Prostaglandin E₂ and prostaglandin F₂α biosynthesis in human gastric mucosa: effect of chronic alcohol misuse

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Abstract

Background and Aims—The results of experimental studies support the hypothesis that decreased prostaglandin production might play a part in the gastric mucosal injury induced by alcohol. In this study, it was investigated whether alcohol misuse impairs the synthesis of prostaglandin E₂ (PGE₂) and prostaglandin F₂α (PGF₂α) in gastric mucosa.

Patients—Fifty six alcoholic patients and 66 subjects without alcohol misuse were included in the study.

Methods—Mucosal biopsy specimens were obtained from the antrum and body of the stomach. Maximal synthesis rates of PGE₂ and PGF₂α were determined in the microsomal fraction of the biopsy specimens.

Results—The rates of synthesis of both prostaglandins in biopsy specimens from the antrum were not significantly different from those obtained in the body. Synthesis of both prostaglandins was significantly reduced in alcoholic patients who abstained less than five days compared with the non-alcoholic group with normal mucosa (PGE₂-40%, PGF₂α-42% respectively). In non-alcoholic patients with severe gastritis PGE₂ synthesis was increased (+30%, p<0.05) and PGF₂α synthesis was decreased (-42-5%, p<0.025). In alcoholic patients with severe gastritis PGE₂ synthesis was depressed by almost 60% (p<0.001) compared with the non-alcoholic group with severe gastritis. Neither colonisation of Helicobacter pylori nor smoking had a significant influence on the prostaglandin synthesis.

Conclusions—Chronic alcohol misuse is associated with significantly reduced capacity for prostaglandin synthesis in gastric mucosa and this alcohol induced decrease in prostaglandin synthesis is modulated by the presence and degree of gastritis.

Keywords: alcohol misuse, gastric mucosa, gastritis, Helicobacter pylori, prostaglandins E₂ and F₂α, stomach.

Alcohol is the most widely used and misused drug, the acute and chronic ingestion of which was shown to lead to distinct functional disturbances and mucosal injury in the stomach of both animals and humans. Ethanol at concentrations of 5%-10% and more, disrupts the mucosal barrier and causes focal areas of pronounced mucosal hyperaemia, oedema, epithelial necrosis, and mucosal haemorrhage. The mechanism by which alcohol leads to gastric mucosal injury has not yet been clarified.

Endogenous prostaglandins, especially PGE₂ and PGI₂, are assumed to be of importance in maintaining the normal function and structure of the gastric mucosa. Exogenous PGE₂ and its derivatives have been shown to protect the gastric mucosa against various noxious agents. The results of experimental studies support the hypothesis that decreased prostaglandin production might play a part in the mucosal injury induced by alcohol. It has recently been reported that the acute digestion of alcohol, at a concentration comparable to that of wine, significantly reduces the PGE₂ output in gastric juice in healthy subjects.

This study was performed to clarify the question whether or not prostaglandin synthesis is impaired in gastric mucosa of patients with chronic alcohol misuse. Because gastritis might influence prostaglandin synthesis, the synthesis of PGE₂ and PGF₂α was studied in gastric biopsy specimens of alcoholic patients and in non-alcoholic controls with normal gastric mucosa and mild or severe gastritis.

Smoking has been shown to influence prostaglandin metabolism in the mucosa of the stomach. Because smoking is often associated with alcohol misuse, the effect of smoking on prostaglandin synthesis was evaluated separately.

Methods

Patients

One hundred and twenty two patients in whom an upper gastrointestinal diagnostic endoscopy was performed were included in the study. In all cases, the examination was carried out to investigate upper abdominal complaints, such as bloating, or pain, or heartburn. Fifty six were alcohol misusers and had been consuming an average daily amount of alcohol in
excess of 60 g for more than two years. In 46 of
these patients the period of abstinence
before the endoscopy was less than one week.
On histological examination 17 had normal
gastric mucosa, 19 had mild gastritis, and 10
had severe gastritis in accordance with the
classification proposed by Wyatt and Dixon.14
In these patients the gastroscope was per-
formed within five days after the last alcohol
intake. In addition, another group of 10 alco-
holic patients who had been abstinent for at
least 12 days before endoscopy were studied
(six patients with normal mucosa and four
patients with mild gastritis on histology. Sixty
six patients with no history of alcohol misuse
(mean alcohol consumption <20 g/day) served
as controls (non-alcoholic group). Eleven of
these had a normal gastric mucosa, 38 had
mild gastritis, and 17 had severe gastritis on
histology. Criteria for exclusion from the study
were: use of non-steroidal anti-inflammatory
drugs, glucocorticoids, antacids or carbo-
anhdydrase blockers within the last two weeks.
Patients with cirrhosis or gastric or duodenal
ulcer were also excluded from the study.
Table I summarises the data on the distribution
of age, sex, smoking, and the presence of Helicobacter pylori.
The study was approved by the ethics
committee of the Robert-Bosch-Hospital, and
all the patients gave their informed consent.

