The role of cyclooxygenase enzymes in the growth of human gall bladder cancer cells

Erik M.Grossman, Walter E.Longo, Ninder Panesar, John E.Mazuski and Donald L.Kaminski

Department of Surgery, Saint Louis University School of Medicine, Theodore Cooper Surgical Research Institute, Grand Boulevard at Vista Avenue, PO Box 15250, St Louis, MO 63110-0250, USA

1To whom correspondence should addressed
Email: kaminsdl@slu.edu

Information suggests that the cyclooxygenase (COX) metabolites, the prostanooids, play a role in gall bladder physiology and disease. Non-steroidal anti-inflammatory drugs which inhibit COX enzymes have been shown in vivo and in vitro to alter the growth patterns of intestinal epithelial cells, and specific COX-2 inhibitors have been shown to decrease mitogenesis in intestinal epithelial cells. The present study was intended to evaluate the effect of specific COX inhibitors on the growth patterns of gall bladder cancer cells. Employing a human gall bladder cancer cell line, mitogenesis, apoptosis and prostaglandin formation were evaluated in response to serum and hepatocyte growth factor and transforming growth factor stimulation in the presence and absence of specific COX-1 and -2 inhibitors. The effect of the mitogens on COX enzyme expression was also evaluated. Serum and the growth factors increased COX enzyme expression and mitogenesis, and decreased apoptosis as evaluated by the percentage of cells that were floating in culture media rather than attached. There was more DNA degradation in floating than in attached cells. The specific COX-2 inhibitor, but not the COX-1 inhibitor, decreased mitogenesis and increased gall bladder cell apoptosis as evaluated by the number of floating versus attached cells and the number of floating cells in the terminal phase of apoptosis or dead. The inhibition of mitogenesis and the increased apoptosis produced by the COX-2 inhibitor was associated with decreased PGE2 production. The inhibition of replication of gall bladder cancer cells and the increase in apoptosis produced by the selective COX-2 inhibitor suggests that the COX enzymes and the prostanooids may play a role in the development of gall bladder cancer and that the COX-2 inhibitors may have a therapeutic role in the prevention of gall bladder neoplasms.

Introduction

Arachidonic acid metabolites play a significant role in gall bladder physiology and disease. The prostanooids have been shown to be involved in gall bladder muscle contraction, mucosal water transport and mucous secretion (1–3). Prostanooids have been shown to mediate gall bladder inflammatory responses (4) and non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to decrease gall bladder inflammation (5). Prostanooids mediate the pain response associated with gall bladder disease (6) and NSAIDs are the drugs of choice to treat the pain associated with gall bladder disease (7). The enzymes that catalyze the formation of the precursors of the prostanooids, prostaglandin G2 (PGG2) from arachidonic acid followed by the conversion of PGG2 to prostaglandin H2 (PGH2) were identified as cyclooxygenase-1 (COX-1) and COX-2 [prostaglandin endoperoxidase synthase, PGH synthase (8)]. COX-1 is usually found to be a constitutive enzyme associated with unstimulated prostanoid formation, and COX-2 is frequently inducible and produces prostanooids in response to various inflammatory and mitogenic stimuli (9,10). We have shown in normal, freshly isolated human gall bladder mucosal cells and cells from a human gall bladder cancer cell line, that COX-2 is induced by the inflammatory agent lysophosphatidylcholine and cytokines and that prostanoid formation in stimulated cells is primarily through a COX-2 metabolic pathway (11).

Prostanooids have been of interest in cancer for many years following the demonstration of high levels of prostanooids in various tumors (12). Additionally, various prostanooids were shown to abrogate the growth of tumors in vivo and in various cancer cell lines (13–15). The possible role of the COX enzymes and prostanooids in carcinogenesis has been dramatically emphasized by the accumulating evidence indicating that NSAIDs decrease the number of colonic polyps in humans with adenomatous polyposis (16) and can reduce the incidence of colorectal cancers in experimental animals and humans (17).

Gall bladder cancer and colon cancer have several analogous characteristics. Colon cancer has an important association with inflammatory bowel disease (18) and gall bladder cancer primarily occurs in diseased gall bladders containing stones (19). Both colon cancer and gall bladder cancer appear to originate in polypoid disease in most patients (16,20). Recent information has indicated that COX enzymes are present in gall bladder cancer cells (11) and others have demonstrated increased COX enzymes in human gall bladder cancer (21).

