Molecular Mechanism of Adaptive Cytoprotection Induced by Ethanol in Human Gastric Cells

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Adaptive cytoprotection is the process by which the pretreatment of cells with low concentrations of a noxious agent prevents the damage caused by a subsequent exposure of those cells to higher concentrations of that same agent. In this study, a human gastric carcinoma cell line was used to examine the molecular mechanism of adaptive cytoprotection induced by ethanol. Pretreatment of cells with 1%–4% ethanol made cells resistant to a subsequent exposure to 8% ethanol. This adaptive cytoprotection was accompanied by an increase in prostaglandin E_2 synthesis and was partially inhibited by inhibitors of cyclooxygenase-2, but not by an inhibitor of cyclooxygenase-1. Furthermore, the adaptive cytoprotection was not dependent on newly synthesized proteins and was inhibited by a protein tyrosine kinase inhibitor. Based on these results, it is proposed that the stimulation of cyclooxygenase-2-dependent prostaglandin E_2 synthesis, which is regulated post-translationally by protein tyrosine phosphorylation, plays an important role in adaptive cytoprotection induced by ethanol in gastric cells. Exp Biol Med 228:1089–1095, 2003

Key words: prostaglandin E_2; protein tyrosine phosphorylation; post-translational regulation

The gastric mucosa is frequently exposed to various types of irritants (such as alcohol, acid, oxidative irritants, drugs, and bacteria), resulting in gastric mucosal cell death through both necrosis and apoptosis (1–4). Cell death under such conditions can cause gastropathy, which is manifested in the form of conditions such as gastric ulcer. Therefore, methods that make gastric mucosal cells resistant to gastric irritants are important for establishing clinical protocols to overcome gastritis caused by gastric irritants. From this point of view, the phenomenon called adaptive cytoprotection has been paid much attention.

Cyclooxygenase (COX) is a rate-limiting enzyme for prostaglandin (PG) synthesis. COX exists in two subtypes, COX-1 and COX-2. In the gastric mucosa, COX-1 is constitutively expressed in normal tissues, whereas COX-2 is induced under pathogenic conditions, such as inflammation (5). However, a low but significant expression of COX-2 in normal gastric mucosa was recently reported (6). It has also been reported that both COX-1 and COX-2 mRNA are expressed in the human gastric carcinoma cell line AGS (7). The COX-2 gene is known to be induced by various stimuli at a transcriptional level (8, 9). Recently, the post-translational regulation of COX-2 by tyrosine phosphorylation in cerebral endothelial cells was reported (10).

Adaptive cytoprotection refers to a phenomenon in which the gastric mucosa develops enhanced resistance to a gastric irritant by preadministration of low doses of the irritant (mild irritant) *in vivo* (11). As for the mechanism of adaptive cytoprotection *in vivo,* the stimulation of PG synthesis, especially PGE_2, was initially thought to be able to fully account for the phenomenon. PGs, especially PGE_2, have cytoprotective effects on gastric mucosa as a consequence of various physiological mechanisms that include increased epithelial mucus, bicarbonate secretion, and inhibition of apoptosis (12–14). Some mild irritants stimulate PG synthesis (11, 15). Indomethacin, an inhibitor of both COX-1 and COX-2, abolishes adaptive cytoprotection (11, 15, 16). Furthermore, it was recently reported that adaptive cytoprotection is inhibited in knockout mice for one of the PG receptors, EP1 (17). Together, these results support the concept that PGs play a major role in adaptive cytoprotection *in vivo.* However, this theory has been challenged by a number of investigators who showed that protective doses of a mild irritant did not always stimulate PG synthesis or that some adaptive cytoprotection was not completely inhibited by indomethacin (18, 19). Furthermore, other factors have been suggested to be involved in adaptive cytoprotection, such as nitric oxide, heat shock proteins, Ca^{2+}, neuronal pathways, salivary agents, and blood flow (20–23).

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Therefore, the molecular mechanism governing adaptive cytoprotection *in vivo* remains to be fully elucidated.

Adaptive cytoprotection of gastric mucosal cells is also observed *in vitro*; pretreatment of cultured gastric mucosal cells with low concentrations of an irritant (mild irritant) induces a cell phenotype that is resistant to subsequent exposure of the cells to high concentrations of the irritant (24–27). Adaptive cytoprotection *in vivo* and *in vitro* are not closely related to each other because the *in vitro* form is simplified and does not involve neural input, mucosal blood flow, salivary factors, or the immune system. Only a few papers have described the characterization of adaptive cytoprotection *in vitro*. In these previous reports, the stimulation of PG synthesis by mild irritants was suggested to be not involved in adaptive cytoprotection *in vitro*, but rather mucin release and Ca^{2+} influx were proposed to be key factors in this process (24–27).

