Clinicopathologic Association of Cyclooxygenase 1 and Cyclooxygenase 2 Expression in Gastric Adenocarcinoma

Chiung-Nien Chen, MD,* Chia-Tung Sung, MD,† Ming-Tsan Lin, MD, PhD,* Po-Huang Lee, MD, PhD,* and King-Jen Chang, MD, PhD*

From the Departments of *Surgery and †Forensic Medicine, National Taiwan University Hospital, Taipei, Taiwan

Objective

To evaluate the association of clinicopathologic factors and prognostic value with the expression of cyclooxygenase 1 and 2 in patients with gastric adenocarcinoma.

Summary Background Data

Epidemiologic studies have indicated that nonsteroidal antiinflammatory drugs reduce the risk of colon cancer by as much as 40% and also decrease the risk of gastric cancer. Recently, gastric cancer was found to express constitutive cyclooxygenase 1 and inducible cyclooxygenase 2 isoenzymes. Nonsteroidal antiinflammatories, which may function as cyclooxygenase inhibitors, inhibited the growth of gastric cancer cells. These two isoenzymes' expressions associated with traditional clinicopathologic factors have not been fully evaluated, and their prognostic value for determining survival in patients remains to be clarified.

Methods

Seventy-one specimens resected from patients with gastric adenocarcinoma were investigated by immunohistochemical stain against cyclooxygenase 1 and 2. The 71 specimens were divided into stain-positive and stain-negative groups. Correlations between cyclooxygenase 1 and 2 expression, various clinicopathologic factors *including vascular invasion and *Helicobacter pylori* infection), and prognosis were studied.

Results

The cyclooxygenase 2-positive group was significantly correlated with vascular invasion and *H. pylori* infection by univariate and multivariate analysis. In patients with cyclooxygenase 2-positive cancer, the prognosis was significantly poorer than in those with cyclooxygenase 2-negative cancer. However, multivariate analysis showed that vascular invasion, serosal invasion, and lymph node metastasis were independent prognostic factors for patients with gastric cancer, but cyclooxygenase 2 expression was not. There was no significant correlation between cyclooxygenase 1 expression and clinicopathologic factors and prognosis.

Conclusions

Upregulated cyclooxygenase 2 expression was associated with *H. pylori* infection in gastric cancer and was also strongly correlated with positive vascular invasion, which was an independent prognostic factor for poorer survival in this study. The usefulness of cyclooxygenase 2 inhibitors in the prevention or treatment of gastric cancer remains undetermined but deserves further investigation.

Gastric cancer is a major cause of death throughout the world[1] and is the fourth most common malignancy in Taiwanese men and the sixth in women.[2] Gastric cancer recurred in 39% of patients who had undergone curative resection,[3] and many chemotherapeutic drugs have failed to provide complete reduction of gastric cancer in the patients.[4]

Recent epidemiologic studies have shown that chronic intake of nonsteroidal antiinflammatory drugs (NSAIDs), which function as cyclooxygenase inhibitors, reduced the incidence of colon cancer and polyps by as much as 40% to 50%.[5,6] In a large prospective study, the use of aspirin was also associated with a reduced risk of gastric and esophageal cancer.[7,8]

The best-known target of NSAIDs is cyclooxygenase, the

key enzyme in the conversion of arachidonic acid to prostanoids. There are two isoforms, cyclooxygenase 1 and 2.[9,10] Cyclooxygenase 1 is considered to be a housekeeping gene and is thought to be related to the cytoprotection of gastric mucosa. Cyclooxygenase 2 is an inducible intermediate-early gene, and its roles have been connected to inflammation and carcinogenesis.[11] A recent study showed that expression of cyclooxygenase 1 and 2 was detected in human gastric cancer.[12] Approximately 70% of gastric cancer specimens expressed cyclooxygenase 2 protein in the study by Murata et al.[13] Therefore, it is interesting to correlate clinicopathologic factors and prognosis of gastric cancer with cyclooxygenase 1 and 2 protein expression, and this may be helpful in the prevention and therapy of gastric cancer.

METHODS

Resected specimens from 71 patients with gastric cancer who underwent curative gastrectomy at our institution from 1995 to 1996 were studied. The patients comprised 48 men and 23 women (age range 35–81 years, average age 62.3). Thirty-eight specimens were obtained from the antrum, 18 from the body, and 14 from the cardia. Normal (nontumor) specimens were collected from accompanying stomach tissues, which were apart from gastric cancer in each case. Paraffin sections of gastric cancer were submitted for cyclooxygenase 1 and 2 immunohistochemical staining.