The purpose of the present study was to evaluate the potential role of COX enzymes in the growth of a gall bladder cancer cell line. It was intended to determine factors associated with the growth of gall bladder cancer cells and to ascertain if the growth pattern was altered by specific COX-1 and -2 inhibitors.

Materials and methods

Cells from a well-differentiated gall bladder mucosal carcinoma were employed for these studies. The origination, characterization and maintenance of this cell line has been described previously (11). For the experiments freshly thawed cells were cultured in Dulbecco’s modified high glucose media supplemented with 100 000 IU/l penicillin, 100 000 IU/l streptomycin sulfate and 100 µg/l Na pyruvate (all from Sigma, St Louis, MO). The cells were cultured on collagen-coated culture dishes (Costar, Cambridge, MA) at a density of 1×10⁶ cells/well and allowed to grow to confluence (~3 days),

© Oxford University Press
The cells were morphologically homogeneous and uniformly stained positive for anticytokeratin 19 antibody, consistent with human gall bladder epithelial cells (22).

During the experiments the cells were cultured in serum-free media or media containing 10% fetal bovine serum (FBS; Sigma). Growth characteristics were also evaluated in response to varying doses of transforming growth factors (TGF) α and β, hepatocyte growth factor (HGF), epidermal growth factor (EGF) (all from Santa Cruz Biotechnology, Santa Cruz, CA; 10, 20 and 40 ng/ml) and phorbol myristate acetate (Sigma; 0.01, 0.05, 0.1 μg/ml) in the presence and absence of serum. Significant increased replication was only induced by serum, hepatocyte growth factor and TGFβ. The other mitogens were subsequently not evaluated.

Mitogenesis was initially evaluated by quantifying [3H]thymidine uptake. The cells were seeded onto 96-well collagen-coated culture plates (Costar) in 100 μl of Dulbecco’s minimum essential medium (DMEM) in 10% FBS at a density of 10,000 cells/well. After 48 h at ~70% confluence, the cells were washed with PBS (Sigma) and treated for 24 h with serum-free medium, serum-supplemented medium, serum-supplemented medium with HGF or TGFβ (20 ng/ml) or serum-supplemented medium with and without growth factors and COX inhibitors. The COX-1 inhibitor employed was 50 μM valeryl salicylic acid (VSA; Cayman, Ann Arbor, MI) (23) and the COX-2 inhibitor was 50 μM SC 58125 (1-[4-(methylsulfonyl)-phenyl]-3-trifluoromethylbenzene-1-sulfonamide; May and Baker, Basildon, UK). [3H]thymidine (ICN, Costa Mesa, CA) was added to the cells and incubated for 7 h. The cells were then lysed with 1 ml of 1 M NaOH and 1 ml of 100 μM (Sigma) and the lysates precipitated with 20% trichloroacetic acid (TCA; Sigma). The precipitates were harvested in 5% TCA onto 240-1 glass-fiber filters (Cambridge, Watertown, MA). The amount of isotope incorporated into DNA was determined by liquid scintillation counting using a Beckman LS 100 instrument.

To confirm the results of the above experiments the study was repeated employing bromodeoxyuridine (BrdU) incorporation into DNA to estimate replication (24). Using the Biotrak cell proliferation ELISA assay system (Amersham, Arlington Heights, IL) the above-described experimental protocol was repeated. BrdU (0.1 μg/ml) was added to the cells and incubated for 7 h. The culture medium was removed and the cells fixed with the DNA denatured by adding the fixative. The peroxidase-labeled BrdU antibody was added and the immune complexes detected by reading at 450 nm in a microtiter plate spectrophotometer. The absorbance values correlate directly with the amount of DNA synthesis and thereby to the number of proliferating cells in culture.

The effect of COX inhibitors on apoptosis was also evaluated. It has been demonstrated by DNA ladder and cell morphology that the majority of cells grown on culture plates floating in the medium are apoptotic (28–30). Apoptosis was determined by counting the number of floating and attached cells. Gall bladder cancer cells were plated at a density of 5×10^4 cells/well. The cells were maintained with and without serum; serum-supplemented cells were treated with and without TGFβ; and TGFβ-treated cells were treated with and without SC 58125 for 96 h. The cells were washed twice with PBS to obtain the floating cells and the attached cells were obtained with trypsin–EDTA treatment; attached and floating cell numbers were quantitated separately by hemacytometry. The ratio of the attached to floating cells multiplied by 100 represents the ‘apoptotic ratio.’

