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COMPARATIVE VIABILITY STUDIES ON ISOLATED GASTRIC MUCOSAL MIXED CELLS AND HEPATOMA AND MYELOMA CELL LINES WITH ETHANOL, INDOMETHACIN AND THEIR COMBINATION

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ABSTRACT

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The toxic effects of ethanol (EtOH), indomethacin (IND) and their combination were studied in vitro. The experiments were performed on freshly isolated gastric mucosal mixed cells and two types of stable cultured cells: Sp2/0-Ag14, which is a non-secreting mouse myeloma cell line, and Hep G2, which is a human hepatocellular carcinoma cell line. EtOH decreased the viability of all types of cells in a concentration-dependent manner. At all concentrations, the EtOH caused a greater decrease in the viability of gastric mucosal cells than in the viability of Sp2/0-Ag14 cells. IND had no effect on the viability of the cultured cells, when this was employed without any other aggressive factor, such as EtOH. When used in combination, IND aggravated the EtOH-induced cell injury. These results show that the endogenous prostaglandins may play a role in the maintenance of cell integrity in all three types of cells.

Keywords: isolated rat gastric mucosal cells, Sp2/0-Ag14 cell line, Hep G2 cell line, ethanol, indomethacin

INTRODUCTION

The meaning of the original term 'gastric cytoprotection', which was described by Robert in 1979 [1,2], is limited at the level of the cell, and new concepts have been defined, such as organoprotection and gastroprotection, which are widely accepted and have been studied recently in in-vivo experiments. In these investigations, many environmental factors, e.g. central nervous system [3], vagal nerve [4,5], blood flow [6,7], vascular permeability [6] and rapid epithelial restitution of neck cell [8] have been studied as defence mechanisms of gastric mucosa.

The in-vivo and in-vitro experiments essentially differ from each other. Investigations on isolated cells have many advantages: all the effects originating from other organs or tissues can be eliminated, so the behaviour of a single cell can be examined.

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Unfortunately, these kinds of studies might have harmful consequences. Acutely isolated cells are less suitable for pharmacological studies of the gastric mucosa because of their isolation procedure [9]. Stress may reduce the cell responsiveness, and surface receptors are damaged during the digestive stages of the isolation procedure. These effects may be eliminated by using stable cultured cells in the experiment, though their number is small and some properties of the original tumour can be seen in their behaviour.

Ethanol (EtOH) is widely used as a toxic agent in gastric cytoprotection investigations in vivo [9]. Indomethacin (IND) is also generally used for experiments [10–12]. It inhibits prostaglandin synthesis, which is responsible for the maintenance of gastric mucosal integrity due to stimulation of mucosal blood flow, preservation of cellular ion transport and protection of the mucosal proliferative zone [13]. These chemical agents have been studied widely in in-vivo experiments, but only a few experiments have been carried out in vitro on isolated cells with these toxic agents.

The aims of this study were:

1. To analyse the toxic effects of 5 min EtOH treatment in vitro on acutely isolated mixed gastric mucosal cells (GMC);

2. To compare the differences between the GMC and the stable cultured cells;

3. To evaluate the differences between the myeloma (Sp2/0-Ag14) and the hepatoma (Hep G2) cell lines;

4. To study the effect of IND on GMC and Sp2/0-Ag14 cells;

5. To examine the combined effect of EtOH and IND on these two types of cells.

MATERIALS AND METHODS

Preparation of mixed gastric mucosal cells

Gastric mucosal cells from Sprague–Dawley rats were isolated by the method of Nagy et al. [9]. The segments of the glandular stomach were separated from the blood vessels and the surrounding connective tissue and were incubated in a physiological solution containing 0.5 mg/ml pronase E (type XXV, Sigma Chemical Co.) and 10^{-3} mol/L EGTA. After several washings, the cells were resuspended in a solution (0.157 mol/L, pH 7.4) produced freshly with the following ingredients: 98.0 mmol/L NaCl, 5.8 mmol/L KCl, 2.5 mmol/L Na_2PO_4, 5.1 mmol/L sodium pyruvate, 6.9 mmol/L sodium fumarate, 2.0 mmol/L glutamine, 24.5 mmol/L HEPES Na, 1.0 mmol/L Trizma base, 11.1 mmol/L D-glucose, 1.0 mmol/L $CaCl_2$, 1.0 mmol/L $MgCl_2$, and 2.0 mg/ml (w/v) bovine serum albumin. All examinations were carried out in this solution.