Immunohistochemical Staining

Tissue immunohistochemical staining was performed with monoclonal anticyclooxygenase 2 antibody and polyclonal anticyclooxygenase 1 antibody (Cayman Chemical Co., Ann Arbor, MI) and the avidin–biotin peroxidase complex kit (Zymed, San Francisco, CA). Resected gastric tissues were fixed in 10% buffered formalin. Specimens were embedded in paraffin, serially sectioned onto microscope slides at a thickness of 4 to 5 μm, and then deparaffinized. The slides were immersed for 45 minutes in 0.3% peroxide in methanol to deplete the activity of endogenous peroxidase. Nonspecific binding sites were saturated with 0.3% bovine serum albumin. Normal goat serum was diluted to 1:66.7 in phosphate-buffered saline for 20 minutes. Primary antibodies against cyclooxygenase 1, cyclooxygenase 2, or control normal rabbit serum (Vector) were used at a dilution of 1:40 and applied to tissue sections and incubated in a humidified chamber at room temperature for 30 minutes. The sections were then washed with phosphate-buffered saline for 10 minutes, and biotinylated goat anti-rabbit IgG (Vector, Burlingame, CA) was applied to the tissue sections and incubated at room temperature for 30 minutes. After being washed with phosphate-buffered saline for 10 minutes, these tissues were incubated with avidin DH-biotinylated peroxidase (Vector) for 45 minutes. The color was developed by immersing sections in freshly pre-pared diamino-benzidine for 2 to 5 minutes. The sections were counterstained with 0.5% light green (Sigma, St. Louis, MO). The normal gastric mucosa was used as a positive control, and the primary antibody replaced by the normal serum was used as a negative control. For the results, negative immunoreactivity implied that staining was absent throughout the specimen. Positive immunoreactivity of cancer cells implied that brownish granular staining was present primarily in cytoplasm and the perinuclear area of the cell, and the background staining was light green. Specificity was determined by preadsorption of anticyclooxygenase 1 or 2 with the cyclooxygenase 1 or 2 synthetic polypeptide (1 mg/mL) before staining.

Clinicopathologic factors including age, sex, gross types of tumors (Borrmann classification), histologic types of tumors (Lauren classification), depth of tumor invasion, status of lymph node metastasis, vascular invasion, and *Helicobacter pylori* infection documented with histologic findings were reviewed in the database. Vascular invasion was considered to be definite only when tumor cells and red blood cells were noted together in an endothelium-lined vascular space or when tumor cells were found in an endothelium-lined vascular space with a definite smooth muscle layer. The tissue was considered positive for *H. pylori* if faintly blue-staining, curved bacilli were seen in the mucus of crypts just adjacent to the tumor using hematoxylin and eosin staining. The follow-up period ranged from 3 to 40 months after surgery.

Statistics

The Fisher exact probability test and the chi-square test were used for the statistical analysis that related the expression of cyclooxygenase 1 and 2 proteins and the traditional clinicopathologic factors. A logistic regression model was used to test the correlation between cyclooxygenase 2 protein expression and clinicopathologic factors by multivariate analysis, and was tested by the Wald chi-square test. Survival rates were calculated using the Kaplan-Meier method and analyzed by the log-rank test. The influence of each variable on survival was assessed by the Cox proportional hazard regression model. $P < .05$ was considered significant.

RESULTS

Immunostaining appeared to be localized in the cytosolic and perinuclear regions in cyclooxygenase-positive cancer cells, consistent with endoplasmic reticulum localization of cyclooxygenase enzymes. In general, cyclooxygenase 1 was expressed in normal gastric epithelial cells and cyclooxygenase 2 was not (Fig. 1). Cyclooxygenase 1 and 2 protein was positively stained in 61% (43/71) and 68% (48/71) of the gastric cancer specimens, respectively.