As has been described by others (31,32) we were interested in determining the cells that were in the terminal phases of apoptosis or necrotic in attached and floating cell populations in the presence and absence of a COX-2 inhibitor. Gall bladder cancer cells (1×10^5) were plated as described previously and maintained for 72 h with or without serum. Experimental protocols were also performed on serum-supplemented cells were evaluated with and without SC 58125 (2.0, 10 and 50 μM). Six wells per variable were evaluated and the percentage of attached and floating cells not excluding trypan blue was determined 100 cells/well.

In addition it was intended to evaluate apoptosis in attached and floating cells by DNA agarose gel electrophoresis. Gall bladder cancer cells were maintained in tissue culture in serum-free and serum-supplemented culture media and harvested at 0, 24, 48 and 72 h. Subsequently, gall bladder cells were maintained in serum-free and serum-supplemented media for 0 and 72 h and serum-supplemented cells were cultured for 72 h in the presence of 2, 10 and 50 μM SC 58125. Six wells per variable were employed. Attached and floating cells were harvested and DNA degradation was assessed by extracting genomic DNA from the attached and floating cells.

Cells were homogenized in digestion buffer (DNAzol; Molecular Research, Cincinnati, OH). Following centrifugation the top layer was transferred to another tube and absolute ethanol added. Following centrifugation, the precipitate was washed twice with 95% ethanol and DNA was dissolved in Tris–EDTA buffer, pH 8.0. DNA (10 μg) was pipetted onto a 1.8% agarose gel and electrophoresis was performed. Following electrophoresis the gels were stained for 1 h in Tris–borate–EDTA buffer containing SYBR Gold Nucleic Acid Gel Stain (Molecular Probes, Eugene, OR).

It was intended to ascertain if the mitogenic response produced was associated with induction of COX-1 or -2 enzyme formation. For western blotting of COX protein, 1×10^5 cells were plated onto 12-well culture plates (Costar) in 1 ml DMEM. At 70% confluence, 10% FBS, 20 ng/ml HGF or 20 ng/ml TGFβ was added to the appropriate wells and the cells incubated for 8 h. The washed cells were washed twice with PBS and the cells detached with trypsin–EDTA and collected by centrifugation. The cell lysates were prepared and separated by SDS–PAGE (11). COX enzymes were identified using antibodies and standard protein obtained from Oxford Biomedical (Clarkston, MI) (11).

The effect of the mitogenic stimuli and the COX inhibitors on COX activity was determined by quantifying prostaglandin E2 (PGE2) formation. The cells were plated at a density of 1×10^5 cells/well on collagen-coated culture dishes (Costar). Cells were treated with and without serum and serum-treated cells were then treated with HGF and TGFβ with and without the COX inhibitors for 4 h. Following incubation the cells and media were separated and the media centrifuged to remove any cells and then the media was frozen at −70°C. The cell pellets were washed with PBS solution and 1 ml collagenase solution (Sigma) was added to each well and incubated for 20 min. The cells were scraped from the wells, centrifuged at 200 g for 10 min and the pellet was stored at −70°C. Cell protein was determined by the method of Bradford (33) on specimens which were solubilized with 0.1 N NaOH for 1 h at 37°C and sonicated for 10 s. Bovine serum albumin was utilized as the standard. The media samples were thawed and aliquots used for PGE2 measurements without extraction using RIA kits obtained from New England Nuclear (Du Pont, Boston, MA) as described previously (11). Prostanoid concentrations were determined in duplicate/mg cell protein.

Statistical analysis was performed using a STATISTICAL program to perform analysis of variance. If the F-value was significant, differences between mean values were evaluated by employing Fischer’s least significant difference. As used throughout the text, ‘significant’ indicates P < 0.05.

### Results

Initially, the effect of various mitogens on cell replication was evaluated. The incorporation of [3H]thymidine was significantly increased by incubation of cells in 10% FBS (Table I). Various other mitogens were evaluated, including phorbol, EGF and TGFβ; however, only HGF and TGFβ stimulated mitogenesis in serum-supplemented cells. The COX-1 inhibitor, VSA, had no effect on serum-stimulated or serum-

### Table I. The effect of selective cyclooxygenase inhibitors on DNA synthesis as evaluated by [3H]thymidine incorporation (c.p.m.) in human gall bladder cancer cells stimulated by serum and growth factors.

