Cigarette smoking reduces human gastric luminal prostaglandin E₂

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SUMMARY The effect of smoking three cigarettes on the release of prostaglandin E₂ (PGE₂) by the gastric mucosa was studied in seven healthy smokers. Smoking caused the expected increases in pulse rate, blood pressure, plasma glucose, and carboxyhaemoglobin. In addition, smoking resulted in a significant ($p < 0.05$) reduction in the volume of pentagastrin stimulated gastric juice from 76.1 ± 4.4 to 54.1 ± 4.6 ml/15 min and PGE₂ output from 22.8 ± 4.9 to 12.2 ± 3.8 ng/15 min but did not alter acid output. It is concluded that smoking reduces the amount of PGE₂ in the gastric lumen and that this may explain why it is a risk factor for peptic ulcer.

Cigarette smoking is endemic in our society and is a major risk factor for the development¹ and recurrence² of peptic ulcer. This association cannot be explained on the basis of an effect on gastric secretion of acid or pepsin,³ blood flow⁴ or even pancreatic secretion.⁵ It is possible that smoking may adversely affect one or more of the mucosal defence mechanisms. Prostaglandins, particularly PGE₂, may play a key role in the maintenance of gastric mucosal integrity.⁶ There is some evidence that smoking may alter prostanoid synthesis.⁷ This study was designed to determine the effect of smoking on gastric mucosal PGE₂ in healthy smokers.

Methods

SUBJECTS

Seven healthy (weight 76.9 ± 6.4 kg) subjects (five men and two women) aged 21-32 years participated in the study which was approved by the University of Toronto Human Experimentation Committee. All subjects provided informed written consent. None had a significant past medical history nor any symptoms related to the gastrointestinal tract. All were smokers who usually consumed 10-25 cigarettes per day. No alcohol, aspirin, or other medication were ingested for at least one week before or during the study.

DESIGN OF EXPERIMENT

The subjects were studied twice (smoking and control) at least five and not more than 14 days apart. Each experiment was conducted at the same time of day and the sequence was randomised. In the control experiment a Pasteur pipette was 'smoked'.

Subjects fasted and abstained from smoking for eight hours. A 14 F gauge double lumen nasogastric tube (Salem Sump, Argyle Co) was passed so that its tip lay in the most dependent portion of the stomach. The gastric contents were aspirated and discarded. Gastric juice was then collected using continuous low pressure suction. Juice was collected in 15 minute aliquots in a specimen flask (Cheeseborough Ponds Inc) placed in an ice-water bath. Saliva was expectorated.

Throughout every experiment pentagastrin $6 \mu\text{g/kg/h}$ was given in normal saline by continuous intravenous infusion to obtain maximal gastric secretion. A catheter was placed intravenously in the opposite arm for blood sampling. A plateau of secretion was reached within 15 minutes. The first 15 minute aliquot was discarded. The next 45 minutes was designated as the 'baseline' period, after which the subjects smoked three cigarettes (DuMaurier King-Size Filter tipped), each of which contained 1.2 mg nicotine and 16 mg 'tar', at the rate of one cigarette per 10 minutes. They were instructed to inhale the cigarette for two seconds, hold the smoke for three seconds, exhale, and then repeat the sequence every 30 seconds. The cigarette was smoked until reduced to a length of 2.5 cm.

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Gastric juice from the 'smoking' period consisted of the two aliquots during actual smoking plus the next 15 minute aliquot. Gastric secretion was collected for a further 45 minutes defined as the 'postsmoking' period. Blood was drawn from the intravenous catheter 0, 60, 75, 90, 120, and 150 minutes after the pentagastrin infusion was started. Blood pressure and heart rate were recorded every 15 minutes.

MEASUREMENTS

The volume of each aliquot was measured and its H^+ concentration determined by titration to pH 7.0 with 0.01 M NaOH using an automatic titrimer (Radiometer, Copenhagen). PGE_2 concentration was measured by radioimmunoassay after extraction and column chromatography as previously described.⁸ Briefly, a 1 ml aliquot of gastric juice was titrated with 1 M NaOH solution to a pH between 3.0 and 3.5. Tritiated PGE_2 was added to determine recovery. It was extracted twice with 4 ml ethyl acetate, dried at 37°C under a stream of nitrogen, and stored at -70°C until silicic acid chromatography and radioimmunoassay. The radioimmunoassay utilised antibody from the Pasteur Institute and achieved 50% binding at 9.1 ± 0.3 pg PGE_2 per assay tube. Tritiated PGE_2 was obtained from New England Nuclear. PGE_2 for the standards was a generous gift from Dr John Pike, Upjohn, Kalamazoo. Interassay and intraassay coefficients of variation were consistently less than 10%. Plasma glucose concentrations were determined by the oxidase method⁹ using the Beckman Glucose Analyser II. Blood for carboxyhaemoglobin was drawn into heparinised syringes, kept on ice, and analysed by co-oximeter (Model 282, Instrumentation Laboratory).

