Gastric cancer is the second leading cause of cancer related death worldwide. Notwithstanding the global declining incidence of gastric cancer, mortality is still rising in Asian countries. To date, there is no effective measure to prevent development of gastric cancer. Although Helicobacter pylori infection has been identified as the most important causative factor, there is little evidence to substantiate the fact that eradication of the bacterium alone can stop the process of gastric carcinogenesis.

Since the observation from the Physician’s Health Study that usage of aspirin may reduce the risk of colorectal cancer, intense interest has been directed towards investigation of the anticancer properties of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs). There are at least 12 published observational studies showing the protective effects of NSAIDs against colorectal cancer. More recently, studies in colon cancer show that induction of cyclooxygenase 2 (COX-2) is associated with inhibition of prostaglandin E2 level was found in the indomethacin treated group, suggesting that the chemopreventive effect of celecoxib may be mediated by a COX independent pathway.

COX-2 expression is upregulated in H pylori induced mucosal inflammation. It is frequently expressed in gastric cancer as well as in premalignant gastric lesions. Inhibition of COX-2 in vitro results in growth inhibition of gastric cancer cells. Furthermore, the use of COX-2 inhibitors has been shown to suppress the growth of gastric cancer xenografts in nude mice. Unlike colorectal cancers, however, there are a lack of animal and human data demonstrating the effectiveness of COX-2 inhibition and NSAIDs in the prevention of gastric cancer.

In this study, we evaluated the use of celecoxib and indomethacin in the prevention of N-methyl-N’-nitro-N’-nitrosoguanidine (MNNG) induced gastric cancer in rats.
Histopathology
For histological examination, the stomach was fixed in 10% neutral buffered formalin. Paraffin embedded sections (5 μm) were cut and stained with haematoxylin and eosin for histological examination by a pathologist who was unaware of the treatment assignments. Adenocarcinoma, as defined by the presence of atypical glands that locally invaded the submucosa, muscularis propria, or serosa, was recorded.19

RNA extraction and quantitative PCR
Gastric tissue specimens were homogenised with an ultrasonic homogeniser. Total RNA was extracted by RNA Tri reagents (CINNA/MRC; Cincinnati, Ohio, USA). Total RNA (1 μg) was reverse transcribed into cDNA using dNTPs (200 μM), 5× reverse transcription buffer (500 mM Tris HCl, pH 8.3, 250 mM KCl, 50 mM MgCl₂, and 50 mM DTT), 16 units RNasin, and 2.5 units of AMV reverse transcriptase (GibcoBRL, Life Technologies Gaithersburg, Maryland, USA).

Real time quantitative polymerase chain reaction (PCR) was performed on a ABI PRISM 7000 sequence detection system using Sybrgreen, PCR mastermix (Perkin Elmer, Branchburgh, New Jersey), and primers. Primer sequences were designed from the Genbank as follows: COX-2 (L25925), (forward, 1408–1435) 5′-ACAGGAGAGAAAGAATGTGC-3′; (reverse, 1598–1573) 5′-CAGATTTGAGGAGAACAGATGGATT-3′; and β-actin (NM-031144), (forward, 476–500), 5′-TCACCCACACTGTGCCCATCTATGA-3′; (reverse, 633–610) 5′-GTACCGAGCAATTTCCCTCAGC-3′. A 24 μl reaction mix was aliquotted with 1 μl replicates of cDNA. A DNase free template control (containing water) was included and each sample was added in duplicate. Reaction tubes were sealed with optical caps, and the PCR reaction was run at 50°C for two minutes, 95°C for 10 minutes, followed by 40 cycles at 96°C for 45 seconds, 60°C for 45 seconds, and 72°C for one minute. The specificity of the PCR products was characterised by melting curve analysis and followed by gel electrophoresis. Quantification was determined by the threshold cycle. Actin was used as a housekeeping gene to normalise mRNA levels and compared against mRNA expression levels in normal control stomach.

