Investigation of gastroprotective compounds at subcellular level in isolated gastric mucosal cells

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Nagy, Lajos, Romeo E. Morales, Martin Beinborn, Peter Vattay, and Sandor Szabo. Investigation of gastroprotective compounds at subcellular level in isolated gastric mucosal cells. Am J Physiol Gastrointest Liver Physiol 279: G1201–G1208, 2000.—We tested the hypothesis that recognized gastroprotective agents exert direct protection against ethanol-induced injury in isolated rat gastric mucosal cells in vitro. If protection exists, we also wanted to identify subcellular targets in the reversible and/or irreversible stages of cell injury. Ethanol-induced cell injury was quantified by measuring plasma membrane leakage (trypan blue exclusion and lactate dehydrogenase release), mitochondrial integrity (succinic dehydrogenase), and nuclear damage (ethidium bromide-DNA fluorescence). Initial cell viability and responsiveness were estimated by the effects of carbachol, carbachol + atropine, or 16,16-dimethyl-PGE<sub>1</sub>, on chief cell pepticinogen secretion. Enriched parietal cells were stimulated by histamine, carbachol, or histamine + IBMX. Preincubation of cells with PG, sucrose octasulfate, or the sulfhydryl compounds N-acetylcysteine, tauroine, or cysteine increased cell resistance ≥21% against ethanol. Similar protection was found with low histamine concentrations, but a higher concentration aggravated ethanol toxicity. Other occurring or synthetic gastroprotective agents offered partial protection or aggravated ethanol-induced cell injury. Only a few in vivo gastroprotective agents demonstrated in vitro direct cytoprotection, which involved mainly the reversible stage of cell injury (e.g., plasma membrane changes) and, less often, irreversible (e.g., mitochondrial and nuclear) damage. Our findings also indicate that a major part of the beneficial effect of gastroprotective agents is expressed at the tissue level.

ethanol-induced cell injury; plasma membrane; mitochondrial; nuclear damage; direct cytoprotection

THE TERM “CYTOPROTECTION” was introduced into gastrointestinal pathophysiology and pharmacology by A. Robert to describe the prevention of acute hemorrhagic gastric erosions produced by concentrated ethanol, hydrochloric acid, sodium hydroxide, sodium chloride, or boiling water by non-antisecretory doses of PGs in rats (28, 29). Although the basic biochemical mechanisms of gastric mucosal injury and protection remain unclear and controversial, the list of natural and synthetic “cytoprotective” compounds has been increasing. In vivo, chemically induced hemorrhagic mucosal lesions (HML) were also decreased by sulfhydryl (SH) compounds (10, 30, 39, 42), protease inhibitors (23, 39, 40), somatostatin (43), growth factors (14, 47), histamine (26), dopamine and related drugs (17, 27), gangliosides (35, 37), carotenoids (22), sucralfate and its derivatives (12, 36), antacids (24, 44), and spasmyloitics (11, 32).

The original concept of cytoprotection has been criticized because of the incomplete protection by exogenous PG against chemically induced gastric HML. That is, histological and electron microscopic investigations revealed that only the deep hemorrhagic erosions could be reduced by PG or other cytoprotective compounds, whereas the surface cell damage was not decreased (10, 12, 16, 30, 44). Thus the terms “histoprotection” and “organoprotection” were suggested to reflect protection at the tissue and organ levels (41, 43). Tarnawski and colleagues (45) found a limited but statistically significant direct protection by PG of human isolated gastric glands against indomethacin- or ethanol-induced damage. Ivey and co-workers (31, 46) demonstrated that the chemically induced damage was diminished by PG and SH derivatives in cultured and transformed epithelial surface cells. Because the gastric mucosa contains numerous cell types, data are lacking concerning the possible direct protection of parietal, chief, and neuroendocrine cells by cytoprotective agents. Thus we tested the hypothesis that old and new cytoprotective compounds might exert direct protection on a mixed population of gastric mucosal cells (GMC). We also wanted to identify subcellular targets in the interaction of gastroprotective agents and ethanol. For this purpose, a method was adapted in our laboratory for the isolation of a mixed population of rat GMC with long viability and preserved membrane receptor sensitivity using minimal amounts of pronase and calcium-binding EGTA (13, 25). The new method not only includes the measurement of plasma membrane damage by the usual dye exclusion tests, e.g., by trypan blue (TB) exclusion, leakage of lactate dehydrogenase (LDH), or total protein from cytosol, but we...
can also assess mitochondrial integrity by measuring the activity of mitochondrial succinic dehydrogenase (SDH) and nuclear damage by fluorescence induced by ethidium bromide (EB)-DNA binding (25).

