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The protective effects of a prostaglandin without antisecretory properties against ethanol-induced injury in the rat stomach: a histologic study

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Summary. This study examined the effect of 2-acetyl-2-decarboxy-15(S)-15 methyl $PGF_{2\alpha}$ ($PGF_{2\alpha}$) on ethanol (EtOH) induced injury in the rat stomach to determine if a PG analogue devoid of antisecretory properties could confer full or partial gastric mucosal protection. Rats were orally administered saline or $PGF_{2\alpha}$ in a dose of 0.5 or 5.0 mg/Kg. Thirty minutes later animals received varying concentrations (i.e. 25%, 50%, and 100%) of EtOH orally. Five minutes following EtOH exposure, they were killed and samples taken from identical regions of the glandular mucosa for microscopic evaluation. All concentrations of EtOH tested damaged the gastric epithelium. The injury induced by 25% EtOH was almost exclusively confined to the surface epithelium and was not altered by either dose of $PGF_{2\alpha}$ pretreatment. In contrast, both 50% and 100% EtOH elicited comparable damage to the gastric mucosa involving both the deep and superficial mucosa of virtually the entire epithelium. The deep injury induced by these two EtOH concentrations was prevented by both the low and high dose of $PGF_{2\alpha}$. Of particular importance the 5.0 mg dose of $PGF_{2\alpha}$ provided complete protection (i.e. both superficial and deep) to as much as 50% of the mucosa exposed to 50% or 100% ethanol. These findings indicate that $PGF_{2\alpha}$ possesses «cytoprotective» properties involving both the superficial and deep epithelium that are dose related.

Key words: Gastric injury - Prostaglandin $F_{2\alpha}$ - Ethanol - Cytoprotection

Introduction

In recent years a number of carefully conducted histologic studies (Lacy and Ito, 1982; Guth et al., 1984; Schmidt et al., 1985; Tarnawski et al., 1985) have shown that prostaglandins (PGs) administered both orally and subcutaneously to rats protect the deep but not the superficial gastric epithelium from injury following exposure to absolute ethanol (Ethanol and alcohol will be used interchangeably throughout this paper). To date, these studies have uniformly used the synthetic prostaglandin analogue, 16, 16 dimethyl PGE_2 (PGE_2). Although the doses of this agent employed in these studies have generally been less than those required to inhibit stimulated acid secretion (Robert et al., 1976), the fact that PGE_2 possesses potent antisecretory properties may, nonetheless, have contributed to its protective action.

The present study utilized an analogue of $PGF_{2\alpha}$, namely 2-acetyl-2-decarboxy-15(S)-15-methyl $PGF_{2\alpha}$ ($PGF_{2\alpha}$), to determine if a prostaglandin other than PGE_2 possesses protective effects against alcohol injury when evaluated histologically in a systematic fashion, and whether this protection is also limited to the deep epithelium. A PG of the $F_{2\alpha}$ type was further chosen for study because it has been previously shown to be devoid of antisecretory properties (Robert et al., 1982). Thus, if inhibition of acid secretion is important in mediating the protective effects of PGs, the ability of $PGF_{2\alpha}$ to confer protection against alcohol injury, if demonstrable, should be less than that previously observed with PGE_2 under similar experimental conditions.

Materials and methods

Female Sprague-Dawley rats with an average weight of 200 g were housed in cages with wire mesh bottoms to prevent coprophagia. The animals were fasted 24 hours and allowed free access to water. On the day of experimentation, animals were randomized. Each animal received 1.0 ml of either 0.5 mg/kg or 5.0 mg/kg of $PGF_{2\alpha}$, or an equivalent volume of physiologic saline via orogastric tube. Thirty minutes later, animals were administered an oral bolus of 1.0 ml aqueous 25%, 50%, or 100% ethanol (EtOH) (v/v). Five minutes after EtOH administration, each animal was sacrificed to obtain

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gastric mucosal samples for histologic evaluation. This sacrifice time was chosen because it was previously shown that 100% EtOH severely damages rat gastric mucosa within 5 min following exposure, and that prostaglandin, if protective, prevents such injury in a similar time frame (Guth et al., 1984; Schmidt et al., 1985; Tarnawski et al., 1985).