In this study, we used AGS cells to systematically examine adaptive cytoprotection induced by ethanol, which is the most typical external gastric irritant. This cell line has been shown to be morphologically and functionally consistent with normal gastric mucosal cells (24, 28, 29). Our data suggest that stimulation of PG synthesis by low concentrations of ethanol is partly involved in adaptive cytoprotection in this model. Furthermore, using inhibitors of protein tyrosine kinase, protein synthesis, and COX, we suggest that the post-translational regulation of COX-2 by protein tyrosine phosphorylation is partly involved in this adaptive cytoprotection.

Materials and Methods

Chemicals and Media. Fetal bovine serum was from Gibco (Grand Island, NY). Ham's F12 medium was from Nissui (Tokyo, Japan). Indomethacin, aspirin, and NS-398 were from Wako Co. (Tokyo, Japan). Ibuprofen, diclofenac, cycloheximide, actinomycin-D, genistein, and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma Co. (Tokyo, Japan). The enzyme immunoassay (EIA) kit for PGE_2 and SC-560 were from Cayman Chemical Co. (Ann Arbor, MI).

Assay of Cell Injury by Ethanol. AGS cells were cultured in Ham's F12 medium containing 10% fetal bovine serum, as described previously (24, 28, 29). AGS cells (2×10^5 cells per well in a 24-well plate) were cultured for 24 hr and used for experiments. Cell viability was determined by the MTT method as previously described (4, 30).

Determination of PGE_2 in Culture Media. Determination of PGE_2 levels in culture media was done by EIA as previously described (31). Briefly, samples were loaded on microtiter plates coated with goat polyclonal anti-mouse IgG and incubated with PGE_2 acetylcholinesterase tracer and PGE_2 monoclonal antibody at 4°C for 18 hr. After washing, Ellman's reagent was added and the medium incubated for 1 hr at room temperature. The activity of acetylcholinesterase trapped in each well was monitored on a microtiter plate reader at a wavelength of 412 nm.

Statistical Analysis. All values are expressed as the mean ± SEM. One-way analysis of variance (ANOVA) of variance followed by Scheffe multiple comparison was used for evaluation of differences between the groups in Table I and Figures 4, 5, and 6. Two-way ANOVA of variance followed by Student's *t* test was used for evaluation of results in Figure 3.

Results

Dose- and Incubation Period–Dependent Adaptive Cytoprotection Induced by Ethanol. We first measured the sensitivity of AGS cells to ethanol. Treatment

Table I. Effect of Nonsteroidal Anti-Inflammatory Drugs on the Adaptive Cytoprotection Induced by Ethanol

NSAID in preincubation	Specificity for COX-1 [COX-2]/[COX-1]	Preincubation (3% EtOH)	Incubation (8% EtOH)	Relative viability (%)
—		−	−	100.0 ± 2.3
—		−	+	3.6 ± 1.1
—		+	+	60.6 ± 2.4
Aspirin (10 m*M*)	1.5	+	+	24.3 ± 2.1[a]
Diclofenac (100 μ*M*)	0.34	+	+	28.6 ± 1.7[a]
Ibuprofen (100 μ*M*)	6.7	+	+	29.9 ± 1.5[a]
Indomethacin (200 μ*M*)	3.3	+	+	27.6 ± 2.5[a]
SC-560 (100 μ*M*)	290	+	+	59.8 ± 1.8
NS-398 (100 μ*M*)	0.045	+	+	31.3 ± 1.1[a]
Aspirin (10 m*M*)	1.5	+	−	97.9 ± 0.9
Diclofenac (100 μ*M*)	0.34	+	−	100.0 + 1.8
Ibuprofen (100 μ*M*)	6.7	+	−	97.4 + 2.0
Indomethacin (200 μ*M*)	3.3	+	−	103.6 + 1.4
SC-560 (100 μ*M*)	290	+	−	104.1 + 2.8
NS-398 (100 μ*M*)	0.045	+	−	106.2 + 0.8

AGS cells were preincubated with for 1 hr with or without each nonsteroidal anti-inflammatory drug (NSAID) as indicated, then with or without 3% ethanol (EtOH) for 1 hr, and finally incubed with or without 8% EtOH for 1 hr. Cell viability was determined by the MTT method. Values are expressed as mean ± SEM.

[a] $P < 0.001$. Specificity for COX-1 (expressed as the ratio of IC_{50} (median inhibitory concentration) for [COX-2]/[COX-1] of each COX inhibitor was from previous papers (41–43).

of cells with 8% ethanol for 1 hr decreased the cell viability to less than 10% (Fig. 1). AGS cells were pretreated with low concentrations (0.5%–4%) of ethanol for 0.5–5 hr and subsequently treated with 8% ethanol for 1 hr (Fig. 2). The pretreatment alone did not affect cell viability (data not shown), suggesting that these low concentrations of ethanol serve only as a "mild irritant" for AGS cells. As shown in Figure 2, the pretreatment of cells with 1%–4% ethanol for 0.5–5 hr clearly attenuated the cell death caused by subsequent treatment of cells with 8% ethanol for 1 hr. We considered these findings to be indicative of adaptive cytoprotection and will refer to this process as such in the subsequent sections of this paper. The adaptive cytoprotection measured here was dependent on both the concentrations of ethanol used and the duration of the pretreatment (Fig. 2), with the most apparent adaptive cytoprotection observed when the pretreatment was performed with 3% ethanol for 2 hr (Fig. 2). We used this 3% ethanol concentration as the pretreatment concentration in the following experiments.