BIOPSY SPECIMENS

The endoscopy was performed by two
tained for routine histology and a rapid
urease test, three specimens were obtained
from the antrum 3–4 cm from the pyloric ring
(mean (SD)) fresh weight 14.6 (6.3) mg for
measurement of prostaglandin formation. To
be able to compare prostaglandin synthesis
in different parts of the stomach, additional
biopsy specimens were taken from the upper
part of the body of the stomach in 10 non-
alcoholic patients (seven with normal gastric
mucosa, three with mild gastritis).

STUDY DESIGN

Specimens for histology were fixed in formalin
buffered saline and embedded in paraffin wax,

and 5 mm sections were prepared for light
microscopy in the standard manner. Sections
were stained for H pylori with haematoxylin
and eosin or cresyl violet.15 In addition, H pylori
colonisation was determined by a gel based
rapid urease test (CLO-test, ASTRA Chemicals
GmbH, Wedel). A positive result was
indicated by a colour change at four hours.

Biopsy specimens taken for measurement of
prostaglandin synthesis were immediately
transferred to an ice cold 1:15% solution of
KCl. Microsomes were prepared within 90
minutes, and then suspended in 200 ml Krebs-
Ringer-HEPES buffer, pH 7.4 (100 mM NaCl,
4.78 mM KCl, 1.15 mM K2HPO4, 8.8 mM
HEPES).10 Incubation, prostaglandin extrac-
tion and estimation of PGE2 and PGF2α were
performed as described earlier.10 Briefly, to
achieve optimal synthesis of prostaglandins,
glutathione (5 mM), haem (1 mM), MgSO4
(3 mM) and CaCl2 (5 mM) were added to the
incubation medium.

After preincubation at 37°C for three
minutes, the reaction was started by the addi-
tion of 50 ml arachidonic acid (100 mg/ml) and
stopped after 0 (blank value), 2, 5, or five
minutes by adding 50 ml acetylsaliclyc acid
(10 mM) and immediately heating in a boiling
water bath for five minutes. The assay of
prostaglandin synthesis was linear between 0
and five minutes. All tests were done in dupli-
cate. The PGE2 and PGF2α contents of the
samples were determined by radioimmunoassay
as described recently.11 The recovery rate of
PGE2 was 85%–95% and that of PGF2α was
95%–100%; the intra-assay variations were
3.5% and 5.4% respectively.12 The DNA con-
tent of samples of the homogenates was deter-
mined by the method of Fiszer-Szafarz et al.16
Determination of DNA was chosen as the refer-
cence variable so as to avoid such confounding
factors as variations in the amounts of mucus
and fluid adhering to the biopsy specimens.

STATISTICS

Group results are expressed as means (SEM).
For between group comparisons the unpaired
t test or its non-parametric analogue, the
Wilcoxon two sample test, were used.

TABLE 1. Patient characteristics and H pylori colonisation
of alcohol misusers and controls without alcohol misuse

<table>
<thead>
<tr>
<th></th>
<th>Non-</th>
<th>Alcohol misusers</th>
<th>Duration of abstinence</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>alcoholic</td>
<td>group</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;5 days</td>
<td>&gt;12 days</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>66</td>
<td>46</td>
<td>10</td>
</tr>
<tr>
<td>Male:female</td>
<td>36:30</td>
<td>20:16</td>
<td>8:2</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>55 (15)*</td>
<td>52 (13)*</td>
<td>48 (6)*</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>18/2</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>H pylori colonisation (%)</td>
<td>56</td>
<td>52</td>
<td>40</td>
</tr>
<tr>
<td>Histology: normal mucosa (n)*</td>
<td>11 (4)</td>
<td>17 (4)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Mild gastritis (n)*</td>
<td>13 (10)</td>
<td>19 (13)</td>
<td>12 (2)</td>
</tr>
<tr>
<td>Severe gastritis (n)*</td>
<td>17 (15)</td>
<td>10 (7)</td>
<td></td>
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</tbody>
</table>

*Number of patients H pylori positive are given in parentheses.