At the time of sacrifice, animals were killed by cervical dislocation and a midline laparotomy was performed. The pylorus and esophagus were ligated and 2.0 ml of half-strength Karnovsky's fixative (Karnovsky, 1965) was injected via syringe into the gastric lumen through a small puncture wound in the forestomach. The stomach was excised, immersed in fixative, and assigned a code number. Twenty-four hours later, stomachs were opened along the lesser curvature and two tissue blocks measuring 2 mm × 1 cm were excised from the gastric corpus. The blocks were removed at right angles to the long axis of the stomach at sites located 2 and 12 mm distal to the limiting ridge. Hematoxylin and eosin stained, paraffin-embedded sections were prepared for light microscopy using standard techniques. All glass slides were coded in such a way that the observer (K.L.S.) was unaware of the experimental protocol. Each tissue section was assessed along the entire mucosal surface using a graduated microscope eyepiece.

Tissue sections were graded as normal or Type 1 through 4 injury, with Type 1 representing the most superficial and Type 4 the most extensive mucosal damage. A detailed discussion of the light microscope criteria for cell and tissue injury and the grading technique has been published elsewhere (Schmidt et al., 1985). Briefly, surface mucous cell damage and/or necrosis was indicated by cytoplasmic vacuolization or swelling, or nuclear pyknosis and/or swelling, with margination of nuclear chromatin. Lucent cytoplasm and pyknotic nuclei indicated parietal cell damage. Gland dilation, hyperemic vessels, and hemorrhage were indications of deeper glandular injury. Type 1 damage involved interfoveolar surface mucous cells only. If gastric pit surface mucous cells were also involved, classification was Type 2. Injury extending from luminal cells through gastric pit cells and including up to 1/3 the depth of the gastric glands was classified as Type 3. The most severe necrotic injury which extended deeper into the gastric glands below the upper third was designated Type 4. After analysis, the individual grades from a single section were summated and an overall percentage of the mucosal surface calculated for normal and each grade of injury. Percentage values for the two tissue samples from each stomach were then averaged to yield the mean percentage for normal and each grade of injury for that stomach. Slides were decoded following analysis.

To confirm that $PGF_{2\alpha}$ is indeed devoid of antisecretory activity an additional series of experiments were performed in pylorus-ligated rats. Under ether anesthesia, the abdomen was incised and the pylorus was ligated. Three hours later, the animals were killed and the gastric contents were analyzed for volume and acidity. Acidity was determined by automatic titration of the gastric juice to pH 7.0 with 0.1 N NaOH (Auroburette, Radiometer, Copenhagen, Denmark). Titratable acid output was expressed as microequivalents of acid per total volume of gastric juice collected. The higher dose of $PGF_{2\alpha}$ (5 mg/kg) was administered orally by gastric intubation 5 min after pylorus ligation in a volume of 1 ml of normal saline. It was assumed that if this dose of $PGF_{2\alpha}$ was devoid of antisecretory activity, any protection against alcohol injury rendered by this PG was independent of effects on acid secretion. Control animals received a comparable volume of saline alone administered by the same route.

All data were subjected to statistical evaluation using analysis of variance. A p value < 0.05 was considered statistically significant.

Results

Gastric secretory studies

Pylorus ligation led to an accumulation of 6.2 ± 1.6 ml of gastric juice 3 hours later in saline control animals (n=6). In animals (n=6) receiving $PGF_{2\alpha}$ this volume increased slightly to 7.9 ± 1.1 ml, but the difference between the two groups was not statistically significant. Acid content in this volume was 744 ± 197 μEq in saline control animals versus 739 ± 135 μEq in PG treated animals. Differences between these two groups in terms of acid content were also insignificant.