PGE₂ assay
Prostaglandin E₂ (PGE₂) levels were measured in snap frozen tissue specimens using an ELISA based assay (Amersham Pharmacia Biotech), and the column was washed with distilled water and hexane. PGE₂ was eluted with two 0.75 ml volumes of ethyl acetate. This fraction was evaporated to dryness under nitrogen and stored at -80°C. Samples were resuspended in 1 ml of buffer and assayed in 96 well plates. The quantity of PGE₂ in supernatants was determined using ELISA.

Statistics
Body weight, tumour incidence (percentage of animals with tumour development), tumour multiplicity (mean number of tumours per animal), mean tumour volume (mean volume of tumour in tumour bearing rats), COX-2, and PGE₂ levels were compared among animals fed MNNG control alone, indomethacin, and celecoxib. Parametric data were analysed by ANOVA with Bonferroni’s multiple comparison; non-parametric data were computed by χ² test or Fisher’s exact test with Bonferroni’s correction. A p value of <0.05 was considered to be statistically significant.

RESULTS
General observation
Body weights of group A control animals were higher than those of the other groups in the early phase of the study (fig 1). However, there was no significant difference in body weight among other treatment groups during the whole study period. There were in total 26 deaths during the study period: none in group A, six in group B, six in group C, five in group D, six in group E, and three in group F. The causes of death are listed in table 1. Most animals died from gastric (n = 14) and small bowel (n = 8) cancer. Two animals died from intestinal haemorrhage after receiving the high dose (20 mg/kg/day) of celecoxib. Two animals died from non-digestive tract diseases.

Tumour incidence
Table 2 summarises the incidences of MNNG induced gastric tumours in the six treatment groups. Seventy five per cent of rats treated with MNNG developed gastric cancer at the end of this study whereas none of the control rats in group A had a gastric tumour. There was a significant difference in tumour incidences among different treatment groups (p = 0.002). Rats treated with celecoxib 10 mg/kg/day (group E) had the lowest tumour incidence (18.8%) which was significantly lower than the MNNG group (75.0%; p = 0.004). The tumour incidence of group F rats (celecoxib 20 mg/kg/day) also tended to be lower than the other treated groups (group B) (31.3% v 75%; p = 0.052). The absolute risk reductions of gastric cancer in animals treated with celecoxib 10 mg/kg/day and 20 mg/kg/day were 56.3% (95% confidence interval (CI) 16.7–80.4%) and 43.8% (95% CI 3.9–71.8%), respectively. On the other hand, the numbers of gastric tumours were comparable between the other treatment groups (indomethacin or celecoxib at 5 mg/kg/day) and the MNNG control group. All gastric tumours were confirmed to be adenocarcinomas (fig 2) and the majority (90.7%) were high grade tumours.

Moreover, premalignant gastric lesions such as dysplasia were frequently detected in MNNG treated rats. Of the 10 remaining viable rats in group B, nine had dysplasia on histological examination of the gastric mucosa. In contrast, only four of the 10 remaining rats in group E (celecoxib 10 mg/kg/day) had gastric dysplasia (p = 0.23). The frequencies of gastric dysplasia in rats treated with indomethacin and other doses of celecoxib were comparable with group B.

Tumour multiplicity
In addition to tumour incidence, there was a significant difference in tumour multiplicity, or number of cancers per rat, among the different treatment groups (p = 0.001, table 2).
Celecoxib prevents gastric cancer in rats

Figure 1 Body weight of animals in the different treatment groups. The body weight of group A control rats was higher than in the N'-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treated groups in the initial phase of the experiments. However, there was no significant difference in body weight among all MNNG treated groups (groups B–F).

Compared with the MNNG group, rats fed celecoxib 10 mg/kg/day or 20 mg/kg/day had significantly lower body weight among all MNNG treated groups (groups B–F). However, there was no significant difference in body weight of group A control rats was higher than in the MNNG control group (group B). Mean tumour volume was significantly different among the different treatment groups.