Involvement of PG and COX-2 in the Adaptive Cytoprotection Induced by Ethanol. The level of PGE_2 in the culture medium was measured by EIA (Fig. 3) and was found to significantly increase within 30 min of treatment of cells with 3% ethanol (ANOVA, $F = 112$, $P = 0.000177$). This high level of PGE_2 in the medium was still present 5 hr later, suggesting that PG synthesis was stimulated by the ethanol treatment.

As shown in Figure 4B, the adaptive cytoprotection caused by the short-term (1-hr) ethanol pretreatment was partially attenuated by indomethacin (200 μM) (ANOVA, $F = 8.55$, $P = 0.0175$). This concentration of indomethacin also partially inhibited the stimulation of PG synthesis caused by the short-term ethanol pretreatment (ANOVA, $F = 9.20$, $P = 0.0149$) (Fig. 4A). We have no clear explanation as to why indomethacin could not completely inhibit the PG synthesis but were unable to evaluate the effect

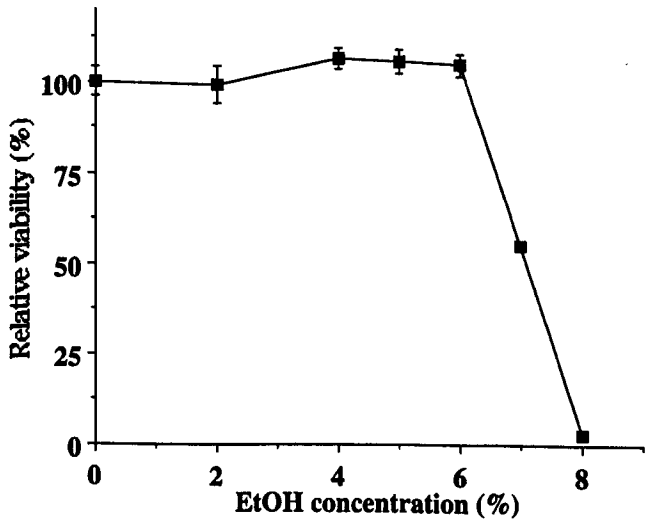

Figure 1. Cell death induced by exposure of AGS cells to ethanol for 1 hr. AGS cells were treated with indicated concentrations of ethanol (EtOH) for 1 hr. Cell viability was determined by the MTT method. Values are expressed as mean ± SEM ($n = 3$).

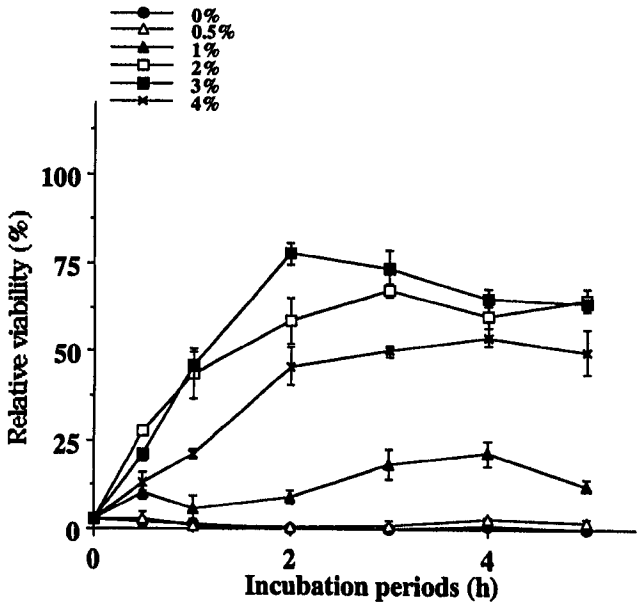

Figure 2. Adaptive cytoprotection in AGS cells induced by ethanol. AGS cells were pretreated with indicated concentrations of ethanol for indicated periods. Cells were further treated with 8% ethanol for 1 hr. Cell viability was determined by the MTT method. Values are expressed as mean ± SEM ($n = 3$).

