Effect of 16,16 dimethyl prostaglandin E\textsubscript{2} on aspirin induced damage to rat gastric epithelial cells in tissue culture

A TERANO, T MACH, J STACHURA, A TARNAWSKI, AND K J IVEY

From The Department of Medicine, Long Beach Veterans Administration Medical Center, Long Beach, and University of California, Irvine, California, USA

SUMMARY Prostaglandins (PGs) protect gastric mucosa against damage produced by acetylsalicylic acid (ASA). Whether this effect of prostaglandins is truly cytoprotective and whether cAMP plays an important role in this effect is uncertain. We studied the effect of: (1) 16,16 dimethyl prostaglandin E\textsubscript{2} (dmPGE\textsubscript{2}), isobutylmethyl xanthine (IMX), and dibutyryl cAMP (DBcAMP) on ASA-induced damage to monolayer cultures of rat gastric mucosa composed primarily of mucus cells; (2) dmPGE\textsubscript{2} on ASA absorption into the cultured cells. Cell damage was quantitated by \textsuperscript{51}Cr-release and trypsin blue staining. Ten millimoles ASA significantly increased \textsuperscript{51}Cr-release (indicating cell damage) at pH 5-0, but not at pH 7-4. DmPGE\textsubscript{2} significantly reduced ASA-induced increase of \textsuperscript{51}Cr-release. Isobutylmethyl xanthine did not change the rate of \textsuperscript{51}Cr-release caused by ASA plus dmPGE\textsubscript{2}. Dibutyryl cAMP did not significantly alter \textsuperscript{51}Cr-release caused by ASA. A dose response study of ASA damage showed close correlation between \textsuperscript{51}Cr-release and trypsin blue staining (r=0-93). Dimethyl prostaglandin E\textsubscript{2} did not affect \textsuperscript{14}C-ASA incorporation by the cells at either pH 7-4 or pH 5-0. We conclude that: (1) dmPGE\textsubscript{2} exerts a cytoprotective effect on cultured rat gastric cells; (2) cAMP does not play an important role in such cytoprotection; (3) this protection is not because of interference with ASA absorption by prostaglandin.

The mechanism by which prostaglandins protect the gastric mucosa in vivo against a variety of ulcerogenic agents such as aspirin, ethanol, or taurocholate is unknown.\textsuperscript{1}\textsuperscript{-9} Whether prostaglandins exert a truly 'cyto'-protective effect on gastric mucosal epithelial cells remains controversial:\textsuperscript{4}\textsuperscript{10} further, there is debate as to whether cyclic AMP (cAMP) is a mediator of the assumed cytoprotective effect of prostaglandins.\textsuperscript{7}\textsuperscript{11}\textsuperscript{12}

The present study was undertaken to determine: (1) if prostaglandins protect the gastric epithelial cells against acetylsalicylic acid (ASA) induced injury in cultured rat gastric epithelial cells; (2) the role of cAMP in any protective effect of prostaglandins in vitro; (3) if this protection is due to interference with absorption of aspirin into gastric cells in vitro.

Methods

MATERIALS

Animals

Five to ten day old rats (Sprague-Dawley, Sasco, Neb). Medium 1: Coon's modified Ham's F-12 medium (KC Biological Inc, Lenexa, KS) containing 0.1 g/dl collagenase (120 U/mg, GIBCO, Grand Island, NY) and 0.05 g/dl hyaluronidase (type I-S, 270 U/mg, Sigma Chemical Co, St Louis, MO), 100 U/ml of penicillin, 100 \mu g/ml of streptomycin and 100 \mu g/ml of gentamicin (all antibiotics were obtained from Sigma). Medium 2: F-12 medium supplemented with heat inactivated (56°C for 30 minutes) 10 g/dl fetal bovine serum (GIBCO), 15 mM Hepes buffer (Sigma), fibronectin (2 \mu g/ml, Sigma), and antibiotics as described above. Acetyl
The method has been described in detail elsewhere. The intact corpus (oxyntic glandular mucosa) area was excised from the antrum and fore-stomach of the rat stomach and the full wall thickness minced into 2-3 mm³ pieces. The minced tissues were incubated in medium 1 at 37°C for 60 minutes, in an atmosphere of 5% CO₂ and 95% O₂. The tissues were pipetted several times and filtered through a nylon mesh. The filtrate was washed by centrifugation at 600 rpm for 5 minutes in Hank's balanced salt solution. The cultures were maintained at 37°C with 5% CO₂ in the air in a humidified atmosphere. Cells from three day old cultures, which were at the confluent state, were studied.