**Tumour volume**

Mean tumour volume was significantly different among the treatment groups (p = 0.009). Specifically, rats treated with celecoxib had a markedly reduced tumour volume compared with the MNNG control group (group B). Mean tumour volumes were significantly lower in animals treated with celecoxib 5 mg/kg/day (group D) (188.5 (377.8) mm³; p = 0.004) and 0.3 (0.5) v 1.0 (0.7), p = 0.025) compared with those treated with MNNG alone (0.2 (0.4) mm³, p = 0.022) and 20 mg/kg/day (group F) (38.9 (110.5) mm³; p = 0.025) compared with those treated with MNNG alone (0.2 (0.4) mm³, p = 0.022) and 20 mg/kg/day (group F) (38.9 (110.5) mm³; p = 0.025) compared with those treated with MNNG alone (0.2 (0.4) mm³, p = 0.022) and 20 mg/kg/day (group F) (38.9 (110.5) mm³; p = 0.025). However, treatment with indomethacin (3 mg/kg/day) or high doses of celecoxib (>10 mg/kg/day) were associated with mildly reduced tumour PGE2 levels, but the difference did not reach statistical significance. Moreover, there was no significant difference in PGE2 levels of normal tissues among the different treatment groups.

**DISCUSSION**

In this study, we determined the role of COX-2 inhibition in the prevention of sodium chloride enhanced gastric carcinogenesis induced by MNNG in Wistar rats. MNNG induced gastric cancer is a well established animal model of stomach carcinogenesis.22 The mutagen, when given in drinking water, induces intestinal metaplasia and adenocarcinoma in the pyloric mucosa of Wistar rats.16 20 The histology of this induced gastric malignancy resembles the differentiated type of stomach cancer in humans. To enhance the carcinogenic effects of MNNG, highly concentrated sodium chloride solution was given to these animals in the initial six weeks.17 In the present study, 75% of MNNG treated animals developed gastric cancer at the end of 48 weeks, confirming that this is a highly successful model of gastric tumorigenesis.

Although the exact mechanism underlying MNNG induced gastric cancer remains poorly understood, previous studies showed that the genetic makeup of the animals may play a role.23 For example, ACI/N rats are highly susceptible to MNNG induced stomach carcinogenesis but BUF/Nac rats are relatively resistant.22 Recently, COX-2 and Bcl-2 were found to be coexpressed in the glandular corpus epithelium of rats treated with MNNG.23 This upregulated expression is associated with cell proliferation, atrophy, and intestinal metaplasia of the stomach. It is therefore logical to anticipate that treatment with a COX-2 inhibitor may have an antiproliferative and hence chemopreventive effect on MNNG induced gastric cancer.

The results of this study showed, for the first time, that both the incidence and multiplicity of MNNG induced gastric cancer can be significantly reduced in rats treated with celecoxib. The chemopreventive effect of celecoxib was demonstrated when a moderate dose (10 mg/kg/day) was given to these animals. With the use of celecoxib 10 mg/kg/ day, there was an approximate 56% reduction in tumour incidence, 80% reduction in tumour multiplicity, and 1169-fold reduction in tumour volume. This remarkable degree of tumour suppression by celecoxib is comparable with that reported in the azoxymethane induced colon cancer model in rats.24 Moreover, it exceeds that previously reported in MNNG induced gastric cancer by other agents, such as

<table>
<thead>
<tr>
<th>Group</th>
<th>No of rats</th>
<th>Treatment</th>
<th>Causes of death (No of animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>Control</td>
<td>Gastric cancer (6)</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>MNNG alone</td>
<td>Gastric cancer (2), small bowel cancer (3), unknown (1)</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>MNNG+indomethacin (3 mg/kg/day)</td>
<td>Gastric cancer (5)</td>
</tr>
<tr>
<td>D</td>
<td>17</td>
<td>MNNG+celecoxib (5 mg/kg/day)</td>
<td>Small bowel cancer (5), lung cancer (1)</td>
</tr>
<tr>
<td>E</td>
<td>16</td>
<td>MNNG+celecoxib (10 mg/kg/day)</td>
<td>Gastric cancer (1), intestinal haemorrhage (2)</td>
</tr>
<tr>
<td>F</td>
<td>16</td>
<td>MNNG+celecoxib (20 mg/kg/day)</td>
<td>Gastric cancer (1)</td>
</tr>
</tbody>
</table>

