cells show a sheet like organised growth pattern and form a confluent monolayer of cells tightly attached to one another. No cells stained with trypan blue (dead cells) are seen. (B) Monolayer treated with sodium taurocholate 10 mmol/l. Taurocholate caused the death of a large percentage of cultured cells as shown by the large 'holes' (closed arrows) in the confluent monolayer. Solid white arrows indicate areas of lysed cells, still attached. (C) Monolayer treated with cysteamine 10 mmol/l then with taurocholate 10 mmol/l. The structure of the monolayer is relatively preserved with smaller areas of detached cells (closed arrows) compared to (B) and some areas of lysed cells (solid white arrows). (D) Monolayer treated with indomethacin 5 mmol/l. Indomethacin damaged a large number of cells which stained with trypan blue (short closed arrows). Solid white arrow shows an area of lysed cells; the small 'hole' (long closed arrow) indicates an area formed from cells which died and detached. (E) Monolayer treated with cysteamine 10 mmol/l before the addition of indomethacin 5 mmol/l. Appreciable reduction in number of dead cells (arrows); no cell detachment; no lysed areas.

kit was obtained from New England Nuclear, Boston, MA. High performance liquid chromatography grade methanol was purchased from Fisher Scientific, Pittsburgh, PA. Sodium octyl sulphate was obtained from Eastman Kodak, Rochester, NY and perchloric acid from Aldrich Chemical, Milwaukee, WI.

CELL CULTURE

The MKN 28 cell line was derived from a well differentiated human gastric tubular adenocarcinoma. For our study MKN 28 cells were grown as monolayers in a 1:1 mixture of DME and F-12 medium supplemented with 15 mmol/l Hapes buffer, 1.2 g/l NaHCO3, 10% fetal bovine serum, and 1% antibiotic-antimycotic solution. Confluent monolayers were subcultured every four days by treatment with 0.1% trypsin and 0.9 mmol/l EDTA in Ca++ and Mg++ free phosphate buffered saline. After trypsin treatment cells were plated onto 35×10 mm culture dishes (Corning, Corning, NY). About 6×10⁶ cells in 1 ml of medium were inoculated per dish. Cultures were maintained in a seriti-cult incubator (Forma Scientific, Marietta, OH) at 37°C in humidified atmosphere of 5% CO2 in air. Confluent monolayers, usually from four to five day old cultures, were studied. Cultured cells were examined daily with an inverted phase contrast tissue culture microscope (Nikon Diaphot, Nikon, Garden City, NY). In all drug experiments medium was used which was not supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution.

³⁵Cr RELEASE ASSAY

The method for³⁵Cr release assay was as described previously. Media were discarded and monolayers were washed three times with 1 ml of medium (37°C) to remove floating cells in culture. Cells were then incubated in medium containing 5 µCi/ml³⁵Cr for 60 minutes in the culture condition. Labelled cells were washed five times with medium (4°C) to remove excess isotope and then 1 ml of medium (37°C) containing test reagents, as described under experimental studies below, was added to monolayers. After incubation for the experimental period in the culture condition, cell free supernatant was collected. Monolayers were dissolved in 1M NaOH for 24 hours. The radioactivity of the cells and of the supernatants was counted with an automatic gamma counter (Beckman 7000, Beckman Instruments, Fullerton, CA). The percentage of³⁵Cr released per sample was expressed as:

\[ \text{³⁵Cr release (%) =} \]
\[ \frac{\text{cpm supernatant}}{\text{cpm supernatant + cpm cells}} \times 100 \]

or as: Specific³⁵Cr release (%) (release due to drug action)=³⁵Cr release (%) in the presence of the test drug –³⁵Cr release (%) in the control (monolayers incubated with medium only).

TRYPAN BLUE DYE EXCLUSION TEST

Trypan blue dye exclusion test was performed as described elsewhere. Briefly, after incubation with test drugs monolayers were washed three times with DME-F12 medium. Washed monolayers were kept in 1 ml of DME-F12 medium until the test was performed. One tenth of a milliliter of trypan blue (0-4%) was then added to monolayers and mixed. Ten minutes later monolayers were observed in an inverted phase contrast microscope by an observer unaware of treatment.
LACTATE DEHYDROGENASE RELEASE ASSAY
Monolayers were washed three times with DME-F12 medium and incubated with sodium taurocholate 1-15 mmol for 30 minutes. Supernatant medium was then collected and 1 ml of DME-F12 medium was added to monolayers. Cells were scraped off the dishes, collected, and disrupted by sonication with a Branson Sonifier 450 (Branson). Lactate dehydrogenase content of supernatant and cells was determined kinetically in the ultraviolet wave length region making use of absorption changes at 340 nm using the pyridine nucleotide forms of nicotinamide adenine dinucleotide (NAD) and (NADH) on a Beckman Astra 8 Automated Stat/Routine Analyst. The amount of lactate dehydrogenase (LDH) released per sample was expressed as:

LDH release%:

\[
\frac{\text{LDH supernatant}}{\text{LDH supernatant} + \text{LDH cells}} \times 100
\]

or as: specific LDH release % (LDH release due to drug action)=LDH release in the presence of the test drug−LDH release in control monolayers (monolayers incubated with medium only).

RADIOIMMUNOASSAY OF PGE2
Monolayers were washed three times with medium (37°C) and incubated for 30 minutes for further washing. This medium was discarded and monolayers were incubated with medium containing test reagents or medium only (control) for 60 minutes. The medium was collected and centrifuged for five minutes at 2000 rpm and 4°C. The supernatant was collected and stored at −70°C until assayed. In separate experiments indomethacin 10⁻⁴ was added to the medium after collection and before centrifugation to rule out the possibility that some detached cells still synthesizing and releasing PGE₂ could produce a high PGE₂ background. No difference was found in PGE₂ production between the two groups—that is, with and without indomethacin. Protein content of cells was determined with the dye binding test according to Bradford.19 PGE₂ was measured directly in duplicate with 1²⁵I radioimmunoassay kit. Standards in the range of 0-2 to 50 pg/0.1 ml were treated in the same way as samples and the curve was calculated on semilogarithmic paper after counting in a Beckman 7000 gammacounter. The limit of detection was 0.2 pg/0.1 ml per assay tube. Cross reactivity with PGE₁ was 3.7% and with all other prostaglandins less than 0.4%. Cross reactivity between medium (with or without test drugs) and antiserum to PGE₂ was evaluated in each assay experiment and found to be not significant. The medium used in PGE₂ assays was not supplemented with fetal bovine serum and was antibiotic-antimycotic free.

DETERMINATION OF NON-PROTEIN GLUTATHIONE AND NON-PROTEIN CYSTEINE
Determination has been described.19 Cells
Cysteamine protection in gastric cell culture

were grown in T 75 tissue culture flasks (Corning). Confluent monolayers were incubated with medium containing test reagents as described under experimental studies below. Cells were collected after trypsinisation, washed three times with Hank's balanced salt solution (4°C), and stored frozen until assayed. On the assay day cells were homogenised at 50 000 rpm for 5 seconds to disseminate any large particles and to form an even suspension; 250 μl of dH2O were added to a 50 μl aliquot of the suspension for osmotic lysis of the cells; 10 μl of perchloric acid 1N were added to precipitate protein. This gave a final perchlorate concentration of 0.25 N and a sample dilution factor of 8. Samples were then filtered through 0.2 μm membrane filter (Altech Associate, Deerfield, IL) and 20 μl injected on the chromatography column. The concentration of free glutathione and cysteine were measured by high performance liquid chromatography with electrochemical detector. Chromatography was on a C-18 reverse phase 5 μm column (Bioanalytical Systems, West Lafayette, IN), the mobile phase being 0.05 mol/l monochloroacetic acid, pH 3, with 1 mmol/l sodium octylsulphate as the ion pairing reagent, and 2–6% methanol to control retention parameters. Detection was with a Bioanalytical Systems LC-4B amperometric detector using a gold/mercury electrode at an applied potential of +0.15 V v Ag/AgCl reference electrode. Thiol concentrations were quantified from glutathione and cysteine standard curves and expressed as nmol/mg of protein.

EXPERIMENTAL STUDIES

Correlation between 51Cr release assay and lactate dehydrogenase release assay

Monolayers were incubated with sodium taurocholate 1, 5, 10, 12.5, 15 mmol/l or medium only (control) for 30 minutes.

Taurocholate or indomethacin induced damage to cultured cells
Cells were incubated in medium containing 5–15 mmol/l sodium taurocholate or 2.5–10 mmol/l indomethacin or medium only (control) for 30 and 60 minutes respectively.

Effect of cysteamine on taurocholate or indomethacin induced cell damage

After preincubation for 60 minutes in medium containing 1–20 mmol/l cysteamine or medium only (control), cells were incubated with sodium taurocholate 10 mmol/l or indomethacin 5 mmol/l for 30 and 60 minutes, respectively.