The main purpose of this study was to evaluate the possible direct cellular effects of established and newly synthesized gastroprotective compounds in isolated mixed rat GMC at the levels of the plasma membrane, mitochondria, or nuclei alone or against a moderate uniform cell injury after a short incubation with ethanol.

MATERIALS AND METHODS

Reagents and Chemicals

The following reagents were purchased from Sigma Chemical (St. Louis, MO): N-acetyl-L-cysteine (NAC), L-alanine, d-arginine, l-arginine, atropine sulfate, brilliant cresyl blue (BCB), BSA, bovine hemoglobin, carbamycholine chloride (carbachol), DMSO, EB, EGTA, Folin and Ciocalteau’s phenol reagent spectrophotometrically (A = 578 nm) with a Gillford 2400–2 spectrophotometer, calculated from a peptin (Merek, Rahway, NJ) dose-response curve and expressed as micrograms of peptin per minute per 5 × 10⁶ GMC.

Enrichment of parietal cells and measurement of [14C]Caminopyrine accumulation. Freshly isolated and dispersed rat GMC (1–2 × 10⁸) were separated in a Beckman 5.0 elutria system (600 rpm, 10°C) in Hanks’ balanced salt solution supplemented with 0.1% (wt/vol) BSA. Parietal cells (60–65% of total GMC) were collected by continuous Percoll density gradient centrifugation at a density of 1.06 mg/ml (15). The implication of HCl production in enriched rat parietal cells is based on accumulation of 14C-labeled aminopyrine ((14C)AP) in acidic tubulovesicles of parietal cells on stimulation (3, 33, 34). Parietal cells (10⁶/ml) were suspended in Hanks’ balanced salt solution (containing 10⁶ cells/ml 0.1% albumin) and incubated at 37°C in an orbital shaker (100 oscillations/min). Incubations were carried out in a total volume of 1.0 ml with 0.05 Ci of [14C]AP in the absence or presence of receptor stimuli such as carbachol (10⁻⁴ M), histamine (10⁻⁴ M), IBMX (10⁻⁴ M), or their combination. The washed cells were dissolved in 1.0 M NaOH, and neutralized aliquots were used for liquid scintillation counting.

Preparation of Mixed GMC

GMC from one or two nonfasted Sprague-Dawley rats (180–210 g; Taconic Farms, Germantown, NY) were obtained by sequential incubation with a low concentration of pronase E in calcium-free medium (EGTA) as we previously described (13, 25). Cells were counted in an improved Neubauer counting chamber (Hemocytometer; Fisher Scientific), dispersed, and kept in warm (37°C) HEPES-buffered salt solution (0.159 M, pH 7.4) produced fresh in our laboratory with the following ingredients (in mM): 98.0 NaCl, 5.8 KCl, 2.54 NaH₂PO₄, 5.1 Na pyruvate, 6.9 Na fumarate, 2.0 L-glutamine, 24.5 HEPES-Na, 1.0 Tris base, 11.1 D-glucose, and 1.0 CaCl₂ with 2.0 mg/ml (wt/vol) BSA. The volume of GMC suspension was adjusted to a density of 1.5 × 10⁷ cells/ml for incubations, TB, and biochemical assays. The numbers of viable and nonviable cells indicating initial cell viability (CV) were also counted by TB exclusion test (% of unstained cells) (25).

Secretory Studies

The responsiveness of rat mixed GMC and the membrane receptor sensitivity of both chief and parietal cells were also measured in separate experiments using secretory agents.
Table 1. Effects of carbachol and carbachol + atropine on pepsinogen secretion of isolated rat gastric mucosal cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Carbachol, M</th>
<th>Pepsinogen Secretion, ( \mu g \cdot min^{-1} \cdot 5 \times 10^6 ) cells (^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>3.15 ± 0.56</td>
</tr>
<tr>
<td>B</td>
<td>10(^{-4})</td>
<td>5.11 ± 0.68</td>
</tr>
<tr>
<td>C</td>
<td>10(^{-3})</td>
<td>5.51 ± 0.41</td>
</tr>
<tr>
<td>D</td>
<td>10(^{-2})</td>
<td>6.21 ± 0.71†</td>
</tr>
<tr>
<td>E</td>
<td>10(^{-1})</td>
<td>7.50 ± 0.75†</td>
</tr>
<tr>
<td>F</td>
<td>10(^{-1})</td>
<td>3.95 ± 0.49‡</td>
</tr>
</tbody>
</table>