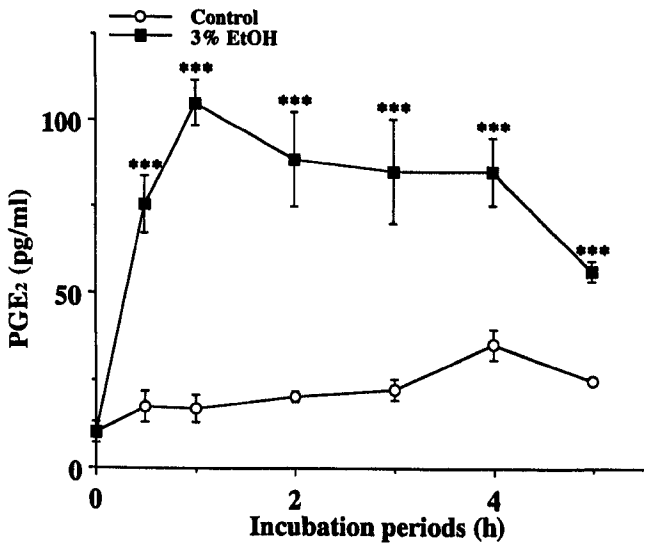

Figure 3. Stimulation of PG synthesis by treatment of AGS cells with 3% ethanol. AGS cells were treated with or without 3% ethanol (EtOH) for indicated periods. The amount of PGE_2 in the culture medium was determined by EIA. Values are expressed as mean ± SEM ($n = 3$). ***$P < 0.001$.

of higher concentrations of indomethacin on PG synthesis due to its cytotoxicity (data not shown). The inhibition by indomethacin was not so apparent for the adaptive cytoprotection brought about by the long-term (2–5 hr) ethanol pretreatment (data not shown).

Next, we used various COX inhibitors, including those specific for COX-1 or COX-2, for examining their effects on the adaptive cytoprotection. As shown in Table I, nonspecific COX inhibitors (aspirin, diclofenac, and ibuprofen) partially attenuated the adaptive cytoprotection induced by

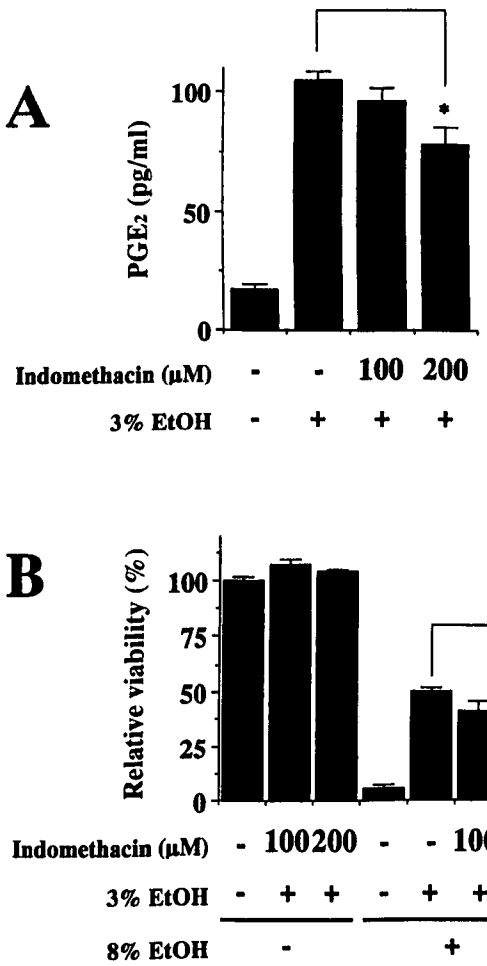

Figure 4. Effect of indomethacin on the adaptive cytoprotection induced by ethanol (EtOH). AGS cells were initially treated with or without indicated concentrations of indomethacin for 1 hr, then with or without 3% EtOH for 1 hr, and finally with or without 8% EtOH for 1 hr. The amount of PGE_2 in the culture medium was determined by EIA (**A**). Cell viability was determined by the MTT method (**B**). Values are expressed as mean ± SEM ($n = 3$). $^*P < 0.05$.

ethanol to a similar extent as did indomethacin (ANOVA, $F = 113$, $P = 4.7 \times 10^{-11}$). The suppression of adaptive cytoprotection was observed with a COX inhibitor specific for COX-2 (NS-398), but not with an inhibitor specific for COX-1 (SC-560). None of the COX inhibitors showed cytotoxicity at the concentrations used (Table I).

We also examined the effects of COX inhibitors on the stimulation of PG synthesis caused by low concentrations of ethanol. As shown in Figure 5, most of the COX inhibitors used (except SC-560) were able to partially inhibit the stimulation of PG synthesis caused by low concentrations of ethanol (ANOVA, $F = 9.29$, $P = 0.000321$) in a manner consistent with the results reported in Table I. The fact that SC-560 was the only inhibitor that did not affect PG synthesis in the presence of low concentrations of ethanol suggests that COX-2 but not COX-1 is primarily responsible for PG synthesis under the conditions examined. These results suggest that COX-2 is responsible for the adaptive cytoprotection.