Effect of indomethacin on PGE2 production by cultured cells
Cells were incubated with indomethacin 10−4 to 10−6 mol/l medium only (control) for 60 minutes. Cell-free supernatant was then collected and assayed for PGE2.

Effect of iodoacetamide on non-protein glutathione and non-protein cysteine levels in cultured cells
Cells were incubated for 30 minutes in medium containing iodoacetamide 0.01–1 mmol/l or medium only (control).
Figure 5: Effect of cysteamine on taurocholate induced cell damage. Mean (SEM) of seven cultures. Cysteamine 10 mmol/l decreased taurocholate induced specific ${^{51}}$Cr release from 28.9% to 17.8% (11.1 point reduction equal to 39% decrease of cell damage).

Figure 6: Effect of cysteamine on indomethacin induced damage. Mean (SEM) of eight cultures. Cysteamine reduced indomethacin induced specific ${^{51}}$Cr release from 31.9% to 14.5% (17.4 point reduction equal to 55% decrease of cell damage).

**Effect of indomethacin or iodoacetamide on protection by cysteamine**

After preincubation with the sulphhydryl blocker iodoacetamide 0.1 mmol/l for 30 minutes or the prostaglandin synthesis inhibitor indomethacin 10$^{-4}$ mol/l for 60 minutes or medium only (control) cells were incubated with cysteamine 10 mmol/l and then with sodium taurocholate 10 mmol/l or indomethacin 5 mmol/l. Indomethacin 10$^{-4}$ mol/l has been shown to inhibit by more than 50% PGE$_2$ production by MKN 28 cells. 

**Effect of cysteamine on PGE$_2$ production by cultured cells**

Cells were incubated with cysteamine 0.1-10 mmol/l or medium only (control) for 60 minutes and cell free supernatant was collected and assayed for PGE$_2$.

**STATISTICAL ANALYSIS**

Results are expressed as mean (SEM). 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. 

Dose dependency was assessed by regression analysis. Differences were considered significant at p<0.05.

**Results**

**CELL CULTURE AND DRUG-INDUCED MORPHOLOGICAL CHANGES**

Figure 1A shows a phase contrast photomicrograph of a four day old culture. Cells were initially plated in isolated clumps and then spread out to form a monolayer consisting of large polygonal shaped cells with a sheet like organised growth pattern. Cells were identified as mucus producing cells by positive periodic acid-Schiff reaction and electron microscopic characteristics of mucus granules. 

Figure 1 A-E show the light microscopic changes and viability of cultured cells after incubation with medium only (control) (A), sodium taurocholate 10 mmol/l alone (B), or after pretreatment with cysteamine 10 mmol/l (C), indomethacin 5 mmol/l alone (D), or after pretreatment with cysteamine 10 mmol/l (E). Preincubation with cysteamine clearly reduced the cell damage induced by taurocholate and indomethacin.

**CORRELATION BETWEEN ${^{51}}$CR RELEASE ASSAY AND LACTATE DEHYDROGENASE RELEASE ASSAY**

Figure 2 shows the quantitative assessment of taurocholate induced cell damage by using ${^{51}}$Cr release assay and lactate dehydrogenase release assay. The two methods were highly correlated (r=0.979, p<0.001). $^{51}$Cr release assay was used to assess cell damage in the subsequent experiments.

**EFFECT OF SODIUM TAUROCHOLATE OR INDOMETHACIN ON CELL VIABILITY**

Figures 3 and 4 show that sodium taurocholate
Cysteamine protection in gastric cell culture

**Figure 7:** Effect of cysteamine on damage induced by indomethacin 7.5 mmol/l and taurocholate 15 mmol/l. Cysteamine significantly decreased 15 mmol/l taurocholate induced damage (mean (SEM) of nine cultures), but did not protect against 7.5 mmol/l indomethacin induced cell injury (mean (SEM) of six cultures).

(5–15 mmol/l) and indomethacin (2.5–10 mmol/l) damaged cells in a dose dependent manner over the concentration range studied ($r=0.96$, $p<0.01$; $r=0.97$, $p<0.01$, respectively).