Correlations between the expression of cyclooxygenase 1 and 2 protein and the clinicopathologic factors are shown in
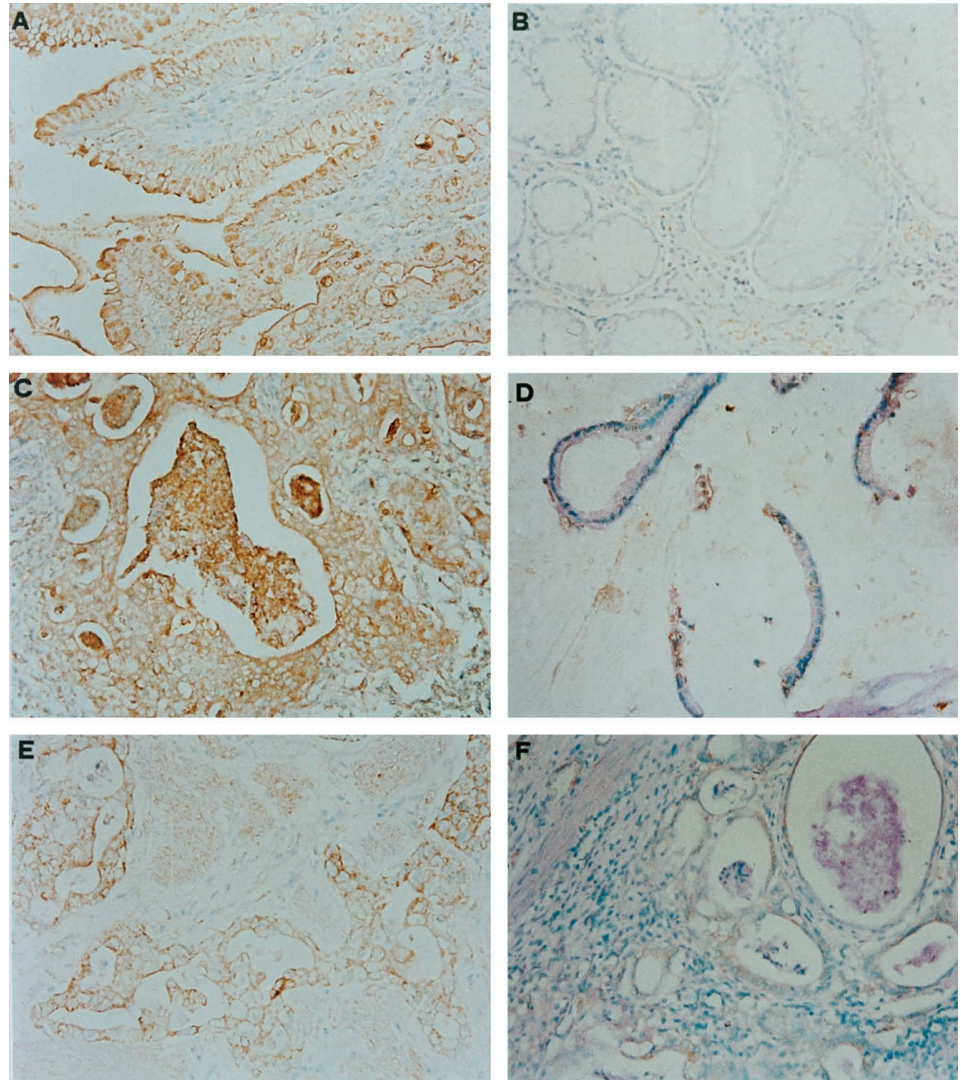
Figure 1. In normal gastric epithelial cells, immunostaining was positive for cyclooxygenase 1 (A) and negative for cyclooxygenase 2 (B). Representative gastric cancer tissues were positively (C) or negatively (D) immunostained with anticyclooxygenase 1 antibodies. Gastric cancer tissues were positively (E) or negatively (F) immunostained with anticyclooxygenase 2 antibodies.

Tables 1 and 2. There was no correlation between cyclooxygenase 1 expression and clinicopathologic factors. A significant correlation was found between cyclooxygenase 2 protein expression and cancer vascular invasion ($P = .028$) and *H. pylori* infection ($P = .011$) by univariate analysis. Multivariate analysis also showed a significant correlation between cyclooxygenase 2 protein expression and vascular invasion ($P = .0296$) and *H. pylori* infection ($P = .0277$) (Table 3).

Univariate analysis showed that five variables were significant adverse prognostic factors for survival after curative resection of gastric cancer: serosal invasion, lymph node metastasis, vascular invasion, *H. pylori* infection, and cyclooxygenase 2 protein expression. Cyclooxygenase 1 protein expression was found to have no prognostic value (Table 4). Multivariate analysis showed that serosal invasion, lymph node metastasis, and vascular invasion were independent prognostic factors. Cyclooxygenase 2 protein expression and *H. pylori* infection were not independent prognostic factors (Table 5).