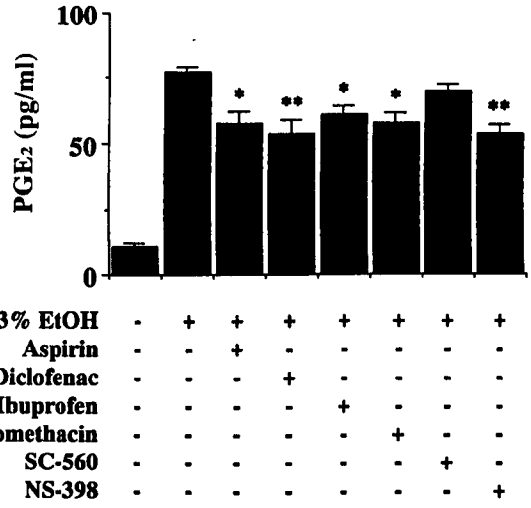

Figure 5. Effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on PG synthesis induced by ethanol (EtOH). AGS cells were initially treated for 1 hr with or without each NSAID as indicated, then with or without 3% EtOH for 1 hr. The amount of PGE_2 in the culture medium was determined by EIA. Values are expressed as mean ± SEM ($n = 3$). $^*P < 0.05$, $^{**}P < 0.01$.

Involvement of Post-Translational Regulation of COX-2 by Tyrosine Phosphorylation in the Adaptive Cytoprotection Induced by Ethanol. We used the quantitative reverse transcription polymerase chain reaction method to measure COX-2 mRNA levels under various experimental conditions. The level of COX-2 mRNA was not increased by the pretreatment of cells with 3% ethanol (data not shown). We further examined the effect of an inhibitor of transcription (actinomycin-D) or translation (cycloheximide) on the adaptive cytoprotection induced by ethanol. Neither actinomycin-D nor cycloheximide inhibited the adaptive cytoprotection (data not shown).

We used genistein (an inhibitor of protein tyrosine kinase) at concentrations of 50 and 100 μM in accordance with previous reports of its use (10, 32). As shown in Figure 6A, pretreatment of cells with 100 μM genistein partially attenuated the stimulation of PG synthesis caused by the ethanol pretreatment (ANOVA, $F = 16.9$, $P = 0.00342$). We also examined the effect of this inhibitor on the adaptive cytoprotection. As shown in Figure 6B, the pretreatment of cells with 50 or 100 μM of genistein partially attenuated the adaptive cytoprotection caused by the ethanol pretreatment (ANOVA, $F = 147$, $P = 7.99 \times 10^{-6}$). Genistein alone at these concentrations did not affect the viability of control cells (i.e., in the absence of the 8% ethanol treatment) (Fig. 6B).

Discussion

In this study, we examined the molecular mechanism of the adaptive cytoprotection induced *in vitro* by exposure of AGS cells to ethanol. The adaptive cytoprotection induced by the pretreatment of cells with low concentrations of ethanol was accompanied by the stimulation of PG synthesis and was partially inhibited by inhibitors of COX-2, suggest-

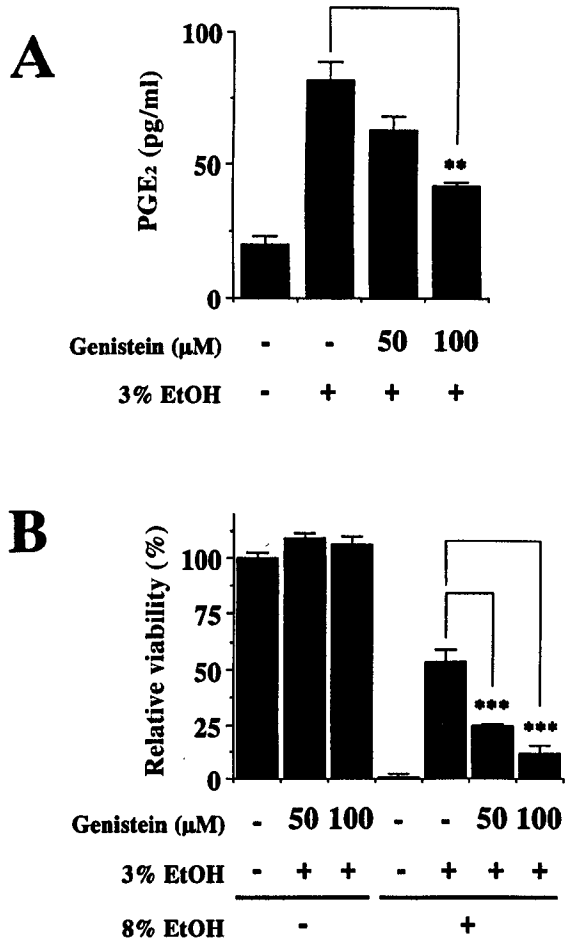

Figure 6. Effect of an inhibitor of tyrosine kinase on both PG synthesis and adaptive cytoprotection induced by ethanol (EtOH). AGS cells were initially treated with or without indicated concentrations of genistein for 1 hr, then with or without 3% EtOH for 1 hr, and finally with or without 8% EtOH for 1 hr. The amount of PGE_2 in the culture medium was determined by EIA (**A**). Cell viability was determined by the MTT method (**B**). Values are expressed as mean ± SEM ($n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$.