<table>
<thead>
<tr>
<th>Serum</th>
<th>VSA SC 58125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum free</td>
<td>15402 ± 1809</td>
</tr>
<tr>
<td>10% FCS</td>
<td>37654 ± 2001</td>
</tr>
<tr>
<td>Serum + HGF</td>
<td>52580 ± 2285</td>
</tr>
<tr>
<td>Serum free</td>
<td>11531 ± 1842</td>
</tr>
<tr>
<td>10% FCS</td>
<td>23477 ± 2288</td>
</tr>
<tr>
<td>Serum + TGFβ</td>
<td>33148 ± 5794</td>
</tr>
</tbody>
</table>

DNA synthesis was evaluated by determining the incorporation of [3H]thymidine into TCA precipitable DNA quantified by liquid scintillation counting (c.p.m.). Cells were plated at a density of 10,000 cells/well, allowed to incorporate labeled thymidine for 24 h, exposed to the experimental variables indicated for 24 h and then harvested. Each value represents the mean ± SEM of six values obtained from duplicate measurements from six wells for each variable.

SEM, significantly different from the value obtained from the serum-starved cells.

The incorporation of [3H]thymidine was significantly different from the value obtained from the serum-starved cells.

SEM of six values obtained from duplicate measurements from six wells for each variable.

SEM of six values obtained from duplicate measurements from six wells for each variable.
Mitogenesis was determined by evaluating the incorporation of BrdU into DNA. Cells were plated at a density of 10,000 cells/well, loaded with BrdU for 24 h and exposed to the experimental variables indicated for 24 h and then harvested. Each value represents the mean ± SEM for six values obtained from duplicate measurements from six wells for each variable.

Table II. The effect of selective COX inhibitors on mitogenesis as evaluated by BrdU incorporation (absorbance × 10³) in human gall bladder cancer cells stimulated by serum and growth factors

<table>
<thead>
<tr>
<th></th>
<th>VSA</th>
<th>SC58125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum free</td>
<td>282 ± 30</td>
<td></td>
</tr>
<tr>
<td>10% FCS</td>
<td>850 ± 40²</td>
<td>940 ± 50</td>
</tr>
<tr>
<td>Serum + HGF</td>
<td>1141 ± 33²</td>
<td>1129 ± 36</td>
</tr>
<tr>
<td>Serum free</td>
<td>450 ± 24</td>
<td></td>
</tr>
<tr>
<td>10% FCS</td>
<td>987 ± 40²</td>
<td>803 ± 49</td>
</tr>
<tr>
<td>Serum + TGFα</td>
<td>1481 ± 49²</td>
<td>1193 ± 36</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of five values obtained from three separate experiments on 5 × 10⁵ gall bladder epithelial cells/well maintained in culture medium for 96 h. The number of floating cells as a proportion of the total multiplied by 100 was quantitated.

Cox-1 inhibitor: VSA did not alter the response, whereas the COX-2 inhibitor significantly decreased mitogenesis stimulated by serum and by growth factors as evaluated by BrdU incorporation (Table II).

Cell growth may also be affected by rates of apoptosis or programmed cell death. We were interested in determining if COX inhibitors altered apoptosis. We evaluated the number of apoptotic cells by counting them using an alternatively methods to evaluate mitogenesis in gall bladder cancer cells. The results presented in Table II confirm the results obtained with [³H]thymidine incorporation. Serum, HGF and TGFα stimulated BrdU incorporation. The COX-1 inhibitor VSA did not alter the response, whereas the COX-2 inhibitor significantly decreased mitogenesis stimulated by serum and by growth factors as evaluated by BrdU incorporation (Table II).

<table>
<thead>
<tr>
<th></th>
<th>VSA</th>
<th>SC58125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% FCS</td>
<td>850 ± 40²</td>
<td>940 ± 50</td>
</tr>
<tr>
<td>Serum + HGF</td>
<td>1141 ± 33²</td>
<td>1129 ± 36</td>
</tr>
<tr>
<td>Serum free</td>
<td>450 ± 24</td>
<td></td>
</tr>
<tr>
<td>10% FCS</td>
<td>987 ± 40²</td>
<td>803 ± 49</td>
</tr>
<tr>
<td>Serum + TGFα</td>
<td>1481 ± 49²</td>
<td>1193 ± 36</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of five values obtained from three separate experiments on 5 × 10⁵ gall bladder epithelial cells/well maintained in culture medium for 96 h. The number of floating cells as a proportion of the total multiplied by 100 was quantitated.