**EFFECT OF CYSTEAMINE ON TAUROCHOLATE OR INDOMETHACIN INDUCED DAMAGE**

Cysteamine alone, up to 20 mmol/l, did not affect cell viability (Figs 5 and 6). Sodium taurocholate 10 mmol/l and indomethacin 5 mmol/l induced about 30% damage as quantitatively assessed by $^{51}$Cr release assay (Figs 5 and 6). Cysteamine 1–20 mmol/l significantly prevented cell damage induced by taurocholate and indomethacin (Fig 5 and 6). Cysteamine 10 mmol/l, the level at which maximum protection occurred, decreased taurocholate or indomethacin induced specific $^{51}$Cr release from (mean (SEM)) 28.9 (2)% to 17.8 (1.7)% (11.1 point reduction, equal to 39% decrease of cell damage, $p<0.01$) and from 31.9 (1)% to 14.5 (1.5)% (17.4 point reduction equal to 55% decrease of cell damage, $p<0.01$), respectively (Figs 5 and 6). To evaluate over what range of damage cysteamine does or does not give protection, we also tested the effect of cysteamine on cell damage induced by higher doses of taurocholate or indomethacin. Cysteamine 10 and 20 mmol/l decreased 15 mmol/l taurocholate induced specific $^{51}$Cr release from 74.5 (1)% to 65.8 (2)% (8.7 point reduction equal to 12% decrease of cell damage, $p<0.01$) and to 62.7 (1)% (11.8 point reduction, equal to 16% decrease of cell damage, $p<0.01$), respectively (Fig 7). Cysteamine did not give appreciable protection against damage induced by indomethacin 7.5 mmol/l.

**EFFECT OF INDOMETHACIN OR IODOACETAMIDE ON PROTECTION BY CYSTEAMINE**

To assess whether cysteamine induced protection was affected by endogenous prostaglandin or sulphhydryl production, we evaluated the effect of the prostaglandin synthesis inhibitor indomethacin and the sulphhydryl blocker iodoacetamide on the protection given by cysteamine. Indomethacin $10^{-4}$ to $10^{-4}$ mol/l decreased PGE$_2$ production by cultured cells in a dose dependent manner (Fig 8). The concentration of indomethacin $10^{-4}$ mol/l reduced PGE$_2$ production by cultured cells by 60%. The cellular content of non-protein glutathione and non-protein cysteine was (mean (SEM)) 66.1 (4.5) nmol/mg protein, and 19.1 (2.5) nmol/mg protein, respectively. Iodoacetamide 0.05 mmol to 1 mmol/l decreased the intracellular levels of non-protein sulphhydryls in a dose dependent manner (Fig 9). Iodoacetamide 0.1 mmol/l decreased by approximately 50% the levels of...
Figure 9: Effect of iodoacetamide on non-protein glutathione and non-protein cysteine content of cultured gastric mucosal cells. Mean (SEM) of five cultures. Iodoacetamide decreased sulphydryl content of cultured cells in a dose dependent manner. Iodoacetamide 0-1 mmol/l decreased by approximately 50% the levels of non-protein sulphydryls in MKN 28 cells.

non-protein glutathione in MKN 28 cells (Fig 9). Pretreatment with the prostaglandin synthesis inhibitor indomethacin in the concentration of 10⁻⁴ mol/l did not prevent the protection given by cysteamine (Figs 10 and 11). On the contrary, the sulphydryl blocker agent iodoacetamide significantly prevented the protection afforded by cysteamine 10 mmol/l. Neither cysteamine (10 mmol/l), indomethacin (10⁻⁴ mol/l), nor iodoacetamide (0-1 mmol/l) produced significant cell damage.

EFFECT OF CYSTEAMINE ON PGE₂ PRODUCTION BY CULTURED CELLS
To further determine the role of endogenous prostaglandins in cysteamine induced protection, we evaluated the effect of cysteamine on the generation of PGE₂ by cultured cells. Cultured cells produced PGE₂ in the amount of (mean (SEM)) 280 (50) pg/mg protein/h. Cysteamine 1 and 10 mmol/l significantly decreased the amount of PGE₂ produced by MKN 28 monolayers (p<0-05 and p<0-01, respectively) (Fig 12).

Discussion
The present study shows that the sulphydryl containing drug cysteamine protects gastric epithelial cell monolayers against drug induced damage in vitro in conditions independent of systemic vascular, neural, and hormonal factors. This protection occurs against an agent which damages cell membranes without affecting the cellular metabolism of prostanoids (taurocholate) and one which alters the internal cellular metabolism of prostaglandins (indomethacin). While only prostaglandins have previously been shown to protect against both these agents in vitro, cysteamine protection in vitro occurs despite partial inhibition of endogenous prostaglandin synthesis.