Dye exclusion test
Trypan blue exclusion test was performed according to a modification of the method of Phillips. The culture media were discarded and the monolayers were washed three times with Hank's balanced salt solution. The cultures were incubated in Hank's balanced salt solution containing test agents for 60 minutes in the culture condition. After incubation, Hank's balanced salt solution was discarded and the cells were washed with Hank's balanced salt solution. The washed monolayers were kept in 1 ml of Hank's balanced salt solution until the test was performed. Trypan blue 0.1 ml (0.4 g/ml), was added directly to the Hank's balanced salt solution and mixed. Within 10 minutes the number of stained or non-stained cells was counted in an inverted microscope at 200× magnification. Cell viability was expressed as follows:

Viability (%) = non-stained cells/stained cells + non-stained cells × 100

All experiments were performed by counting approximately 300 cells in each culture.

Isotope (⁵¹Cr)-release assay
The media were discarded and the monolayers were washed three times with 1 ml Hank's balanced salt solution (4°C) to remove floating cells in culture. The cells were then incubated in Hank's balanced salt solution containing 10 μCi/ml of ⁵¹Cr for two hours in the culture condition. The labelled cells were washed five times with Hank's balanced salt solution to remove excess isotope and then 1 ml Hank's balanced salt solution (37°C) containing test drugs as described under 'experimental design' was added to the monolayer. After incubation for experimental periods in the culture condition, Hank's balanced salt solution (1) was decanted and the monolayers were washed with 1 ml Hank's balanced salt solution (4°C) (2). Hank's balanced salt solution of (1) and (2) were combined and centrifuged at 2000 rpm for 15 minutes. The pellets were combined with washed monolayers and dissolved in 1M NaOH for 24 hours. ⁵¹Cr radioactivity of the cells and of the supernatant was counted with a well type Automatic Gamma Counting System (Searle Analytical Inc, Des Plaines, IL).

Calculation
The percentage of ⁵¹Cr released per sample was expressed as:

Release (%) =

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

or specific release (%) due to drug action = (percent of release in the presence of test drug) – (percent of release in the control).

¹⁴C-ASA absorption into rat gastric cultured cells
In order to study the effect of pH and dmPGE₂ on ASA absorption into the gastric cells, uptake of ¹⁴C-ASA into the cultured cells was determined.

On day 2 of culture, the cultured cells were incubated in medium 2 containing 0.1 μCi/ml of ¹⁴C-ASA in both groups at pH 5.0 and 7.4. Before incubation of the cells, dmPGE₂ (1.0 μM) or saline was added to the medium. After incubation for two hours, the medium was discarded and the monolayers were washed with Hank's balanced salt solution three times. After dissolving the cells with 1M NaOH, the radioactivity of ¹⁴C-ASA in the cells was measured in a liquid scintillation counter (Packard Instr Co, Downers Grove, IL). Total protein of the cells was measured according to the method of Bradford. The result was expressed as cpm/mg protein.

EXPERIMENTAL DESIGN
ASA-induced damage to gastric mucosal cells
(a) Time course of ⁵¹Cr-release: prelabelled cells were incubated in Hank's balanced salt solution containing saline (controls at pH 7.4 and 5.0) or 10
Prostaglandin protection of cultured gastric cells

mM ASA (pH 5-0) for periods up to three hours. (b) Effect of ASA at various pH concentrations on $^{51}$Cr-release: prelabelled cells with $^{51}$Cr were incubated in Hank's balanced salt solution containing 10 mM ASA or saline at various pH concentrations (7.4, 6.0, 5.0, 4.0) for two hours. (c) Dose response study of ASA and correlation between specific $^{51}$Cr-release and dye exclusion test: saline at pH 5-0 (control) and ASA 1, 5, 10, and 20 mM at pH 5-0 was added to the culture for two hours. Subsequent specific $^{51}$Cr-release and cell viability (dye exclusion test) were determined and compared.

**Effect of dmPGE$_2$ on ASA-induced cell damage**

Prelabelled cells for $^{51}$Cr-release study and non-labelled cells for the dye exclusion test were incubated in Hank's balanced salt solution containing the following agents at pH 5-0: (a) 10 mM ASA, (b) 10 mM ASA plus dmPGE$_2$ (0.1, 1.0, 10.0 $\mu$M), (c) saline (control), (d) saline plus dmPGE$_2$ (1.0 $\mu$M).

**Effect of DBcAMP or IMX on cell viability**

In order to investigate the role of cAMP in prostaglandin cytoprotection, DBcAMP (1.0 mM) or dmPGE$_2$ (1.0 $\mu$M) plus IMX (0.1 mM) was added to the medium containing 10 mM ASA. These cells were processed for $^{51}$Cr-release assay or dye exclusion.