Gastric damage studies

The damaging effects of the different ethanol concentrations following saline pretreatment are summarized in Table 1. As one would expect, damage was noted to be concentration-related with 25% ethanol eliciting the least damage. With this concentration, nearly 45% of the mucosa was noted to be normal. When injury was present, it was almost exclusively superficial, being confined to Types 1 and 2. A very different pattern of injury emerged when mucosa was exposed to 50% ethanol. As much as 68% of mucosa involved injury extending into the gastric glands; of this, about 25% involved marked damage to the glands (Fig. 1). Only 30% of the mucosa exposed to this alcohol concentration demonstrated superficial injury (Types 1 and 2) and less than 2% was histologically normal. Administration of 100% alcohol following saline pretreatment produced a pattern of injury that was virtually identical to that observed in mucosa exposed to 50% ethanol (Fig. 2). Approximately 33% of the mucosa was superficially injured, 38% was moderately injured (Type 3), and 22% was deeply injured (Type 4); only 7% appeared normal histologically. In fact, there was no statistical difference between the damaging effects of 50% and 100% ethanol with respect to any category of injury.

With the exception of mucosa exposed to 25% ethanol, pretreatment with $PGF_{2\alpha}$ influenced the magnitude of injury induced by ethanol (Table 2). Pretreatment with either the low or high dose of $PGF_{2\alpha}$

Table 1. Damaging effects of different ethanol concentrations

Ethanol Concentration	Category of Injury				
	Normal	Type 1	Type 2	Type 3	Type 4
25% (n = 10)	44.8 ± 6.2	24.4 ± 1.8	26.6 ± 5.4	4.1 ± 1.5	0
50% (n = 10)	1.8 ± 0.6*	8.0 ± 1.3*	22.1 ± 3.2	43.3 ± 3.1*	24.7 ± 5.0*
100% (n = 6)	7.0 ± 5.6*	8.0 ± 5.9**	25.1 ± 1.7	37.6 ± 8.0*	22.1 ± 7.9**

* $p < 0.005$ compared to 25% ethanol.
** $p < 0.05$ compared to 25% ethanol.

Fig 1. Light micrograph of gastric mucosa exposed to oral saline and oral 50% ethanol showing ethanol induced injury. Clusters of exfoliated surface mucous cells (double arrows) are embedded in a layer of thick mucus. Such clusters are lost from interfoveolar sites (single arrows) following ethanol injury. Focal areas of vascular hyperemia (V) are present in the lamina propria. Surface mucous cells adjacent to the gastric lumen and in the pits exhibit pyknotic nuclei (N) indicative of cell injury. Paraffin-embedded, hematoxylin and eosin stain. M, muscularis mucosa; L, lumen. × 245

Inset. Higher magnification of the mucosal surface pictured above showing vascular hyperemia (V) and pyknotic nuclei (N). Cytoplasmic vacuoles (arrows) are additional evidence of cell injury. × 610

Fig. 2. Light micrograph of gastric mucosa exposed to oral saline prior to oral 100% ethanol showing moderate mucosal injury. Groups of surface mucous cells (double arrows) are exfoliated in a thick mat of mucus. Many surface mucous cells still attached to the basement membrane show pyknotic nuclei (N). Glands (G) are dilated. Injured parietal cells and mucous neck cells are present in the glands (single arrows). Paraffin-embedded, hematoxylin and eosin stain. M, muscularis mucosa; L, lumen. × 245

Inset. Higher magnification of the mucosal surface shown above. Surface mucous cells in a variety of viable states are seen. Several cells show cytoplasmic vacuolization (arrows). Other cells contain pyknotic nuclei (N) or are being sloughed from the lamina propria into the gastric lumen (L). Hyperemic vessels (V) are present in the lamina propria. × 610