Stable cultured cells

Sp2/0-Ag14 (CRL 1581) is a non-secreting mouse myeloma; Hep G2 is a human hepatocellular carcinoma cell line obtained from the American Type Culture Collection (ATCC). Cells were cultured and the examinations were carried out in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in a humidified incubator containing 95% air and 5% CO_2 at 37°C.

Toxicological studies

The cells were incubated with different concentrations of EtOH (1, 5, 10, 15, 20 and 50% (v/v)), IND (10^{-8}–10^{-3} mol/L dissolved in 5% $NaHCO_3$, pH 7.4 with 5 N HCl) and their combination (15% EtOH and 10^{-3} mol/L IND) for 5 min in a shaking water bath at 37°C. Each study used 10^5 cells. After 5 min incubation the cells were separated from the supernatant by centrifugation (500*g*, 10 min), washed out (10 min water bath and centrifugation again) and resuspended in a toxic-free medium.

Trypan blue exclusion test

Trypan blue is taken up by damaged cells, staining the cytoplasm blue; viable cells can resist this staining. Trypan blue (0.2%) was mixed with the same volume of cell suspension and, after 5 min latency, the numbers of stained (dead) and unstained (viable) cells were calculated as percentages in a haemocytometer. In the comparison with stable cultured cells, we examined the viability over longer periods (5 min, 60 min, 4 h and 24 h) after the 5-min EtOH incubation.

Statistics

Values in figures and text are expressed as means $\pm$ SEM. Comparisons were performed using the unpaired Student's *t*-test and *p* values were considered significant at $p < 0.05$.

RESULTS

Effect of EtOH on GMC

EtOH (1, 5, 10, 15, 20 and 50%) concentration-dependently decreased the viability of GMC. The EC_{50} was 13.5% (Figure 1).

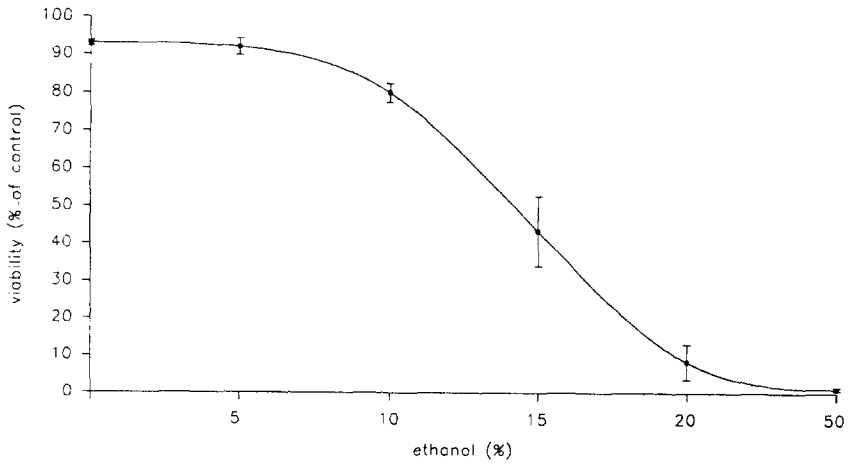

Figure 1. Changes in the viability of acutely isolated rat gastric mucosal cells (GMC) after 5 min incubation with 1–50% ethanol, detected by trypan blue exclusion test (% of control). The results are expressed as means ± SEM ($n = 5$)

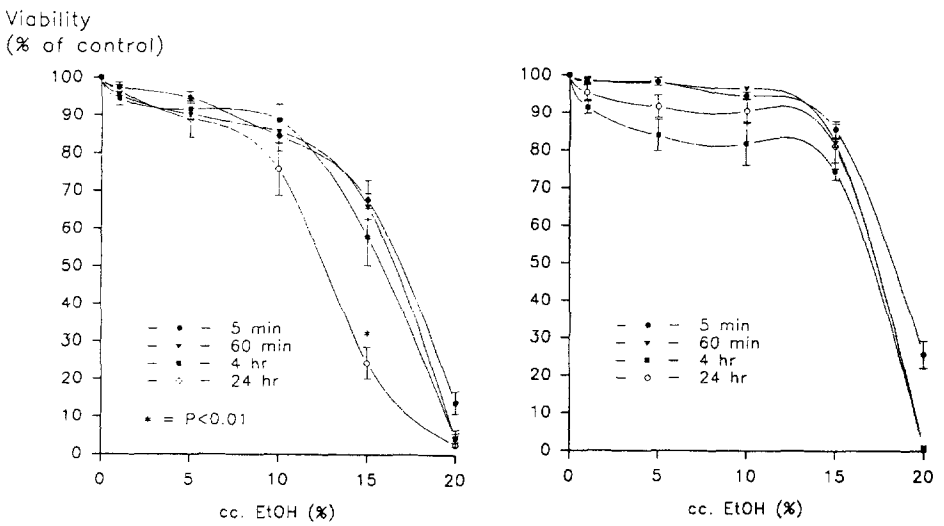

Figure 2. Changes in the viability of the Sp2/0-Ag14 (left) and Hep G2 (right) cell lines at various times (5 min, 60 min, 4 h and 24 h) after incubation with different concentrations of EtOH, detected by trypan blue exclusion test. The results are expressed as means ± SEM ($n = 5$). $^{*}p < 0.01$ compared with the 4-h value