DISCUSSION

Our results showed that cyclooxygenase 1 protein expression was not correlated with traditional clinicopathologic factors and prognostic value in gastric cancer. Cyclooxygenase 2 protein expression was not associated with histologic differentiation (intestinal type or diffuse type of gastric cancer), gross morphology (Borrmann classification), depth of tumor invasion, or lymph node metastasis. However, it was significantly correlated with vascular invasion of cancer and *H. pylori* infection. On univariate analysis, the prognosis of patients with cyclooxygenase 2-positive cancer was poorer than that of those with cyclooxygenase 2-negative cancer, but it was not an independent prognostic indicator. In addition to invasion depth and lymph node metastasis, vascular invasion of cancer was an independent prognostic factor.

Vascular invasion has been reported as an independent prognostic factor in gastric cancer.[14–19] Cyclooxygenase 2 expression of gastric cancer was significantly correlated

Table 1. CYCLOOXYGENASE 1 EXPRESSION AND CLINICOPATHOLOGIC FACTORS: UNIVARIATE ANALYSIS

	Negative	Positive	P Value
Age	60.93 ± 11.00	63.19 ± 11.66	.418
Sex			.767
Men	20	28	
Women	8	15	
Depth of invasion (T)			.538
T1	4	2	
T2	4	8	
T3	18	29	
T4	2	4	
Nodal status (N)			.292
Node negative	9	9	
Node positive	19	34	
Borrmann type			.463
I	0	1	
II	5	5	
III	21	31	
IV	2	6	
Lauren classification			.226
Intestinal type	10	24	
Diffuse type	12	14	
Mixed type	6	5	
Vascular invasion			.207
Negative	12	11	
Positive	16	32	
Helicobacter pylori infection			.09
Negative	18	18	
Positive	10	25	

Table 2. CYCLOOXYGENASE 2 EXPRESSION AND CLINICOPATHOLOGIC FACTORS: UNIVARIATE ANALYSIS

	Negative	Positive	P Value
Age	62.39 ± 9.10	62.25 ± 12.42	.957
Sex			.279
Men	18	30	
Women	5	18	
Depth of invasion (T)			.365
T1	3	3	
T2	5	7	
T3	12	35	
T4	3	3	
Nodal status (N)			.095
Node negative	7	11	
Node positive	16	37	
Borrmann type			.804
I	0	1	
II	3	7	
III	17	35	
IV	3	3	
Lauren classification			.291
Intestinal type	8	26	
Diffuse type	11	15	
Mixed type	4	7	
Vascular invasion			.028
Negative	12	11	
Positive	11	37	
Helicobacter pylori infection			.011
Negative	17	19	
Positive	6	29	

with vascular invasion in this study. Cancer cells with cyclooxygenase 2 overexpression had metastatic potential in vitro because the enzyme enhanced metalloproteinase 2 activity, which could break down the basement membrane, resulting in vascular invasion and metastasis.[20,21] However, cyclooxygenase 2 expression of cancer has been associated with angiogenesis,[22,23] making the vascular invasion step easier to accomplish.[24] These factors may explain why elevated cyclooxygenase 2 expression in gastric cancer was associated with vascular invasion in this study. However, elevated cyclooxygenase 2 expression was not an independent prognostic factor, possibly reflecting the clinical and molecular complexities in gastric cancer; elevated cyclooxygenase 2 expression in gastric cancer may be associated with metastasis and progression but not with survival. A recent study showed that cyclooxygenase 2 could regulate angiogenesis by modulating the production of angiogenic factors in colon cancer cells, a process that could be inhibited by NSAIDs.[25] The effect of NSAIDs on gastric cancer after curative resection remains unknown and needs further investigation with solid clinical trials.

Infection with *H. pylori* is recognized as the primary cause of peptic ulcers and their recurrence. Compelling evidence has also been found linking *H. pylori* infection to gastric cancer and its premalignant lesion.[26–29] The possible mechanisms of *H. pylori*-induced carcinogenesis may be chronic epithelial injury and inflammation caused by the ammonia that is produced by the bacteria in the gastric microenvironment.[30,31] *H. pylori* induces inflammatory cells to produce reactive oxygen metabolites that may damage DNA and promote carcinogenesis,[32,33] and it also increases the proliferative activity of epithelial cells.[34] We found that cyclooxygenase 2 expression of gastric cancer