Results

PROSTAGLANDIN SYNTHESIS

Antrum versus body

Comparison of the rates of prostaglandin syn-
thesis between biopsy specimens from the
antrum and those from the corpus in 10
patients disclosed no significant differences:
PGE2, 97 (14) vs 101 (17) and PGF2α, 42.1 (16)
vs 39 (14) pg/mg DNA×min respectively.

Non-alcoholic patients with and without gastritis
In patients with mild gastritis the PGE2
synthesis did not differ significantly from that
in non-alcoholic group with normal gastric
mucosa (92 (13) and 105.5 (17) pg/mg

By: Robert-Bosch-Hospital, and
all the patients gave their informed consent.

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BIOPSY SPECIMENS

The endoscopy was performed by two
experienced gastroenterologists who together
decided where in the stomach the biopsy
specimens should be obtained from. In
addition to tissue from the antrum, corpus, and
fundus taken for routine histology and a rapid
urease test, three specimens were obtained
from the antrum 3–4 cm from the pyloric ring
(mean (SD)) fresh weight 14.6 (6.3) mg for
measurement of prostaglandin formation. To
be able to compare prostaglandin synthesis
in different parts of the stomach, additional
biopsy specimens were taken from the upper
part of the body of the stomach in 10 non-
alcoholic patients (seven with normal gastric
mucosa, three with mild gastritis).

STUDY DESIGN

Specimens for histology were fixed in formalin
buffered saline and embedded in paraffin wax,
DNA×min respectively), whereas in non-alcoholic group with severe gastritis, PGE<sub>2</sub> synthesis was greatly increased (137 (24), pg/mg DNA×min, p<0.05; Fig 1). Synthesis of PGF<sub>2α</sub> in non-alcoholic patients with mild gastritis was decreased by 23% compared with non-alcoholic patients with normal mucosa (33 (4.0) and 43.5 (6.5) pg/mg DNA×min respectively, p<0.05). The reduction of PGF<sub>2α</sub> synthesis was more pronounced in non-alcoholic patients with severe gastritis (25 (5) pg/mg DNA×min, p<0.025; Fig 2).

**Alcoholic patients with and without gastritis**

The synthesis of PGE<sub>2</sub> was clearly reduced (∼40%) in the alcoholic patients with normal mucosa who continued to drink within five days of their endoscopy compared with the non-alcoholic group with normal mucosa (Fig 1, p<0.05). In alcoholic patients with mild gastritis, PGE<sub>2</sub> synthesis was not significantly decreased compared with the corresponding non-alcoholic group (Fig 1, p>0.05). However, in alcoholic patients with severe gastritis, PGE<sub>2</sub> synthesis was depressed by almost 60% (49 (22) pg/mg DNA×min) compared with the non-alcoholic patients with severe gastritis (137 (24) pg/mg DNA×min; p<0.001).

In the subgroups with normal mucosa, synthesis of PGF<sub>2α</sub> was significantly reduced in the alcoholic patients who continued to drink to within five days of their endoscopy compared with the non-alcoholic patients (Fig 2, p<0.025). Although PGF<sub>2α</sub> synthesis was not influenced by the degree of mucosal inflammation in the alcoholic patients (Fig 2), in the group of alcoholic patients with mild gastritis PGF<sub>2α</sub> synthesis was still significantly lower than in the corresponding control group (p<0.025).

**Effect of alcohol abstinence**

In the group of alcoholic patients who abstained from alcohol for more than 12 days, PGE<sub>2</sub> synthesis exhibited almost normal values (93 (27) pg/mg DNA×min) whereas PGF<sub>2α</sub> synthesis was still diminished (28.5 (19); p<0.05).