ing that a COX-2-dependent stimulation of PG synthesis is partially involved in adaptive cytoprotection in this model. As for the mechanism of the activation of COX-2, because the adaptive cytoprotection caused by the ethanol pretreatment was inhibited by an inhibitor of protein tyrosine kinase, but not of transcription or translation, we consider that the post-translational regulation of COX-2 by tyrosine phosphorylation is partially involved in the adaptive cytoprotection. COX-2 itself is one candidate as a target protein for tyrosine phosphorylation, because it has been suggested that COX-2 is phosphorylated by tyrosine kinase (10), in addition to which some tyrosine residues located close to the active site of COX were shown to be important for the maintenance of COX activity (33–35). However, because there are a number of proteins whose activities are regulated by tyrosine kinase, the target protein of tyrosine phosphorylation in the adaptive cytoprotection process is unknown at present.

As described in the introduction, several papers have been published concerning adaptive cytoprotection in gastric mucosal cells *in vitro*. None of these reports supported the idea that stimulation of PG synthesis is involved in this phenomenon. Mutoh *et al.* (25) used primary cultures of rat gastric mucosal cells to demonstrate the induction of adaptive cytoprotection by ethanol. They showed that the pretreatment of cells with low concentrations of ethanol decreased PG synthesis (25), a finding that is not consistent with our data (Fig. 3). This discrepancy may be due to the difference in cell type (primary culture and cell line). They pointed out the importance of mucin release in the adaptive cytoprotection by showing that changing the culture medium after the pretreatment diminished this phenomenon (25). Although AGS cells produce mucin (36), changing the culture medium after pretreatment with low concentrations of ethanol did not affect the extent to which adaptive cytoprotection was manifested in our model (data not shown). Kokoska *et al.* (24) reported adaptive cytoprotection induced by deoxycholate using AGS cells. They suggested that this effect was not mediated by the stimulation of PG synthesis by showing that the stimulation of PG synthesis required much higher concentrations of deoxycholate than did the adaptive cytoprotection. According to our data, the stimulation of PG synthesis was observed simultaneously with the adaptive cytoprotection phenomenon (Figs. 2 and 3). This discrepancy may be due to the difference in irritant used. It was reported that the mechanism of adaptive cytoprotection *in vivo* differs depending on the irritant involved (37). Differences in the optimal preincubation time for the adaptive cytoprotection (10–20 min for deoxycholate (24) and 2 hr for ethanol (Fig. 2) suggest that the mechanism of the adaptive cytoprotection is different between ethanol and deoxycholate. Kokoska *et al.* (27) suggested that Ca^{2+} influx is a key factor for the adaptive cytoprotection against deoxycholate; however, we found that the adaptive cytoprotection induced by ethanol was not inhibited by the Ca^{2+} chelator EGTA (data not shown), suggesting that Ca^{2+} is not a key factor in this mechanism.

Recently, much attention has been paid to the relationship between COX-2 and cancer. This is because overexpression of COX-2 has been reported in many types of cancer cells, and epidemiological studies have shown that prolonged use of aspirin or other COX inhibitors reduces the risk of cancer (38, 39). It is considered that elevated levels of PGs *in vivo* stimulate the growth of cancer, inhibit apoptosis in cancer cells, and stimulate angiogenesis around cancer cells, resulting in progression of the cancer (40). Because it is believed that COX-2 is regulated only at the level of transcription, much attention has been paid to the regulation of COX-2 transcription in cancer cells. Cancer cells appear to be challenged by various stressors. If these stressors affect cancer cells in a similar manner to that in which ethanol affects AGS cells, then these stressors may activate PG synthesis *via* a post-translational mechanism. From this point of view, to study the relationship between

cancer and COX-2, much attention should be paid to the post-translational regulation of COX-2.

In summary, we have observed here the presence of adaptive cytoprotection induced by ethanol in AGS cells and suggest that stimulation of PG synthesis by low concentrations of ethanol is partly involved in this process. Furthermore, we suggest that the post-translational regulation of COX-2 by protein tyrosine phosphorylation is partly involved in the mechanism of adaptive cytoprotection. However, because inhibition of neither COX nor protein tyrosine kinase is able to completely block ethanol-induced adaptive cytoprotection, other factors might be involved in the process. As described in the introduction, adaptive cytoprotection *in vivo* is not closely related to that *in vitro*. Therefore, at present, it is unclear whether observations in this study can be used to explain adaptive cytoprotection *in vivo*. Furthermore, although AGS cells are morphologically and functionally consistent with normal gastric mucosal cells, they are cells from a cancer cell line. Constitutive high-level expression of the COX-2 gene in AGS cells may be related to the fact that these cells are from a cancer cell line. Therefore, similar experiments to this study should be repeated on normal gastric mucosal cells (such as primary cultures of these cells) before it is concluded that the mechanism of adaptive cytoprotection proposed in this study is involved in adaptive cytoprotection in normal gastric mucosal cells.