Selective COX inhibitor significantly altered the value found in the serum-supplemented cells or the cells exposed to serum and growth factors.

and growth-factor-stimulated mitogenesis. Incorporation of [³H]thymidine by gall bladder cancer cells was significantly inhibited by the COX-2 inhibitor in the presence of serum and in the cells stimulated by serum and growth factors (Table I).

Antibodies generated against the thymidine analog BrdU can produce an immunochemical method of detecting DNA synthesis (27). We employed BrdU detection by antibody techniques as an alternative method to evaluate mitogenesis in gall bladder cancer cells. The results presented in Table II confirm the results obtained with [³H]thymidine incorporation. Serum, HGF and TGFα stimulated BrdU incorporation. The COX-1 inhibitor VSA did not alter the response, whereas the COX-2 inhibitor significantly decreased mitogenesis stimulated by serum and by growth factors as evaluated by BrdU incorporation (Table II).

Addition of serum to cell culture media decreased the number of floating versus attached cells (Table III). When the cells were maintained in a serum-free environment did not exclude trypan blue, more cells maintained in a serum-free environment that were floating appeared to still be viable, as evaluated by their ability to exclude trypan blue, and presumed to be in various stages of apoptosis. This percentage was significantly decreased by the COX-2 inhibitor (Table IV).

The mitogens increased DNA replication as evaluated by [³H]thymidine incorporation and BrdU labeling. Serum and growth factors also decreased apoptosis. We intended to determine if these changes were associated with alterations in COX enzyme levels. As seen in Figure 3, COX-1 enzyme expression was detectable in cells maintained in serum-free media and there was apparent increased expression following exposure to serum or serum and growth factors. COX-2 enzyme was also present in gall bladder cancer cells maintained in serum-free and serum-supplemented media and there was apparent induction of COX-2 protein formation by serum and growth factor stimulation particularly in response to TGFα.

PGE₂ formation by serum-starved human gall bladder cancer cells was low and was significantly increased by exposure to 10% FBS (Table V). Neither growth factor significantly increased PGE₂ formation compared with values produced by serum alone. VSA, the COX-1 inhibitor, had no effect on PGE₂ levels while the COX-2 inhibitor, SC 58125, significantly decreased PGE₂ levels produced in the presence of serum and serum + growth factors.

Discussion

The results of this study suggest that gall bladder cancer cell growth can be stimulated by mitogens, and that mitogens can decrease apoptosis. Serum and mitogens stimulated COX protein induction. The mitogenic stimulus can be decreased by a specific COX-2 inhibitor, and the COX-2 inhibitor can increase apoptosis. The decreased mitogenesis and increased apoptosis produced by the COX-2 inhibitor were associated with decreased PGE₂ formation.
Fig. 1. Genomic DNA was isolated from human gall bladder cancer cells (GBC), attached and floating, with and without serum at various intervals following plating. Each line represents 10 µg DNA pooled from cells from six wells containing 1×10⁶ cells/well.

Table IV. The effect of a COX-2 inhibitor on the death of gall bladder cancer cells

<table>
<thead>
<tr>
<th></th>
<th>Attached</th>
<th>Floating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum free</td>
<td>9 ± 8</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Serum supplemented</td>
<td>2 ± 0.05</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>Serum supplemented +2.0 µM SC 58125</td>
<td>3 ± 0.05</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>+10 µM SC 58125</td>
<td>4 ± 0.05</td>
<td>11 ± 5a</td>
</tr>
<tr>
<td>+50 µM SC 58125</td>
<td>3 ± 0.06</td>
<td>14 ± 6a</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of six values obtained from six wells for each variable. The percentage of cells that were blue was determined at 72 h.

*aValue is significantly different from the control.

The results of this study follow a typical pattern of response associated with the effect of COX-2 inhibitors on cell growth in a number of epithelial cell lines. In a recent study we evaluated a murine colon cancer cell line and found that COX-1 enzyme was induced by serum exposure compared with cells maintained in a serum-free medium (34). COX-2 was not present in cells maintained in serum-free medium but was induced by exposure to serum. Despite these differences in enzyme level, when compared with gall bladder cancer cells, COX-2 enzyme inhibition, but not COX-1 inhibition, decreased mitogenesis and increased apoptosis in the colon cancer cell line, comparable with the results reported in the present study.