† Values are means ± SE. Mixed rat gastric mucosal cells (5 \times 10^6) containing 42% chief cells were used in each tube for 15-min incubations with pharmacological agents at 37°C. Supernatants were assayed for peptic activity at pH 2.0 using hemoglobin as substrate, and digestive products were measured by Folin reagent. * \( P < 0.05; \) † \( P < 0.001 \) as compared with group A; ‡ \( P < 0.001 \) between groups E and F.

TB Dye Exclusion Test

The TB dye exclusion test was carried out as we described previously (25). Briefly, 10\(^6\) cells (0.1 ml of cell suspension) were mixed with 0.1 ml of 0.4% TB, and 5 min later the number of stained (dead) and unstained (viable) cells were counted in a hemocytometer and the yield of viable cells and CV (% of unstained cells) were calculated.

LDH Assay

LDH assay was carried out in samples of both supernatants alone and with 10\(^6\) sonicated cells/0.1 ml (Sonifer cell disruptor 200; Branson, Danbury, CT). The colorimetric assay is based on reduction of 1.5 mM NAD\(^+\) to NADH catalyzed by LDH in the presence of lactate (50 mM) as substrate in a 1.0-ml final volume at 37°C in a 10-min incubation, as described previously (4). A color product was rapidly formed by reductions of PMS (1.6 mM) and INT (0.8 mM) in the same tube and measured spectrophotometrically at 500 nm. The specific LDH activity was measured and calculated as milliunits per minute per microgram of protein.

Succinic Dehydrogenase Assay

Mitochondrial integrity was tested in 2 \times 10^6 previously treated and redispersed GMC at 37°C after incubation (90 min) with MTT (2.4 mM) in buffer (pH 7.4) in 1.0 ml of total reaction volume. The color formazan product was quantified spectrophotometrically at 500 nm after its dissolution in 2.0 ml of DMSO (21). Specific activity of SDH was calculated as nanomoles of formazan per minute per microgram of protein.

EB-DNA Fluorescence Assay

Nuclear damage of GMC was assessed by nuclear fluorescence of cells due to the EB-DNA binding as described previously (9). GMC suspension (1 ml containing 10\(^7\) cells) was mixed with 2.0 ml of 25 \( \mu \)M EB solution, and fluorescence intensity was measured in a Perkin-Elmer fluorescence spectrophotometer (Hitachi Instruments) at 325–585 nm (excitation-emission). The results were expressed as arbitrary fluorescence units/10\(^7\) cells.

Protein Concentration

Protein concentration in the supernatants and sonicated cells was determined by Bradford's method (Bio-Rad Laboratories, Richmond, CA; Ref. 6). Absorption of samples at 595 nm was measured against blanks and known standards of BSA.

Data Presentation and Statistical Evaluation

Data from three to eight experiments were pooled and expressed as means ± SE. Comparisons were performed by Student’s \( t \)-tests (paired or unpaired) and nonparametric Mann-Whitney \( U \)-tests. Values were considered statistically significant at \( P < 0.05 \).

RESULTS

Yield, Composition, and Viability Of Isolated Rat GMC

A large number of mixed GMC (1.2–1.6 \times 10^6) can be isolated from a single rat glandular stomach. The ratio of parietal, chief, and mucous epithelial cells using supravital staining with 0.05% BCB was 23.0 ± 2.1, 42.1 ± 4.9, and 34.1 ± 3.7%, respectively. The average initial CV of 28 cell isolations was 90.5 ± 3.1% measured by TB dye exclusion. High CV (85–95%) was maintained for 7 h after cell isolation using our cell harvesting method and physiological solution at 37°C.

Secretory Studies

Pharmacological investigations with chief cells in mixed rat GMC revealed that the muscarinic receptor agonist carbachol significantly stimulated pepsinogen secretion from 3.15 ± 0.56 to 7.50 ± 0.75 \( \mu g \cdot min^{-1} \cdot 5 \times 10^6 \) GMC\(^{-1} \) \(( P < 0.01)\). Atropine given in combination with carbachol almost totally inhibited carbachol-induced increase in pepsinogen secretion \(( P < 0.001)\) (Table 1). In addition, dmPGE\(_2\) also increased pepsinogen secretion from isolated chief cells from 8.75 ± 0.35 to 10.10 ± 0.30 \( \mu g \cdot min^{-1} \cdot 5 \times 10^6 \) GMC\(^{-1} \) \(( P < 0.01; \) Fig. 1).