**Results**

**CELL CULTURE**

The cultured cells reached confluency on day 2 or 3. Over 90% of the cells were identified as mucus producing epithelial cells; mitoses were seen, the mitotic index on day 2 being 2-0% as previously reported.13

**EFFECT OF ASA ON $^{51}$CR-RELEASE AND CELL VIABILITY**

The time course of $^{51}$Cr-release by cultured cells treated with 10 mM ASA (at pH 5-0) or saline (at pH 7-4 or 5-0) is shown in Fig. 1. In the control group at pH 7-4, only a slight increase of $^{51}$Cr-release was observed up to three hours, whereas at pH 5-0 $^{51}$Cr-release was increased highly significantly at two and three hours compared with pH 7-4. In the group treated with 10 mM ASA at pH 5-0, this value was significantly increased compared with its control group at pH 5-0 at all time periods. Figure 2 shows the effect of pH on $^{51}$Cr-release caused by 10 mM ASA or saline (control). At neutral values (pH 7-4), there was no significant difference in $^{51}$Cr-release between 10 mM ASA and

![Figure 1](http://gut.bmj.com/)

**Fig. 1** Time course of effect of ASA (10 mM) on $^{51}$Cr-release. Each point represents mean ± SEM of four culture (*p<0.05, **p<0.01 control (pH 5-0) values compared with control at pH 7-4; ***p<0.01 ASA values compared with control at pH 5-0).”

![Figure 2](http://gut.bmj.com/)

**Fig. 2** Effect of pH on $^{51}$Cr-release caused by 10 mM ASA (two hour incubation). Each point represents mean ± SEM of four culture (*p<0.05, **p<0.01 compared with control).
control, while, at pH 5-0, 10 mM ASA significantly increased this value compared to control.

Figure 3 illustrates that results of specific release of $^{51}$Cr and dye exclusion test caused by different concentrations of ASA were strongly positively correlated ($r=0.926$, $p<0.001$).

**EFFECT OF DM PGE$_2$ ON SPECIFIC $^{51}$CR-RELEASE OF ASA-TREATED GASTRIC CULTURED CELLS**

Figure 4 shows a dose response study of the effect of dmPGE$_2$ (0-1-10-0 uM) on specific $^{51}$Cr-release of 10 mM ASA-treated rat gastric cultured cells. Addition of dmPGE$_2$ (1-0 and 10-0 uM) significantly diminished the increase of specific $^{51}$Cr-release caused by 10 mM ASA (pH 5-0), whereas 0-1 uM dmPGE$_2$ did not. Dimethyl prostaglandin E$_2$ did not significantly change the effect of pH 5-0 on $^{51}$Cr-release.

A similar degree of protection against 10 mM ASA-induced damage with 1-0 uM dmPGE$_2$ was seen using the dye exclusion test ($p<0.01$, Fig. 5).

**EFFECT OF DBCAMP AND IMX ON CELL VIABILITY**

Dibutyryl cAMP (1-0 mM) did not significantly change ASA-induced decrease of viability (trypan blue exclusion test) or increase of $^{51}$Cr-release (Figs 5 and 6). Isobutylmethyl xanthine did not cause any significant change in the effect of dmPGE$_2$ on ASA-induced decrease of viability (dye exclusion test) or increase cAMP level compared with dmPGE$_2$ alone in rat gastric cultured cells. These results show that cAMP does not play a major role in prostaglandin cytoprotection in vitro.

**EFFECT OF DM PGE$_2$ ON $^{14}$C-ASA ABSORPTION INTO CULTURED CELLS**

Figure 7 shows the results of $^{14}$C-ASA absorption by rat gastric cultured cells at different pH levels (7-4 and 5-0) and after dmPGE$_2$ treatment. $^{14}$C-ASA absorption was significantly enhanced at pH 5-0 compared with pH 7-4. Addition of dmPGE$_2$ (1-0 uM), however, did not significantly change $^{14}$C-ASA absorption into the cells over corresponding control values for either pH 7-4 or 5-0.

**Discussion**

In this study we have shown that dmPGE$_2$ can significantly reduce ASA-induced damage of rat gastric epithelial cells in tissue culture, under conditions independent of systemic factor such as blood flow.