Fig. 3. Light micrograph of gastric mucosa exposed to oral 0.5 mg/kg $PGF_{2\alpha}$ prior to oral 50% ethanol showing a region of mucosal injury. Clusters of exfoliated surface mucous cells (double arrows) are present in a thin, adherent layer of luminal mucus. Surface mucous cells and gland cells demonstrate pyknotic nuclei (single arrows). Although vascular congestion is not prominent, dilation of glandular lumina (G), a criterion of gland injury, is present. Paraffin-embedded, hematoxylin and eosin stain. M, muscularis mucosa; L, lumen. × 245

Inset. Higher magnification of the gastric mucosa shown above. Cells in this inset exhibit features of cell injury and death. Pyknotic nuclei (N) and cytoplasmic vacuoles (V) are visible. Glands (G) are dilated. × 610

Fig. 4. Light micrograph of gastric mucosa exposed to oral 5.0 mg/kg $PGF_{2\alpha}$ prior to oral 50% ethanol showing minimal mucosal injury. A thin veil of mucus (arrows) is present in the gastric lumen. Surface mucous cells remain attached to the basement membrane and have not exfoliated into the gastric lumen. A focal zone of cell injury subjacent to the mucus veil is evidenced by the dilation and staining pallor of the nuclei (N). Glands appear normal. This appearance of the gastric mucosa typified most of the tissue from stomachs exposed to the higher $PGF_{2\alpha}$ dose followed by 50% ethanol. Paraffin-embedded, hematoxylin and eosin stain. M, muscularis mucosa; L, lumen. × 245

Inset. Higher magnification of the gastric mucosa shown above. Most surface mucous cells (double arrows) are normal appearing. Slight vascular (V) congestion is present. × 610