Uptake of \(^{14}\)CAP in enriched (65%) rat parietal cells was stimulated by either histamine or carbachol.

![Fig. 1. Pepsinogen secretion from isolated chief cells in a mixed suspension of rat gastric mucosal cells (GMC) containing peptic cells. Dose response for 16,16-dimethyl-PGE\(_2\) (dmPGE\(_2\)) in a 15-min incubation at 37°C is shown. Peptic activity of GMC medium was assayed at pH 2.0 using hemoglobin as substrate. Results shown are means ± SE of 6 experiments (6 different cell preparations). \( * P < 0.05; \) ** \( P < 0.01 \).](http://ajpgi.physiology.org/)

Downloaded from [http://ajpgi.physiology.org/](http://ajpgi.physiology.org/) by 10.220.33.4 on September 8, 2017
The histamine-induced increase in HCl production in parietal cells was considerably potentiated by the phosphodiesterase inhibitor IBMX (Fig. 2).

**Ethanol-Induced Cellular Injury: An Assay for Cytoprotection**

The average values obtained by all methods used to measure indicators of damage before and after 5-min incubation with ethanol at 37°C are shown in Table 2. Ethanol (15% vol/vol) significantly decreased CV by TB dye exclusion from 90.3 ± 3.1 to 20.9 ± 1.4 (P < 0.001) and increased LDH leakage from cytosol into media via damaged cell membrane (P < 0.001). It induced severe mitochondrial and nuclear lesions as detected by marked decrease in SDH activity from 5.7 ± 0.3 to 1.5 ± 0.1 nmol formazan·min⁻¹·mg protein⁻¹ and elevation of EB-DNA fluorescence intensity from 4.2 ± 0.5 to 17.3 ± 1.1 fluorescence units/10⁷ gastric mucosal cells.

**Effects of Thiol Compounds on Ethanol-Induced Cell Injury**

NAC, taurine, or cysteamine alone did not induce any injury in mixed GMC (data not presented). The effects of 60-min incubation of isolated rat GMC with NAC against ethanol-induced cellular injury are shown in Fig. 3. Cell membrane damage quantified by TB dye exclusion and LDH leakage, mitochondrial SDH activity, and EB-DNA fluorescence revealed a mild (12–21%) protection against ethanol-induced damage (P < 0.05).

As shown in Fig. 4, a similar (10–20%) increase in cell resistance was detected after 60-min preincubation with taurine.

**Table 2. Ethanol-induced injury in isolated gastric mucosal cells after 5-min incubation**

<table>
<thead>
<tr>
<th>Method</th>
<th>Subcellular Targets</th>
<th>Cell Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before ethanol</td>
<td>After ethanol</td>
</tr>
<tr>
<td>TB exclusion</td>
<td>Cell membrane</td>
<td>90.3 ± 3.1</td>
</tr>
<tr>
<td>LDH-media</td>
<td>Cell membrane</td>
<td>17.0 ± 9</td>
</tr>
<tr>
<td>LDH-cells</td>
<td>Cell membrane</td>
<td>19.0 ± 1.5</td>
</tr>
<tr>
<td>SDH</td>
<td>Mitochondria</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>EB-DNA</td>
<td>Nucleus</td>
<td>4.2 ± 0.5</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE of 28 cell isolations and treatments. TB, trypan blue (% of unstained cells); LDH, lactic dehydrogenase (mU·min⁻¹·mg protein⁻¹); SDH, succinic dehydrogenase (nmol formazan·min⁻¹·mg protein⁻¹); EB, ethidium bromide (fluorescence/10⁷ gastric mucosal cells). *P < 0.001.
with taurine (2-aminoethanesulfonic acid) against ethanol injury, and the difference was statistically significant with LDH leakage and EB-DNA fluorescence ($P < 0.05$). The protection of mitochondria and nuclei reached the level of significance ($P < 0.05$).

In contrast, cysteamine moderately aggravated ($P < 0.05$) the ethanol-induced cell membrane and mitochondrial damage measured by TB exclusion and SDH, respectively. However, some concentration-dependent nuclear protection ($P < 0.01$) was measured by EB-DNA fluorescence. No major changes were found in LDH leakage from cysteamine-treated GMC after incubation with 15% ethanol (Fig. 5).