1. Miller TA. Stress erosive gastritis: what is optimal therapy and who should undergo it? Gastroenterology **109:**626–628, 1995.
2. Tomisato W, Tsutsumi S, Rokutan K, Tsuchiya T, Mizushima T. NSAIDs induce both necrosis and apoptosis in guinea pig gastric mucosal cells in primary culture. Am J Physiol Gastrointest Liver Physiol **281:**G1092–G1100, 2001.
3. Tsutsumi S, Tomisato W, Takano T, Rokutan K, Tsuchiya T, Mizushima T. Gastric irritant-induced apoptosis in guinea pig gastric mucosal cells in primary culture. Biochim Biophys Acta **1589:**168–180, 2002.
4. Tomisato W, Tsutsumi S, Tsuchiya T, Mizushima T. Geranylgeranylacetone protects guinea pig gastric mucosal cells from gastric stressor-induced necrosis by induction of heat-shock proteins. Biol Pharm Bull **24:**887–891, 2001.
5. Vane J. Towards a better aspirin. Nature **367:**215–216, 1994.
6. Jackson LM, Wu KC, Mahida YR, Jenkins D, Hawkey CJ. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. Gut **47:**762–770, 2000.
7. Lim JW, Kim H, Kim KH. Nuclear factor-kappaB regulates cyclooxygenase-2 expression and cell proliferation in human gastric cancer cells. Lab Invest **81:**349–360, 2001.
8. Mifflin RC, Saada JI, Di Mari JF, Adegboyega PA, Valentich JD, Powell DW. Regulation of COX-2 expression in human intestinal myofibroblasts: Mechanisms of IL-1-mediated induction. Am J Physiol Cell Physiol **282:**C824–C834, 2002.
9. Pausawasdi N, Ramamoorthy S, Crofford LJ, Askari FK, Todisco A. Regulation and function of COX-2 gene expression in isolated gastric parietal cells. Am J Physiol Gastrointest Liver Physiol **282:**G1069–G1078, 2002.
10. Parfenova H, Balabanova L, Leffler CW. Posttranslational regulation of cyclooxygenase by tyrosine phosphorylation in cerebral endothelial cells. Am J Physiol **274:**C72–C81, 1998.
11. Robert A, Nezamis JE, Lancaster C, Davis JP, Field SO, Hanchar AJ. Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated by prostaglandins. Am J Physiol **245:**G113–G121, 1983.
12. Kauffman GL Jr, Reeve JJ Jr., Grossman MI. Gastric bicarbonate secretion: Effect of topical and intravenous 16,16-dimethyl prostaglandin E2. Am J Physiol **239:**G44–G48, 1980.
13. Keogh JP, Allen A, Garner A. Relationship between gastric mucus synthesis, secretion and surface gel erosion measured in amphibian stomach in vitro. Clin Exp Pharmacol Physiol **24:**844–849, 1997.
14. Hoshino T, Takano T, Tsutsumi S, Tomisato W, Tsuchiya T, Mizushima T. Effects of prostaglandin E2 on gastric irritant-induced apoptosis. Dig Dis Sci **47:**2370–2379, 2002.
15. Konturek SJ, Brzozowski T, Piastucki I, Radecki T, Dembinski A, Dembinska-Kiec A. Role of locally generated prostaglandins in adaptive gastric cytoprotection. Dig Dis Sci **27:**967–971, 1982.
16. Miller TA, Henagan JM. Indomethacin decreases resistance of gastric barrier to disruption by alcohol. Dig Dis Sci **29:**141–149, 1984.
17. Takeuchi K, Araki H, Umeda M, Komoike Y, Suzuki K. Adaptive gastric cytoprotection is mediated by prostaglandin EP1 receptors: A study using rats and knockout mice. J Pharmacol Exp Ther **297:**1160–1165, 2001.
18. Hawkey CJ, Kemp RT, Walt RP, Bhaskar NK, Davies J, Filipowicz B. Evidence that adaptive cytoprotection in rats is not mediated by prostaglandins. Gastroenterology **94:**948–954, 1988.
19. Smith GS, Myers SI, Bartula LL, Miller TA. Adaptive cytoprotection against alcohol injury in the rat stomach is not due to increased prostanoid synthesis. Prostaglandins **41:**207–223, 1991.
20. Ko JK, Cho CH. Co-regulation of mucosal nitric oxide and prostaglandin in gastric adaptive cytoprotection. Inflamm Res **48:**471–478, 1999.
21. Cho CH, Ko JK, Tang XL. The differential mechanisms of mild irritants on adaptive cytoprotection. J Gastroenterol Hepatol **9**(Suppl 1):S24–S28, 1994.
22. Nakamura K, Rokutan K, Marui N, Aoike A, Kawai K. Induction of heat shock proteins and their implication in protection against ethanol-induced damage in cultured guinea pig gastric mucosal cells. Gastroenterology **101:**161–166, 1991.
23. Tepperman BL, Soper BD, Morris GP. Effect of sialoadenectomy on adaptive cytoprotection in the rat. Gastroenterology **97:**123–129, 1989.
24. Kokoska ER, Smith GS, Rieckenberg CL, Deshpande Y, Banan A, Miller TA. Adaptive cytoprotection against deoxycholate-induced injury in human gastric cells in vitro: Is there a role for endogenous prostaglandins? Dig Dis Sci **43:**806–815, 1998.
25. Mutoh H, Ota S, Hiraishi H, Ivey KJ, Terano A, Sugimoto T. Adaptive cytoprotection in cultured rat gastric mucus-producing cells: Role of mucus and prostaglandin synthesis. Dig Dis Sci **40:**872–878, 1995.
26. Kokoska ER, Smith GS, Deshpande Y, Rieckenberg CL, Miller TA. Adaptive cytoprotection induced by ethanol in human intestinal cells: Role of prostaglandins and calcium homeostasis. Ann Surg **228:**123–130, 1998.
27. Kokoska ER, Smith GS, Wolff AB, Deshpande Y, Rieckenberg CL, Banan A, Miller TA. Role of calcium in adaptive cytoprotection and cell injury induced by deoxycholate in human gastric cells. Am J Physiol **275:**G322–G330, 1998.
28. Sharma SA, Tummuru MK, Miller GG, Blaser MJ. Interleukin-8 response of gastric epithelial cell lines to Helicobacter pylori stimulation in vitro. Infect Immun **63:**1681–1687, 1995.
29. Nilius M, Bode G, Buchler M, Malfertheiner P. Adhesion of Helicobacter pylori and Escherichia coli to human and bovine surface mucus cells in vitro. Eur J Clin Invest **24:**454–459, 1994.
30. Tsutsumi S, Tomisato W, Hoshino T, Tsuchiya T, Mizushima T. Transforming growth factor-beta1 is responsible for maturation-dependent spontaneous apoptosis of cultured gastric pit cells. Exp Biol Med **227:**402–411, 2002.
31. Futaki N, Arai I, Hamasaka Y, Takahashi S, Higuchi S, Otomo S.