Eberhart et al. (35) and others (36) demonstrated that COX-2 enzyme is over-expressed in human colorectal tumors compared with adjacent normal colonic mucosa. Employing a specific COX-2 inhibitor, NS398, Tsuji et al. (37,38) demonstrated that proliferation of a gastric cancer cell line and a colon cancer cell line could be inhibited by the COX-2 enzyme inhibitor. Others have subsequently demonstrated that, in a variety of epithelial cell lines, specific COX-2 inhibitors decrease mitogenesis (39,40).

A significant amount of evidence derived from clinical epidemiologic studies and in vitro studies employing non-specific NSAIDs suggested that non-specific COX inhibitors may prevent colon cancer (41). Both in vitro and in vivo studies evaluating the effect of non-specific NSAIDs on colon cancer suggested that an important effect of NSAIDs on colon tumor cells was the induction of apoptosis (42). In colon
cancer cell lines specific COX-2 inhibitors have also been shown to induce apoptosis (30,43). The above information has led to the speculation that the effect of NSAIDs on chemoprevention of epithelial cancers may be due to the COX-2 activity of these agents, and that specific COX-2 inhibitors may have a role in preventing and treating cancers originating in intestinal epithelium (17,44,45). The results of the present study suggest that these same relationships between COX-2 inhibition and suppression of gall bladder cancer growth in vitro exist and that specific COX-2 inhibitors may have a therapeutic role in preventing or treating gall bladder cancer. Based on information in the present study, COX-1 inhibitors do not alter mitogenesis in gall bladder cancer cells stimulated by serum or growth factors.

The mechanism by which COX-2 enzyme inhibition alters mitogenesis in gall bladder cancer cells is unknown. It would seem most likely that the response is due to decreased prostanoid formation and in the present study decreased PGE₂ concentrations correlated with decreased mitogenesis and increased apoptosis.

There was, however, no evident increase in PGE₂ production associated with stimulation of mitogenesis produced by serum or growth factors. We know of no other information related to the effects of prostanoids on the growth patterns of gall bladder mucosal cells and the effect of various prostanoids on mitogenesis and apoptosis in gall bladder cancer will require further study. In other types of intestinal epithelial cells the information is confusing. Previous studies demonstrated that PGE₂ stimulated the growth of cells derived from human colon cancer (13) which would correlate well with the inhibitory response of gall bladder cancer cell mitogenesis produced by the COX-2 inhibitor. Other studies failed to demonstrate an effect of PGE₂ on mitogenesis in colon cancer cell lines (46). It is possible that prostanoid species other than PGE₂ are involved in producing the changes in mitogenesis (13,14). The gall bladder cancer cell line used in this study is known to also produce prostacyclin; however, this prostanoid is produced in relatively small amounts compared with PGE₂ (11).

Since the original work of Santoro et al. (47), the prostanoids have been recognized to have a general inhibitory effect on the growth of tumor cells which has been subsequently demonstrated in numerous studies employing various cell lines (48). PGE₂ and prostaglandin D₂ (PGD₂) decreased mitogenesis in gastric cancer cells (49), and PGD₂ decreased mitogenesis in colon cancer cells (50). There does not appear to be a common universal response in all cancer cells and the application of prostanoids or COX-2 inhibitors would have the potential of promoting one tumor and treating another. In addition, the problem is complicated by the multiple species of eicosanoids and prostanoids, their local interactions and the interaction of eicosanoids and prostanoids with other signaling systems.

The results of these studies replicate other studies that demonstrated that a COX-2 inhibitor increased the number of floating cancer cells with floating cells manifesting increased apoptosis (31). That study along with another study (32) demonstrated a similar response produced by sodium salicylate. As sodium salicylate had little effect on PGE₂ formation compared with the specific COX-2 inhibitor, Santini et al. (31) concluded that COX-2 inhibition may not affect apoptosis by altering prostanoid metabolism. We found significant correlation between the effect of the COX-2 inhibitor on PGE₂ levels and its effect on apoptosis. It is possible that other prostanoids not measured could be mediating the response produced by Santini et al. (31).

The absence of a universal correlation between inhibition of proliferation and decreased prostanoid formation produced by COX-2 inhibitors and the inhibition of proliferation of cancer cells by PGE₂ has generated concern that the alterations in mitogenesis and apoptosis produced by COX-2 inhibition are not due to prostanoid changes (51). It has been postulated that COX-2 may independently be a tumor suppressor gene at 1q23-pter (51). The information presented in this study suggests that the COX-2 enzyme may play a role in the development of gall bladder cancer. Further studies will be required to
References
Inhibition of gall bladder cancer cell growth


Received December 28, 1999; revised March 27, 2000; accepted March 29, 2000