MNNG, N-methyl-N'-nitro-N-nitrosoguanidine.
genistein (a tyrosine kinase inhibitor),\textsuperscript{25} C-erbB-2/neu antisense oligonucleotide,\textsuperscript{26} and curcumin.\textsuperscript{27} However, this effect was not seen in animals treated with a lower dose of celecoxib (5 mg/kg/day), presumably due to suboptimal suppression of COX-2 expression in the gastric mucosa. It is interesting to note that the high dose of celecoxib (20 mg/kg/day) did not produce a further increase in the chemopreventive effect. In keeping with this observation, there was no further reduction in tumour PGE\textsubscript{2} or COX-2 levels with the high dose of celecoxib (20 mg/kg/day) compared with 10 mg/kg/day, suggesting the effect had plateaued. Previous experiments in rat models of inflammation also suggest that the high dose of celecoxib (20 mg/kg/day) did not produce a further increase in the chemopreventive effect. It remains undetermined whether concurrent COX-1 inhibition has a promotional effect on tumour development.

In this study, indomethacin, a non-selective COX inhibitor, showed no apparent chemopreventive effect on MNG induced gastric tumours in rats. There was only a tendency favouring a lower tumour volume in indomethacin treated rats compared with MNG controls. The reason for these discrepancies between indomethacin and celecoxib is unclear. One plausible explanation may be related to the dose of indomethacin used in this study. Our selection of this dose was based on two facts. Firstly, the recommended dose of indomethacin in humans is 1–3 mg/kg/day. Secondly, previous animal studies demonstrated inhibitory effects on the formation of aberrant crypt foci in the colons of dimethyl hydrazine treated rats using a dose of 2 mg/kg/day.\textsuperscript{30} As shown in figure 4, tumour PGE\textsubscript{2} levels in the indomethacin

---

### Table 2 Tumour incidences and multiplicity in the different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No of deaths (%)</th>
<th>No of rats with gastric tumours (%) (incidence)\textsuperscript{†}</th>
<th>No of gastric cancers per rat (SD) (multiplicity)\textsuperscript{†}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>B MNNG alone</td>
<td>6 (37.5)</td>
<td>12 (75.0)</td>
<td>1.0 (0.7)</td>
<td></td>
</tr>
<tr>
<td>C MNNG+indomethacin</td>
<td>6 (37.5)</td>
<td>11 (68.8)</td>
<td>0.8 (0.80)</td>
<td></td>
</tr>
<tr>
<td>D MNNG+celecoxib 5 mg/kg/day</td>
<td>5 (29.4)</td>
<td>12 (70.6)</td>
<td>0.8 (0.6)</td>
<td></td>
</tr>
<tr>
<td>E MNNG+celecoxib 10 mg/kg/day</td>
<td>6 (37.5)</td>
<td>3 (18.8)</td>
<td>0.2 (0.4)</td>
<td></td>
</tr>
<tr>
<td>F MNNG+celecoxib 20 mg/kg/day</td>
<td>3 (18.8)</td>
<td>5 (31.3)</td>
<td>0.3 (0.5)</td>
<td></td>
</tr>
</tbody>
</table>

*\(p=0.002 (\chi\textsuperscript{2}); C\ versus B, \(p=1.00\); D versus B, \(p=1.00\); E versus B, \(p=0.004\); F versus B, \(p=0.052\).*