Selective inhibition of NS-398 on prostanoid production in inflamed tissue in rat carrageenan-air-pouch inflammation. J Pharm Pharmacol **45:**753–755, 1993.
32. Quinn JC, Johnson-Farley NN, Yoon J, Cowen DS. Activation of extracellular-regulated kinase by 5-hydroxytryptamine(2A) receptors in PC12 cells is protein kinase C-independent and requires calmodulin and tyrosine kinases. J Pharmacol Exp Ther **303:**746–752, 2002.
33. Shimokawa T, Kulmacz RJ, DeWitt DL, Smith WL. Tyrosine 385 of prostaglandin endoperoxide synthase is required for cyclooxygenase catalysis. J Biol Chem **265:**20073–20076, 1990.
34. Guo YS, Draviam E, Townsend CM Jr., Singh P. Differential effects of Ca2+ on proliferation of stomach, colonic, and pancreatic cancer cell lines in vitro. Nutr Cancer **14:**149–157, 1990.
35. Lecomte M, Laneuville O, Ji C, DeWitt DL, Smith WL. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. J Biol Chem **269:**13207–13215, 1994.
36. Basque JR, Chenard M, Chailler P, Menard D. Gastric cancer cell lines as models to study human digestive functions. J Cell Biochem **81:**241–251, 2001.
37. Mercer DW, Cross JM, Castaneda AA, Gunter JA. Gastroprotective actions of bombesin, L-DOPA, and mild irritants: Roles of prostaglandins and sensory neurons. Surgery **124:**864–870, 1998.
38. Thun MJ. Aspirin, NSAIDs, and digestive tract cancers. Cancer Metastasis Rev **13:**269–277, 1994.
39. Baron JA, Sandler RS. Nonsteroidal anti-inflammatory drugs and cancer prevention. Annu Rev Med **51:**511–523, 2000.
40. Fosslien E. Molecular pathology of cyclooxygenase-2 in neoplasia. Ann Clin Lab Sci **30:**3–21, 2000.
41. Blanco FJ, Guitian R, Moreno J, de Toro FJ, Galdo F. Effect of antiinflammatory drugs on COX-1 and COX-2 activity in human articular chondrocytes. J Rheumatol **26:**1366–1373, 1999.
42. Kato M, Nishida S, Kitasato H, Sakata N, Kawai S. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of non-steroidal anti-inflammatory drugs: Investigation using human peripheral monocytes. J Pharm Pharmacol **53:**1679–1685, 2001.
43. Kawai S, Nishida S, Kato M, Furumaya Y, Okamoto R, Koshino T, Mizushima Y. Comparison of cyclooxygenase-1 and -2 inhibitory activities of various nonsteroidal anti-inflammatory drugs using human platelets and synovial cells. Eur J Pharmacol **347:**87–94, 1998.