Exogenous sulphydryl compounds have been reported to protect gastric mucosa in vivo against acute injury by ethanol and non-steroidal anti-inflammatory drugs. The role of sulphydryl agents in gastric mucosal injury has, however, been questioned. The prevention of early damage to gastric vascular endothelium may contribute to the ability of sulphydryl compounds to protect gastric mucosa in vivo. Whether sulphydryl containing drugs can protect gastric mucosal cells directly has not been studied. We investigated this by using an experimental model where the influence of vascular and other systemic factors is excluded. In these conditions, cysteamine 10 mmol/l, reduced cell damage induced by indomethacin 5 mmol/l and taurocholate 10 mmol/l by 55% and 39% respectively. To evaluate over what range of damage cysteamine is protective, we also tested the effect of cysteamine on severe damage induced by indomethacin 7-5 mmol/l and taurocholate 15 mmol/l. Cysteamine 10 and 20 mmol/l significantly decreased taurocholate 15 mmol/l induced cell damage by 12% and 16% respectively, but did not protect against damage induced by indomethacin 7-5 mmol/l. These results show that cysteamine has a direct protective effect on gastric mucosal cells, independent of vascular and systemic factors.

Whether prostaglandins play a part in the protective effect of sulphydryl compounds is uncertain. Complete suppression of prostaglandin synthesis with indomethacin in vivo caused only a 50% reduction in the protective effect of cysteamine against ethanol induced damage. Our study shows that pretreatment with indomethacin in vitro did not prevent protection by cysteamine and that incubation with a protective concentration of cysteamine decreased the amount of PGE₂ produced by cultured cells. Our data are in agreement with those in a recent report by Peskar and Lange, who found inhibition of mucosal release of PGE₂ by sulphydryl containing drugs in rats. Never-
effect of cysteamine. On the other hand, pretreatment with the sulphydryl blocker agent indomethacin prevented the protection afforded by cysteamine. Mean (SEM) of seven cultures.

Cysteamine is not the first agent that has been found to exert a protective action on gastric epithelial cells without enhancing prostaglandin production. Protection by cimetidine, ranitidine, and omeprazole against taurocholate induced damage to gastric mucosal cells was not associated with any change in prostaglandin production. On the other hand, somatostatin, which did not protect against taurocholate damage in vitro significantly increased prostaglandin production. 

Suggestive evidence that protection by cysteamine in this study is specifically related to endogenous sulphydryl groups is that the sulphydryl blocker iodoacetamide, in a concentration which decreased by more than 50% the cellular levels of non-protein sulphydryls (without affecting cell viability), significantly prevented the protective effect of cysteamine. It has been suggested that endogenous sulphydryl groups participate in many membrane related processes that involve energy transduction and solute transport which might play a part in the cellular response to injury. Both non-steroidal anti-inflammatory drugs and bile salts may induce damage to gastric cells via toxic oxygen radicals. Sulphydryl agents are potent protectors against toxic oxygen radical induced damage to gastric mucosal cell in vitro. It is possible that cysteamine exerts its protective actions via its sulphydryl group by this mechanism.

No agents to date in vitro can offer complete protection against damaging drugs. By comparison prostaglandins in vitro offer more effective protection against taurocholate induced damage by 30% and indomethacin induced damage by 25%. Recently, 16, 16 dimethyl PGE2 has been shown to decrease ethanol induced damage to dispersed chief cells from guinea pig stomach by 47% as assessed by lactate dehydrogenase release assay. Failure by cysteamine and prostaglandin to protect mucosal cells in vitro completely is consistent with the significant percentage of microscopic mucosal cell damage in vivo after potent damaging agents such as ethanol despite prostaglandin administration; in the latter case microscopic protection of mucosal epithelial cells is of the order of 25%. Moreover, in vivo other protective factors are potentially involved such as vascular, hormonal, neural, motility, and secretory.

Our findings show that (i) the sulphydryl compound cysteamine directly protects gastric epithelial cells in vitro (ii) this protection is against a drug which inhibits prostaglandin production (indomethacin) and one whose damaging effect is not associated with interference with prostanooid metabolism (taurocholate), (iii) cysteamine protection in vitro occurs despite inhibition of PGE2 synthesis (iv) seems to be directly related to the activity of its sulphydryl group possibly against toxic oxygen radicals.

MR was a visiting scientist from the Istituto di Medicina Generale e Metodologia Clinica, 1st Medical School, University of Naples, Italy.


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