Effect of EtOH on Sp2/0-Ag14 and Hep G2 cell lines

EtOH concentration-dependently decreased the viability of stable cultured cells. In the case of Sp2/0-Ag14 cells, there was no significant difference between the viability values counted at 5 and 60 min, but 4 h after incubation with 10% or 15% of EtOH, a significant level of cell destruction could be detected. In the case of the Hep G2 cell line, a greater level of resistance was found up to a concentration of 15% EtOH; above that concentration, a similar level of cell destruction occurred (Figure 2).

Comparing GMC and stable cultured cells

At all concentrations, the EtOH decreased the viability of GMC much more potently than the viability of the stable cultured cells. The EC_{50} for GMC was 13.5%; the EC_{50} for Sp2/0-Ag14 was 16% (Figures 1 and 2).

Effect of IND

Five minutes incubation with 10^{-8}–10^{-3} mol/L IND had no effect on the viability of Sp2/0-Ag14 cells. In the case of GMC, only the highest dose (10^{-3} mol/L) of IND decreased significantly ($p < 0.02$) the number of viable cells (Figure 3).

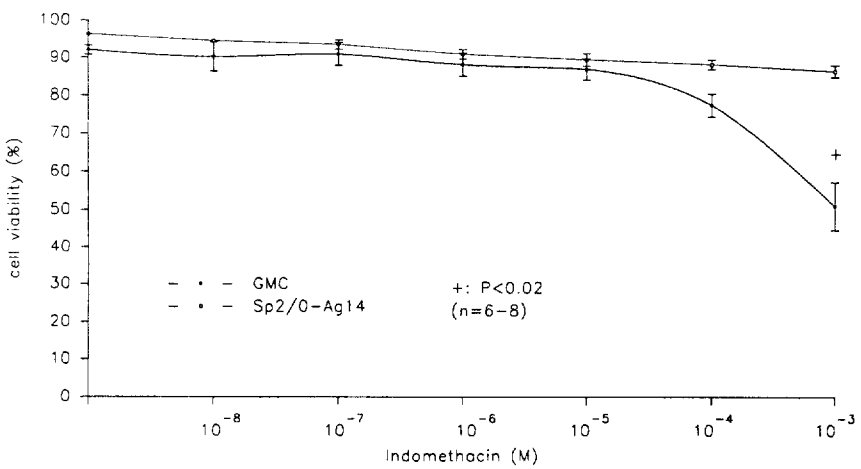

Figure 3. Changes in the viability of GMC and Sp2/0-Ag14 cells after 5-min incubation with 10^{-8}–10^{-3} mol/L indomethacin, detected by trypan blue exclusion test. The results are expressed as means ± SEM ($n = 6$–8). $^{+}p < 0.02$ compared with GMC

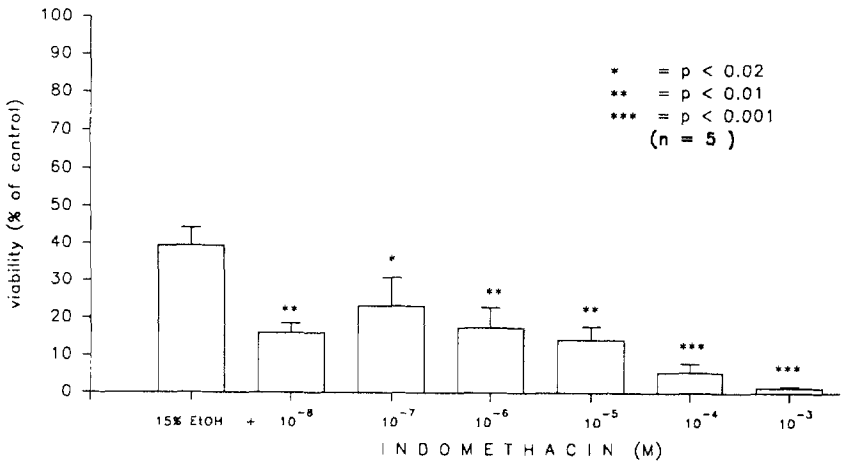

Figure 4. Changes in the viability of GMC after combined incubation with 15% ethanol and 10^{-8}–10^{-3} mol/L indomethacin, detected by trypan blue exclusion test. The results are expressed as means ± SEM ($n = 5$). $^{*}p < 0.02$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with treatment with 15% EtOH alone