**Effect of H pylori colonisation**

A comparison of the non-alcoholic group negative for H pylori with those colonised with the organism disclosed that the rates of synthesis of PGE<sub>2</sub> and PGF<sub>2α</sub> were not different (Table II). In the H pylori positive group of alcoholic patients PGE<sub>2</sub> synthesis was slightly reduced compared with the H pylori negative group (p>0.05), whereas PGF<sub>2α</sub> values did not differ (Table II). Colonisation of H pylori had no significant influence on the prostaglandin synthesis in either subgroup of patients with normal mucosa or mild gastritis (data not shown).

**Effects of smoking**

The mean values of PGE<sub>2</sub> synthesis were slightly lower in smokers, both in the non-alcoholic group and the group of alcoholic patients, but the differences were not significant (p>0.05; Table III). An investigation of the

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Effect of H pylori colonisation on prostaglandin synthesis in antral mucosa</th>
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<tbody>
<tr>
<td></td>
<td>H pylori</td>
</tr>
<tr>
<td>Non-alcoholic group</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
</tr>
<tr>
<td>Alcohol misusers</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>24</td>
</tr>
</tbody>
</table>

Values for PGE<sub>2</sub> and PGF<sub>2α</sub> are pg/μg DNA×min (mean (SEM)).
influence of smoking on PGE$_2$ synthesis in the subgroups with and without gastritis also failed to disclose any significant differences. This applied in particular to the subgroups with severe gastritis (Fig 1), although the number of cases were few (smokers in the non-alcoholic group three of 17, in the alcoholic patients four of 10). The synthesis of PGF$_{2a}$ was not influenced by smoking (Table III).

Discussion
For the purpose of studying factors that might affect prostaglandin metabolism in the human stomach, different approaches have been used. Determination of prostaglandin content in mucosal biopsy specimens (in particular arachidonic acid) in mucosal biopsy specimens has both been applied. Each of these methods has its own particular problems with respect to the interpretation of the data obtained. A major disadvantage of using whole mucosal biopsy specimens or homogenates of mucosal biopsy specimens is the pronounced influence of sample removal and homogenisation on the results. The measurement of prostaglandin formation from the endogenous substrate or from added exogenous arachidonic acid in mucosal biopsy specimens is additionally influenced by the conditions of incubation and the concentration of the substrate. Also, gastric mucosa has a high catalytic capacity for prostaglandins, making interpretation of the results obtained in whole tissue difficult. To overcome these problems, prostaglandin synthesis was assessed in the microsomal fraction, in which the enzymes of prostaglandin formation are localised, but where prostaglandin degradation does not occur.

In earlier investigations into the effect of alcohol on prostaglandin production in gastric mucosa, the main line of approach has been to study the action of acute alcohol administration. Depending on the concentration of the alcohol administered, results have differed. Numerous studies have reported an inhibition of prostaglandin production in gastric mucosa of laboratory animals after the acute administration of concentrated alcohol (50%-100%) as used to produce mucosal necrosis in the stomach. On the other hand, experiments on rats to investigate the effect of the moderate concentrations of alcohol commonly found in alcoholic beverages showed an increase in PGE$_2$ formation and in PGE$_2$-like material has been reported. In humans, acute ingestion of 12-5% alcohol reduced the output of prostaglandin E$_2$ in gastric juice, whereas the output of PGF$_2$ and 6-keto PGF$_{2a}$ remained unchanged.

The effect of chronic administration of moderate concentrations of alcohol has, to date, been investigated only in animals. Feeding rats a liquid diet containing ethanol for one to three months reduced the capacity of gastric mucosa to synthesise PGE$_2$ by 40%-60%. The findings of this study – namely that chronic alcohol misuse was associated with reduced mucosal activity in terms of PGE$_2$ and PGF$_{2a}$ synthesis – accords with the results of several animal experiments.

The relevance of alcohol misuse as a cause of gastric mucosal injury including haemorrhagic erosive gastritis in humans has become apparent from several endoscopy studies. However, the pathomechanism of alcohol-induced mucosal damage has not been clarified. Suspected factors are disturbances in the permeability of the mucosa and in the microcirculation. Moreover, inhibition of protective mechanisms of the gastric mucosa – for example, the breakdown of the mucosal bicarbonate barrier – brought about by the acute action of alcohol, might contribute to mucosal damage. The reduction in the ability to synthesise PGE$_2$ and PGF$_{2a}$ shown in this study might have an influence on several of the factors considered to be of relevance for the pathogenesis of the alcohol induced mucosal injury in the stomach. PGF$_2$ stimulates gastric mucosal blood flow and bicarbonate output, as well as the production of mucus. Also PGE$_2$ inhibits the release of inflammatory mediators from intestinal mucosal mast cells such as platelet activating factor and tumour necrosis factor. Moreover, a trophic effect of PGE$_2$ on mucosal epithelium has also been reported.