**\(tp=0.001\) [ANOVA]; C versus B, \(p=1.00\); D versus B, \(p=1.00\); E versus B, \(p=0.004\); F versus B, \(p=0.025\).**

---

\textsuperscript{†}Significant differences:

- Celecoxib 5, 10, 20, celecoxib 5, 10, and 20 mg/kg/day.
- Celecoxib used in this study may be associated with more toxicity, such as intestinal haemorrhage. Moreover, the COX-2 selectivity of celecoxib may be lost at high doses, resulting in more COX-1 inhibition. Based on our data with high dose celecoxib and indomethacin, concurrent COX-1 inhibition may have a paradoxical effect on chemoprevention. It remains undetermined whether concurrent COX-1 inhibition has a promotional effect on tumour development.

---

**Figure 2**

Macroscopic and microscopic appearance of \(N\)-methyl-\(N\)\'-nitro-\(N\)\''-nitrosoguanidine (MNNG) induced gastric tumour in a rat.\textsubscript{20} (A) Macroscopic view of MNNG induced tumour formation in the distal stomach of a Wistar rat. (B) Haematoxylin-eosin staining of well differentiated gastric adenocarcinoma in the stomach (\(\times20\)).

---

**Figure 3**

Cyclooxygenase 2 (COX-2) mRNA expression levels of tumours and adjacent normal tissues in the different treatment groups. COX-2 mRNA expression levels were determined by quantitative reverse transcription-polymerase chain reaction. Mean (SEM) values are shown. There was upregulation of COX-2 in all tumours compared with adjacent normal tissues (\(p<0.02\)). However, there was no significant difference in COX-2 mRNA levels among tumours in different treatment groups. Adjacent normal tissues from the MNNG treated group had the highest COX-2 levels (\(p<0.0001\); between different tumours, \(p>0.05\); between different normal tissues, \(p<0.0001\); \(tp<0.0001\), MNNG normal versus all other normal tissues.)
Celecoxib prevents gastric cancer in rats

ELISA and mean (SEM) values are shown. PGE2 levels tended to be higher in tumours than in adjacent normal tissues. Treatment with indomethacin (Indo) or celecoxib (Cele) (10 mg/kg/day) tended to reduce PGE2 in tumours but the difference did not reach statistical significance. There was no difference in PGE2 levels among normal tissues of the different treatment groups (p > 0.05). Significant differences: *p = 0.015; between different tumours, p = 0.05; between different normal tissues, p = 0.05.

The Mongolian gerbil was recently found to be a good animal model to study H pylori associated gastric carcinogenesis. Moreover, emerging data show that COX-2 is upregulated in the gerbil stomach after H pylori infection. It will be interesting to characterise the role of COX-2 inhibition in the chemoprevention of gastric cancer in this gerbil model. Another issue that is worth further study is the role of celecoxib in the therapy of established gastric cancer, as this drug was introduced at the same time as the carcinogen in this study. The exact therapeutic role of celecoxib against established cancer remains unknown and a study that introduces celecoxib at different time points may be useful in clarifying this point. Moreover, this type of study may help address the important question of the optimal time of intervention if it is found that celecoxib only prevents gastric cancer development but fails in the treatment of established cancer.

In summary, our study showed that treatment with celecoxib, a specific COX-2 inhibitor, suppressed MNNG induced gastric cancer in rats. This finding lends further support to the use of COX-2 inhibitors in the chemoprevention of gastric cancer. Whether this result can be translated into clinical benefit requires further confirmation in human clinical studies.


Chemoprevention of gastric cancer by celecoxib in rats


Gut 2004 53: 195-200
doi: 10.1136/gut.2003.021477

Updated information and services can be found at:
http://gut.bmj.com/content/53/2/195

These include:

References
This article cites 34 articles, 11 of which you can access for free at:
http://gut.bmj.com/content/53/2/195#BIBL

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

Topic Collections
Articles on similar topics can be found in the following collections
Pancreatic cancer (660)
Stomach and duodenum (1689)

Notes

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

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

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