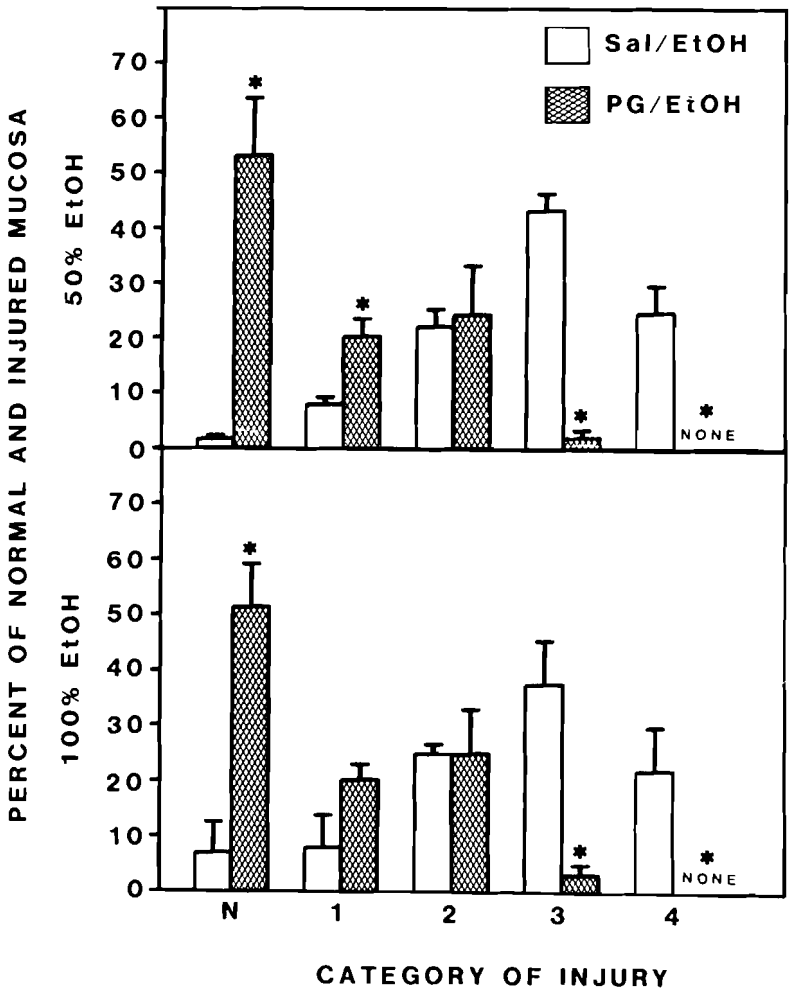

Fig. 5. Effects of prostaglandin or saline treatment on the depth of gastric injury in rats sacrificed 5 min after oral administration of 50% or 100% ethanol. There was no Type 4 injury (i.e none) in any animals pretreated with prostaglandin and subsequently administered 50% or 100% ethanol.
Sal/EtOH = oral saline followed by 50% or 100% ethanol
PG/EtOH = oral 5 mg/kg $PGF_{2\alpha}$ followed by 50% or 100% ethanol
N = histologically normal mucosa
N = 6 for both experimental groups exposed to 100% EtOh;
N = 10 for Sal/50% EtOH group; and N=11 for PG/50% EtOH group.
* $p < 0.05$ compared with Sal/EtOH

failed to alter the pattern of injury induced by 25% ethanol. As noted with mucosa exposed to 25% ethanol following saline pretreatment, injury was confined to Types 1 and 2 in mucosa pretreated with $PGF_{2\alpha}$ and then exposed to this alcohol concentration. In animals pretreated with the low dose of $PGF_{2\alpha}$, superficial injury involved 41% of the mucosa, and with the higher dose it involved 45% of the mucosa. Neither of these values were significantly different from the 51% of mucosa that was superficially injured by 25% ethanol if $PGF_{2\alpha}$ pretreatment had not been rendered.

In mucosa exposed to 50% ethanol, following pretreatment with a low dose of $PGF_{2\alpha}$ (Fig. 3), 71% of the mucosa was only superficially injured, 3% was moderately injured, 0% was deeply injured and greater than 25% was judged to be histologically normal (Table 2). Pretreatment with the high dose of $PGF_{2\alpha}$ (Fig. 4) followed by 50% ethanol exposure resulted in 53% of the mucosal surface being histologically normal, 45% superficially injured, 2% moderately injured, and 0% deeply injured (Table 2, Fig. 5).

Similar results were obtained in mucosa pretreated

Table 2. Effect of $PGF_{2\alpha}$ on ethanol injury. (Percent of Undamaged Mucosa)

Ethanol Concentration	Ethanol Alone	$PGF_{2\alpha}$ 0.5 mg/kg	$PGF_{2\alpha}$ 5.0 mg/kg
25%	44.8 ± 6.2 (n = 10)*	59.4 ± 5.1 (n = 11)*	55.4 ± 6.5 (n = 8)
50%	1.8 ± 0.6 (n = 10)	25.2 ± 7.0 (n = 6)**	53.2 ± 10.4 (n = 11)
100%	7.0 ± 5.6 (n = 6)	6.7 ± 4.0 (n = 6)	51.4 ± 7.9 (n = 6)

* $p < 0.005$ compared to 50% and 100% ethanol.
** $p < 0.05$ compared to 100% ethanol.

Fig. 6. Light micrograph of gastric mucosa exposed to oral 0.5 mg/kg $PGF_{2\alpha}$ followed by oral 100% ethanol showing superficial (Types 1 and 2) injury. Exfoliated surface mucous cells are observed in the gastric lumen (L). Cell injury as indicated by pyknotic nuclei (single arrows) extends to the pit base in most regions. Most glands are normal appearing. Vascular congestion (V) is seen in several vessels of the lamina propria. Exfoliation of the surface mucous cells is accompanied by collapse of the gastric pit (double arrows). Paraffin-embedded, hematoxylin and eosin stain. M, muscularis mucosa. X245

Inset. Higher magnification of the mucosal surface shown above. Virtually all surface mucous cells show injury or death; cytoplasmic vacuoles (arrows) and pyknotic nuclei (N) are prominente. Collapse of the gastric pit is an event that is concomitant with surface mucous cell exfoliation. X610