Combined effect of EtOH and IND

After the combined treatment a greater cell destruction could be detected. Using different concentrations, the 10^{-3} mol/L dose was the most aggressive; the amount of necrotic cell loss was concentration dependent (Figure 4). Comparing the response of the GMC with the myeloma cells, the GMC are much more vulnerable than Sp2/0-Ag14 cells after EtOH treatment, and after the combined treatment too (Figure 5).

DISCUSSION

In these studies freshly isolated rat gastric mucosal cells and two types of stable cultured cells were used to evaluate the effects of EtOH, IND and their combination in toxicological studies. The mixed population of isolated rat GMC contained at least three types of cells: parietal (20–25%), chief (40%), and epithelial (45%) cells. A viability of 80–95% could be maintained for 6–7 h. These cells do not have the potential for proliferation. Cultured cells were always kept in the same conditions, tests are reproducible and cells can survive for a longer time. During the calculation of viability, it must be remembered that the two cell lines have different proliferation rates. In particular, this should be borne in mind during the interpretation of the different viability values of longer incubation times.

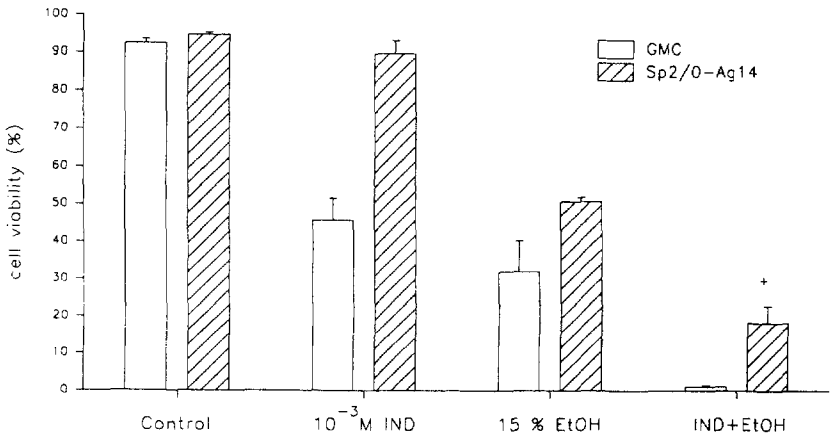

Figure 5. Changes in the viability of GMC and Sp2/0-Ag14 cells after 5-min incubation with 15% EtOH, with 10^{-3} mol/L IND and with 15% EtOH and 10^{-3} mol/L IND combined, detected by trypan blue exclusion test. The results are expressed as means ± SEM ($n = 6$–8). $^{+}p < 0.01$; $^{*}p < 0.05$ compared with 15% EtOH treatment

Our results indicate that the acutely isolated cells are more vulnerable than cultured cells. After EtOH treatment, in the case of Sp2/0-Ag14 cells, the EC_{50} was higher (16%) than that of GMC (13.5%). IND had no damaging effect on stable cultured cells. The combined treatment reduced the viability in both types of cells, but this effect was much smaller in Sp2/0-Ag14 cells; almost all the GMC were destroyed. These results show that cultured cells are more resistant to toxic agents than acutely isolated cells. Though different types of cells were used in this study, the differences in behaviour may derive from the differences in isolation procedure rather than from their differences in type. In-vivo experiments have shown that gastrointestinal ulceration can be produced by IND administration [9,10]. It is known that IND inhibits the activity of cyclo-oxygenase, producing less prostaglandins and excessive vasoconstrictor leukotrienes [14], and decreases the mucosal level of adenosine triphosphate [15]. These factors reduce the gastric mucosal resistance to acid. In the in-vitro study described here, IND was applied without any other aggressive factor, such as EtOH, and was not toxic for these cells. However, after the combination treatment, cell viability was considerably decreased compared with the effect of EtOH alone. It is likely that the decreased levels of endogenous prostaglandins might play a role in the enhanced toxic effect of EtOH. These results are in a good agreement with those of Tarnawski et al. [16] who observed a protective effect of exogenous prostaglandins on human isolated gastric glands against IND and EtOH injury.

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