Robert and colleagues reported that prostaglandins can protect gastric mucosa against various kinds of damaging agents – for example, aspirin, alcohol, or taurocholate. The mechanism of this action of prostaglandins, however, has not been clarified.
Prostaglandin protection of cultured gastric cells

Fig. 5 Effect of ASA (10 mM), 16,16 dimethyl prostaglandin E₂ (dmPGE₂, 1-0 μM), isobutyl methyl xanthine (IMX, 0-1 mM), and dibutyryl cyclic AMP (DBcAMP, 1-0 mM) on cell viability determined by trypan blue exclusion test. Each bar represents mean ± SEM of eight cultures. Probability values are expressed diagrammatically at the top of the figure.

Whether prostaglandins exert a direct protective effect on gastric mucosa independent of systemic influences especially of blood flow⁵-⁸ has been questioned. Chaudhury and Jacobson⁷ reported prostaglandin cytoprotection in isolated canine gastric mucosa and Schiessel et al showed it in isolated frog mucosa.⁹ Controversy, however, exists as to whether prostaglandins are truly 'cyto'-protective against damaging agents to gastric epithelial cells in vitro. In a preliminary report, Lacy and Ito⁴ claimed that prostaglandins did not prevent histological damage of the surface epithelium produced in vivo by ethnol. Blum's group¹⁰ could not show protection by PGE₂ against damage produced by taurocholate in isolated gastric cells.

We tested the effect of dmPGE₂ on ASA-induced damage to rat gastric mucosal cells in an in vitro tissue culture composed predominantly of mucus-producing epithelial cells.¹³ In the present study, ASA considerably decreased cell viability at pH 5-0, but not at pH 7-4. Moreover, absorption of ASA into the gastric cells was significantly increased at pH 5-0 compared with that at pH 7-4. These results were compatible with other studies performed in vitro.¹⁶

As parameters of cellular damage, we used the dye exclusion test and ⁵¹Cr-release. Although the dye exclusion test has been widely used for estimation of cell viability and is a simple method, it also has some disadvantages; it is subjective and time consuming. On the other hand, the quantitative measurement of cell damage, using the release of radioactive isotopes¹⁷-²¹ provides an objective and accurate method which permits the handling of a large number of samples. Out results showed a strongly positive correlation between
Whether cAMP is mediator of this prostaglandin cytoprotection has been controversial. As prostaglandins have been shown to increase cAMP concentration in nonparietal cells, and cAMP stimulates mucus production and ion transport in gastric mucosa (both postulated mechanisms of cytoprotection), it was reasonable to assume that the mechanism of cytoprotection involved cAMP.

In this study we tested the effect of DBcAMP, an analogue of cAMP, on ASA-induced cellular damage measured both by $^{51}$Cr-release and dye exclusion. The data obtained have shown that DBcAMP did not significantly increase the viability of ASA-treated cells. Further, IMX, a phosphodiesterase inhibitor, did not change the effect of dmPGE$_2$ on ASA-induced cellular damage, though it significantly increases cellular cAMP levels caused by dmPGE$_2$. Thus, it does not appear that prostaglandins exert their cytoprotective effect by increasing intracellular cAMP concentration in gastric epithelial cells. These results are consistent with reports of cytoprotective properties of prostaglandins in human gastric mucosal tissue independent of their effect on adenyl cyclase activity.

The effect of dmPGE$_2$ on ASA absorption into gastric cells was studied to test the hypothesis that prostaglandin cytoprotection may be because of interference with absorption of the damaging agent. In our study, ASA absorption into the cultured cells was not significantly different between the groups treated with dmPGE$_2$ (1.0 $\mu$M) or saline (control), whereas this concentration of dmPGE$_2$ exerted a cytoprotective effect on these cells. This confirms results of Guth and Paulsen who reported that dmPGE$_2$, at doses that prevented aspirin induced lesions, did not affect aspirin absorption by the gastric mucosa.

In summary, dmPGE$_2$ diminishes ASA-induced damage to rat gastric epithelial cells cultured in vitro. Thus, dmPGE$_2$ can exert a true cytoprotective effect on gastric mucosal epithelial cells independently of systemic factors such as blood flow.

This work was supported by the Medical Research Service of the Veterans Administration. Dr Terano was a visiting scientist from the Second Department of Medicine, University of Tokyo, Japan. Drs Mach and Stachura were visiting scientists from the Medical Academy, Krakow, Poland. We are grateful to Miss K Raymont for technical assistance and to Mrs Linda Burkhardt-Dowd for typing the manuscript.
Prostaglandin protection of cultured gastric cells

References


Effect of 16,16 dimethyl prostaglandin E2 on aspirin induced damage to rat gastric epithelial cells in tissue culture.
A Terano, T Mach, J Stachura, A Tarnawski and K J Ivey

Gut 1984 25: 19-25
doi: 10.1136/gut.25.1.19

Updated information and services can be found at:
http://gut.bmj.com/content/25/1/19

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/