Fig. 7. Light micrograph of gastric mucosa exposed to 5.0 mg/kg $PGF_{2\alpha}$ prior to oral 100% ethanol showing focal superficial injury. Pyknotic nuclei are seen in focal sites (double arrows) of interfoveolar regions. Cell death with exfoliation is absent. Gland and gastric pit histoarchitecture is normal. Most regions of the mucosa exposed to this protocol displayed a similar histology. Paraffin-embedded, hematoxylin and eosin stain. M, muscularis mucosa; L, lumen. X245

Inset. Higher magnification of gastric surface shown above. Scattered cells contain pyknotic nuclei (arrows) but, otherwise, the tissue is normal. X610

with $PGF_{2\alpha}$ prior to 100% ethanol exposure. The low dose of $PGF_{2\alpha}$ administered prior to 100% ethanol exposure resulted in 7% normal mucosa, 76% superficial, 17% moderate, and 2% deep injury (Fig. 6). If the higher $PGF_{2\alpha}$ pretreatment dose was given prior to absolute alcohol (Fig. 7), 51% of the mucosa was normal histologically, 45% was superficially injured and only 3% exhibited moderate damage; deep injury was absent (Table 2, Fig. 5).

Discussion

This study investigated the efficacy of a prostaglandin not of the PGE_2 series and devoid of antisecretory properties (as shown by our secretory studies) in preventing or attenuating gastric mucosal damage as determined by histologic evaluation in a standard ethanol injury model. A number of important findings were observed. First, all concentrations of alcohol tested in this study induced injury in the glandular epithelium. This damage was concentration-related with 25% ethanol eliciting the least damage. Of particular interest, 50% ethanol was as damaging as 100% to the gastric mucosa morphologically, a circumstance not previously reported.

Second, $PGF_{2\alpha}$ pretreatment produced remarkable protection of mucosa exposed to both 50% and 100% ethanol by sparing a substantial portion of gastric epithelium from any morphologic evidence of injury in contrast to control mucosa in which these concentrations of ethanol almost completely injured the entire epithelial surface. This finding was especially noteworthy with the high dose of $PGF_{2\alpha}$ in which at least 50% of the gastric mucosa was histologically normal following exposure to these ethanol concentrations. Much controversy has been generated recently regarding the appropriateness of the term «cytoprotection» in describing the protective action of PGs since, heretofore, most studies have indicated that only the deep gastric epithelium is spared following ethanol insult (Lacy and Ito, 1982; Guth et al., 1984; Schmidt et al., 1985; Tarnawski et al., 1985). However, the present data indicate that under the conditions of our study protection of superficial as well as deep epithelium was achieved by $PGF_{2\alpha}$ and suggests to us that more information is required to fully resolve the dispute as to whether cytoprotection is a real phenomenon.

Third, it is evident from this study that even ethanol exposure at concentrations as low as 25% induced a certain low level of superficial epithelial damage (i.e. surface mucous cell injury and/or death) in the gastric mucosa. Even though the high does of $PGF_{2\alpha}$ was able to substantially lessen the magnitude of surface cell injury induced by 50% and 100% ethanol, injury to the surface epithelium was never completely eradicated and could not be circumvented with the administration of either dose of $PGF_{2\alpha}$ against any concentration of EtOH tested. This finding seems to underscore the normal function of surface mucous cells in response to an injurious agent. These cells mature as they migrate up the pit wall and once in the interfoveolar zone they normally terminate function by in situ degeneration and extrusion (Zalewsky and Moody, 1979). Substances which stimulate mucus secretion such as acetylcholine chloride (Zalewsky et al., 1983) or damaging agents such as acetylsalicylic acid or isobutyric acid (Morris et al., 1984) induce mucus expulsion by surface mucus cells. A rapid and voluminous mucous release is accompanied by surface mucous cell involution, death, and exfoliation (Zalewsky et al., 1983; Morris et al., 1984) Therefore, one must question whether injury and death of interfoveolar surface mucous cells are reliable markers of mucosal damage since these cells may be programmed to die under normal as well as stressful conditions. Certainly, cells found in the deep pit and gland isthmus are appropriate indicators of mucosal integrity since the pool of cells for resurfacing the basement membrane are known to reside in this zone (Ito and Lacy, 1985; Schmidt et al., 1985; Tarnawski et al., 1985; Schmidt et al., 1986).