**Effects of dmPGE$_2$ and Ethanol**

Concentration-dependent protection (15–21%) against ethanol-induced injury was found after 60-min incubation with dmPGE$_2$ as measured by TB exclusion ($P < 0.05$), LDH release ($P < 0.05$), and changes in activity of SDH from 1.95 ± 0.11 to 3.31 ± 0.21 ($P < 0.05$). Only a slight increase ($P > 0.05$) in nuclear resistance was observed after incubation with dmPGE$_2$ in ethanol-treated GMC (Fig. 6).

**Effects of SOS and Ethanol**

Incubation of GMC for 60 min with SOS dose-dependently decreased the ethanol-induced LDH release and increased CV measured by TB dye exclusion; the differences reached statistical significance ($P < 0.05$) at $10^{-3}$ M of SOS (Fig. 7). The effect of SOS on mitochondrial and nuclear membranes detected by changes in the activity of SDH and EB-DNA binding was not statistically significant.

**Effects of Various Gastroprotective Compounds on Ethanol Toxicity**

The effects of different agents that in vivo exert gastroprotection in isolated GMC with or without incubation with 15% ethanol are summarized in Table 3. Only the statistically significant changes in TB dye exclusion and biochemical assays are listed, indicating cell injury alone or aggravation of ethanol-induced
and D-alanine or in vivo gastroprotective compounds such as L-arginine, D-arginine, glycine, L-alanine, and other agents slightly increased resistance (up to 21%) against ethanol-induced cellular damage. Histamine had a biphasic effect in the rat GMC: low concentration protected but high concentration aggravated the ethanol-induced cell injury.

**DISCUSSION**

Our study demonstrates that preincubation of isolated rat gastric mucosal cells with dmPGE$_2$, SOS, or the SH compounds NAC, taurine, or cysteamine alone did not cause any measurable cell injury, and these agents slightly increased resistance (up to 21%) against ethanol-induced cellular damage. Similar protection was found with a low concentration of histamine, but at a higher concentration it aggravated ethanol toxicity. Partial or general aggravation of ethanol injury was detected on GMC membranes after preincubation with wide concentration ranges of amino acids such as L-arginine, D-arginine, glycine, L-alanine, and D-alanine or in vivo gastroprotective compounds such as nitecapone, ganglioside GM$_1$, the spasmolytic pinaverium, and KC-10667. Some of these agents alone (i.e., without ethanol) exerted some direct cellular toxicity. Our results also revealed that the isolated chief cells (pepsinogen secretion) or enriched parietal cells (HCl production) have an excellent membrane receptor-mediated responsiveness to pharmacological stimuli such as cholinergic and histaminergic stimulators or PG. These results are in accordance with other studies in cultured and isolated parietal cells (3, 33, 34) or peptic cells (2, 8, 18, 19).

Acute gastric mucosal injury is a complex process because of the heterogenous structure and multiple functions of the stomach wall. In the pathways of direct and indirect chemical injury, vascular damage, inflammatory processes, free radicals, and proteases are involved (41, 45). The list of gastroprotective agents has been growing since the introduction of the concept of gastric cytoprotection, without a proportionate increase in our understanding of the mechanisms of gastric mucosal injury and protection. We nevertheless know that most of the mucosal protection is relative and indirect. Namely, despite the initial destruction of superficial epithelial cells in rats given ethanol intragastrically after pretreatment with gastroprotective agents, the histological integrity of the gastric mucosa is restored by rapid epithelial restitution and the organ structure and function are maintained.

One of the new possibilities for assessing the phenomenon of direct gastric cytoprotection is to investigate in vitro the possible protection of isolated GMC by naturally occurring or exogenous compounds against chemically induced and measurable cell damage. We have recently developed and optimized morphological and biochemical methods for harvesting mixed populations of rat GMC to investigate reversible and irreversible cell damage and protection at the level of cell membranes in organelles (13, 25). Selective and parallel assessment of chemical injury and protection of plasma membrane, mitochondria, and nuclei can be investigated with sensitive measurements such as TB exclusion, LDH leakage, SDH activity, or EB-DNA fluorescence, respectively. Only the statistically significant changes ($P < 0.05$) are listed. I, cell injury; A, aggravation of ethanol-induced cell injury; biphasic, concentration-dependent protection or aggravation; no, no effect.