Table 3. CYCLOOXYGENASE 2 EXPRESSION AND CLINICOPATHOLOGIC FACTORS: MULTIVARIATE ANALYSIS

	B	SE	Sig	Exp(B)
Sex	−0.6798	0.7425	0.3599	0.5067
Age	0.0377	0.0329	0.2509	1.0384
Borrmann type	−0.4780	0.4583	0.2669	0.6200
Lauren classification	−0.6225	0.5192	0.2305	0.5366
Depth of invasion	−0.3652	0.5115	0.4752	0.6940
Nodal status	−0.3974	0.37362	0.2875	0.6721
Helicobacter pylori infection	1.7286	0.7853	0.0277	5.6329
Vascular invasion	1.6870	0.7754	0.0296	5.4033

B, β regression coefficient; SE, standard error; Sig, significance; Exp(B), exponent β.

Table 4. CLINICOPATHOLOGIC FACTORS AFFECTING SURVIVAL RATE: UNIVARIATE ANALYSIS

Variable	No. of Patients	Median Survival (months)	3-Year Survival (%)	P Value
Serosal invasion				
Negative	18	23	64.2	.0037
Positive	48	13	29.4	
Lymph node metastasis				
Negative	19	22	79.3	<.0001
Positive	47	13	23.9	
Vascular invasion				
Negative	23	23	92.9	<.0001
Positive	43	10	132	
Helicobacter pylori infection				
Negative	35	22	34.5	.0132
Positive	31	9	24.2	
Cyclooxygenase 1 protein expression				
Negative	27	12	56.9	.3043
Positive	39	15	32.1	
Cyclooxygenase 2 protein expression				
Negative	21	22	74.4	.0016
Positive	45	13	24.3	

was significantly correlated with *H. pylori* infection; this result was consistent with a recent in vitro study showing that *H. pylori* upregulated cyclooxygenase 2 mRNA expression and prostaglandin E2 synthesis in a gastric cancer cell line.[35] Therefore, *H. pylori*-induced gastric carcinogenesis may be associated with the elevated expression of cyclooxygenase 2 in gastric epithelium.

Whether the eradication of *H. pylori* can prevent gastric cancer remains contradictory. Some reports have described regression of preneoplastic changes, such as atrophy or reversal of intestinal metaplasia, with *H. pylori* eradication.[36–39] Uemura et al[40] reported that eradication of *H. pylori* was effective in secondary prevention of tumor recurrence in patients with early gastric cancer who underwent endoscopic resection. In contrast, other investigators found that *H. pylori* eradication had no effect or an effect that was not sustained.[41–44] Cyclooxygenase 2 overexpression leads to phenotypic changes in intestinal epithelial cells that could enhance their tumorigenic potential.[45] However, cyclooxygenase 2 expression remained and was not eliminated in gastric epithelium after successful eradication of *H. pylori*.[46] This may suggest that combined treatment of cyclooxygenase 2 inhibitors and *H. pylori* infection may be more useful in eliminating *H. pylori*-induced cyclooxygenase 2 expression than either anti-*H. pylori* antibiotics or cyclooxygenase 2 inhibitors alone. This issue may deserve further study.

In conclusion, upregulated cyclooxygenase 2 expression was associated with *H. pylori* infection in gastric cancer and was also strongly correlated with positive vascular invasion, which was an independent prognostic factor for poorer survival in this study. Cyclooxygenase 2 may represent a possible therapeutic target in patients with gastric cancer, but the utility of cyclooxygenase 2 inhibitors in the prevention or treatment of gastric cancer remains undetermined and deserves further investigation.

Table 5. CLINICOPATHOLOGIC FACTORS AFFECTING SURVIVAL RATE: MULTIVARIATE ANALYSIS

Variable	B	SE	Sig	Exp(B)
Serosal invasion: negative vs. positive	0.884	0.420	0.0350	2.42
Lymph node metastasis: negative vs. positive	0.623	0.214	0.0035	1.87
Vascular invasion: negative vs. positive	2.648	1.043	0.0110	14.12
Helicobacter pylori infection: negative vs. positive	0.327	0.391	0.4000	1.39
Cyclooxygenase 1 protein expression: negative vs. positive	−0.210	0.447	0.6400	0.81
Cyclooxygenase 2 protein expression: negative vs. positive	1.003	0.620	0.1100	2.73

B, *β* regression coefficient; SE, standard error; Sig, significance; Exp(B), exponent *β*.

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