Fourth, the dose of PG administered may be important in determining the extent to which PGs are protective in a given experimental circumstance. Using a rat model, Whittle and Steel (1985) reported that 20 µg/kg of 16.16 dimethyl PGE_2 administered orally provided full protection to 80% of gastric surface epithelial cells exposed to 98% ethanol although lower doses of this PG did not have this effect. In contrast, other investigators (Tarnawski et al., 1985; Ohno et al., 1985) found no protection against surface cell injury in rats receiving doses of 16.16 dimethyl PGE_2 ranging from 3 to 100 µg/kg orally prior to 50% or 100% ethanol exposure. The reason for these discordant results is unknown. Using a different prostaglandin analogue administered orally, our data corroborate those of Whittle and Steel (1985) and clearly indicate a dose-response between the lower and higher concentrations of $PGF_{2\alpha}$ in terms of mucosal protection.

The sampling of tissues within five minutes following ethanol exposure, as done in the present study, avoids the rapid restitution of epithelium which occurs in the stomach following injury. Ito and Lacy (1985) have shown that intact cells from the gastric isthmic zone begin migrating over a denuded basal lamina within seven minutes following a brief exposure to absolute ethanol. Further, Schmidt and coworkers (1985) and Tarnawski and associates (1985) have reported that pretreatment with 16.16 dimethyl PGE_2 prior to absolute ethanol results in more rapid gastric reepithelialization, presumably by sparing injury to the deep mucosa and thereby allowing normal repair processes to proceed efficiently and smoothly. Thus, early selection of tissue samples circumvents the problem of a disproportionately large percentage of normal tissue resulting as a consequence of repair and obscuring the extent to which a given damaging agent is in fact «damaging» or a protective agent is in fact «protective».

A final consideration that needs to be addressed relates to the observation that $PGF_{2\alpha}$ when administered in a low dose was a more effective protective agent against damage induced by 50% ethanol than when mucosa was

exposed to the 100% ethanol concentration, even though both concentrations of alcohol elicited equivalent damage to the gastric mucosa. The reason for this discrepancy does not seem readily apparent. It has been shown previously that protection by prostaglandins against absolute alcohol injury in the rat stomach still occurs despite uptake of this damaging agent by gastric mucosal cells (Robert et al., 1985). What is not known is whether the efficiency of PG protection is modulated in any way by the concentration of the damaging agent entering cells, independent of inducing cellular damage. Thus, it is entirely possible that alcohol may have direct adverse effects on those processes necessary for PG protection to occur that could be concentration-related. If this notion is correct, 50% ethanol would have less adverse effects than would 100% ethanol, and a low concentration of prostaglandin, such as the 0.5 mg/kg dose of $PGF_{2\alpha}$ used in this study, would conceivably be a more effective protective agent against damage induced by 50% ethanol than that induced by the higher concentration. With a high enough dose of PG, even the adverse effects of 100% ethanol could be partially or perhaps completely overcome. Obviously, support for this hypothesis must await further studies.

In summary, we conclude that cytoprotection (i.e. both superficial and deep mucosa) of as much as half the gastric epithelium following exposure to concentrated ethanols can be demonstrated with an analogue of $PGF_{2\alpha}$, when administered in a high dose. This finding suggest that under appropriate conditions, other PGs may exhibit similar dose-response activities. Further, the fact that $PGF_{2\alpha}$ is devoid of antisecretory properties suggests that its protective effects are mediated by processes independent of modulation of acid secretion.

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