### Table 3. Effects of various gastroprotective compounds alone and in presence of 15% ethanol on isolated rat gastric mucosal cells

<table>
<thead>
<tr>
<th>Compounds, M</th>
<th>Treatments</th>
<th>Plasma membrane</th>
<th>Mitochondria</th>
<th>Nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>+ Ethanol</td>
<td>Biphasic</td>
<td>Biphasic</td>
<td>No</td>
</tr>
<tr>
<td>(2 × 10$^{-3}$–2 × 10$^{-7}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>GM$_1$</td>
<td>+ Ethanol</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>(10$^{-3}$–10$^{-6}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Nitecapone</td>
<td>+ Ethanol</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>(10$^{-5}$–10$^{-7}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Pinaverium</td>
<td>+ Ethanol</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>(10$^{-5}$–10$^{-7}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>KC-10667</td>
<td>+ Ethanol</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>(10$^{-3}$–10$^{-6}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>+ Ethanol</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>(10$^{-3}$–10$^{-6}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>D-Arginine</td>
<td>+ Ethanol</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>(10$^{-3}$–10$^{-6}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>+ Ethanol</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>(10$^{-3}$–10$^{-6}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Glycine</td>
<td>+ Ethanol</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>(10$^{-3}$–10$^{-6}$)</td>
<td>Alone</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Data from 3–6 cell isolations and experiments are pooled and calculated. Plasma membrane, mitochondrial and nuclear membrane integrities were assessed by TB exclusion and LDH leakage, SDH activity, or EB-DNA fluorescence, respectively. Only the statistically significant changes ($P < 0.05$) are listed. I, cell injury; A, aggravation of ethanol-induced cell injury; biphasic, concentration-dependent protection or aggravation; no, no effect.
GMC against ethanol-induced cytotoxicity by preincubation with NAC (in all organelles examined) or taurine (in mitochondria and nuclei). Taurine is a metabolite of L-cysteine. In vivo, it protects several organs against chemical injury, and we recently found gastrointestinal protection against ethanol in rats (unpublished data). Cysteamine, another SH-containing agent, has also been shown to exert in vivo gastroprotection (42). Ronman et al. (31) demonstrated that cysteamine has a direct protective effect in vitro against damage induced by taurocholate or indomethacin in gastric epithelial monolayers derived from a human cell line. In our study with freshly isolated rat GMC, cysteamine did not induce significant enhancement of cell resistance.

SOS, a derivative of sucralfate, had a moderate protective effect against ethanol injury in isolated rat GMC. This direct cellular protection might be one of the mechanisms of acute gastroprotection by sucralfate (36).

GM₃, a ganglioside and sialic acid-containing glycosphingolipid, exerts acute gastric mucosal protection against ethanol (37, 48). Gangliosides play a role in the regulation of transmembrane signaling, cellular differentiation and proliferation, membrane fluidity, and ion transport (35). In the present study, exogenous GM₃ was not able to protect against ethanol-induced damage in isolated GMC.

Amino acids such as L- or D-arginine, glycine, and D- or L-alanine are either modulators of neural transmissions or sources of vasoactive nitric oxide. They are also gastroprotective against ethanol injury in rats (unpublished data), and they reduce tubular cell damage in the kidney (1). In our study they did not have any direct cytoprotective effect in rat GMC against ethanol.

Our findings show the following. 1) A large number of mixed GMC with high and long viability and preserved membrane receptor sensitivity can be isolated from rat stomach. 2) Selective examination of ethanol injury and protection of plasma, mitochondrial, and/or nuclear membranes may be demonstrated biochemically and morphologically. 3) Only a slight or moderate concentration-dependent protection was detected by thiol compounds, dmpGE₂, or SOS. Of the few chemicals that exerted direct cytoprotection in vitro (e.g., NAC, taurine, dmpGE₂, SOS), all decreased plasma membrane damage as revealed by TB exclusion and/or LDH release, whereas only taurine and NAC diminished nuclear damage, i.e., cell death. This implies that it is much easier to offer protection against the reversible than against the irreversible stage of cell injury. 4) In contrast, a large number of naturally occurring or synthetic in vivo gastroprotective compounds have no direct cytoprotective effects against ethanol cytotoxicity in a mixed population of GMC. 5) Consequently, minimal or no correlation could be demonstrated between in vitro cytoprotection and in vivo gastroprotection by most compounds investigated. These findings indicate that a major part of the beneficial effect of gastroprotective agents seems to be mediated via complex mechanisms at the tissue and organ levels.

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REFERENCES