In the control group PGE$_2$ synthesis remained unchanged in patients with mild gastritis, where there was a significant increase in the patients with severe gastritis. On the other hand, gastritis was associated with a decrease in the ability to synthesise PGF$_{2a}$. Other studies on the effect of mucosal inflammation on the prostaglandin synthesis have produced varying results. When the activity of prostaglandin synthesis was measured after incubation of homogenates of gastric biopsy specimens with C-labelled arachidonic acid, no significant differences were seen in the sum of cyclooxygenase products (prostaglandins A2+B2, D2, E$_2$ and F$_2$) in the mucosa of patients with erosive or non-erosive type B gastritis and controls with normal mucosa. Similarly, no difference was found for the combined PGE$_2$ and PGF$_{2a}$ content of biopsy specimens from the antrum in patients with and without mucosal inflammation.

Smoking has been shown to influence prostaglandin metabolism in the mucosa of the stomach. Active smoking reduces PGE$_2$ concentration in gastric juice, and decreases mucosal 6-keto-PGF$_{1a}$ synthesis. In another study significant decreases in mucosal prostaglandin in SDF and F$_2$ synthetase assay in biopsy specimens from the body and antrum of patients who had smoked within the two days immediately

<table>
<thead>
<tr>
<th>Patients</th>
<th>Smoking n</th>
<th>PGE$_2$</th>
<th>PGF$_{2a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-alcoholic patients</td>
<td>no</td>
<td>54</td>
<td>108 (1-21)</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>12</td>
<td>95-2 (19-2)</td>
</tr>
<tr>
<td>Alcoholic patients</td>
<td>no</td>
<td>23</td>
<td>76 (22-3)</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>23</td>
<td>59-6 (24)</td>
</tr>
</tbody>
</table>

Values for PGE$_2$ and PGF$_{2a}$ are pg prostaglandin/μg DNA×min (mean (SEM)).

The findings of this study – namely that chronic alcohol misuse was associated with reduced mucosal activity in terms of PGE$_2$ and PGF$_{2a}$ synthesis – accords with the results of several animal experiments.
Bode, Maute, Bode

preceding endoscopy. Because in the present study the percentage of smokers was higher among alcoholic patients, smoking might contribute to the lower rate of PGE2 production in these patients. However, active smoking did not significantly influence the results in terms of prostaglandin synthesis in the group of alcoholic patients.

The presence of H pylori in the biopsy specimens had no influence on prostaglandin synthesis. This accords with the results of other studies on prostaglandin synthesis in homogenates of antrum specimens of patients with and without H pylori. Moreover, the presence of H pylori did not influence the content of PGE2 in the antrum and body of the stomach. Reduced PGE2 synthesis in the mucosa of the antrum and corpus in H pylori positive patients reported by others might be explained by the type of measurement used, and also by the fact that values were expressed in pg/mg of wet weight.

In patients with alcoholic cirrhosis and portal hypertension the PGE2 content in biopsy specimens from the gastric antral mucosa was shown to be significantly lower than in controls without liver disease. In these studies the patients abstained from alcohol for more than two weeks or eight days respectively. In addition, PGE2 content in cirrhotic patients without portal hypertension were not significantly different from the controls without liver disease. Therefore, it was concluded that the decrease in PGE2 tissue concentrations is related to the presence of portal hypertension. In this study alcoholic patients with cirrhosis were excluded to avoid a potential overlap of the effect of portal hypertension with the effect of chronic alcohol misuse.

In conclusion, chronic alcohol misuse reduces the rate of synthesis of PGE2 and PGE2 in the gastric mucosa in humans. The decreased capacity for prostaglandin production might be a factor in the increased vulnerability of mucosa in alcoholic patients. The reasons for the reduced capacity for prostaglandin synthesis in gastric mucosa in alcoholic patients remain to be clarified.

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