ANALYSIS OF DATA

A two way analysis of variance was used to determine statistical significance. If the initial analysis on the raw data proved significant, further analysis was carried out by transforming the data into 'percentage of baseline'. Student's *t* test was then used to determine the significance of the difference between baseline and subsequent measurements. A *p* value of less than 0.05 was considered significant. In this way the analysis helped factor out differences between individuals. The results are reported conventionally as mean $\pm$ standard error of actual values.

Results

Cigarette smoking had a significant effect on gastric secretion (Fig. 1). The volume of juice was significantly reduced from 76.1 ± 4.4 to 54.1 ± 4.6 ml/15

Fig. 1 Gastric secretion stimulated by pentagastrin in healthy adults. Each point represents the mean 15 min output for seven subjects. The bars represent SE of the means. Subjects smoked three cigarettes during the interval represented by the shaded bar. The asterisks indicate values significantly different from the mean of the first three values (baseline period).

minutes in the smoking period, an average reduction of 29%. In the postsmoking period the volume recovered slightly. PGE_2 output was also significantly reduced by an average of 46%. The baseline value of 22.8 ± 4.9 ng/15 minutes dropped to 12.2 ± 3.8 ng/15 minutes during the smoking period and increased to 17.9 ± 3.0 ng/15 minutes in the post-smoking period. Acid output fell slightly during the smoking period but this was not statistically significant.

Cigarette smoking caused a significant rise in the pulse, and blood pressure (Fig. 2). Plasma glucose was significantly increased from 4.93 ± 0.07 to 5.12 ± 0.06 in the smoking period and 5.31 ± 0.10 mmol/l in the postsmoking period. Carboxyhaemoglobin increased significantly from 0.45 ± 0.10 to 0.61 ± 0.13 and 0.59 ± 0.11 in the smoking and postsmoking periods respectively. The results obtained during the smoking of sham cigarettes are shown in the Table. No significant changes were found nor were there any changes in pulse rate, or blood pressure (Fig. 2).

A comparison of the data obtained during the sham-smoking experiment was made with the data from the smoking experiment using analysis of variance. Smoking caused a significant ($p < 0.05$) reduction in PGE_2 output (12.2 ± 3.8 ng/15 minutes) and of volume of secretion (54.1 ± 4.6 ml/15 minutes) during the smoking period but there was no

Fig. 2 Heart rate and blood pressure values obtained in the smoking and control experiments. Each point represents the mean value for seven subjects. The bars represent SE of the means. Subjects smoked three cigarettes during the interval represented by the shaded bar. The asterisks indicate values significantly different from the mean of the first three values (baseline period).

significant change in acid output (7.9 ± 0.7 mEq/15 minutes).

Discussion

The circumstantial evidence that cigarette smoking has an adverse effect on the gastroduodenal mucosa is considerable. There is not only an epidemiological association between smoking and peptic ulceration¹ but also evidence that stopping smoking promotes ulcer healing.¹⁰ It has been generally assumed that smoking is not a direct cause of ulceration but rather a factor preventing the healing of mucosal lesions indirectly *via* either a secretory effect or an effect on gastric mucosal defence.

The mechanism(s) underlying the harmful effects of cigarette smoking on peptic ulcer are not understood. The effect of smoking on gastric secretion of acid and pepsin is reported to be either inhibitory or nil.^{3 11-15} There is some evidence that cigarette smoking decreases gastric⁵ and pancreatic bicarbon-

ate output.^{5 16 17} This could in part explain the adverse effect of smoking on duodenal and gastric ulcer. There is conflicting data on the effect of smoking on gastric mucus secretion.^{18 19} The other factors putatively involved in mucosal defence such as cell turnover and mucosal blood flow have either not been studied or have been shown not to be altered.⁴

It has been suggested that endogenous gastric mucosal prostaglandins may play an important role in the maintenance of mucosal integrity.²⁰ There is now a large volume of data showing that exogenous prostaglandins are capable of preventing mucosal damage in animals and in man.²¹ There is evidence that endogenous prostaglandin synthesis can be induced by 'mild irritants'^{22 23} and by stress.²⁴ The prostaglandin content of gastric mucosa may be decreased in patients with gastric ulceration or gastric cancer.²⁵ Duodenal ulcer patients may have impaired prostaglandin release by their duodenal mucosa in response to an acid and food load.²⁶ Further, drugs which inhibit gastric mucosal prostaglandin synthesis, for example aspirin and indomethacin, typically injure the gastric mucosa.

Smoking has been shown to alter prostaglandin metabolism in other organ systems. For example, aspirin (a cyclooxygenase inhibitor) prevented the smoking induced platelet aggregate formation in non-smokers.²⁷ Cigarette smoke reduced prostacyclin production by rat aorta *in vitro*.²⁸ The neonates of women who smoked throughout pregnancy had decreased umbilical artery prostacyclin synthesis immediately after delivery.²⁹ It was therefore reasonable to explore the possibility that smoking might impair prostaglandin production by the human gastric mucosa.

This study shows that the smoking of three cigarettes over 30 minutes by healthy adult smokers causes a significant reduction in the total volume of gastric juice but not of acid output. This suggests that smoking inhibits non-parietal cell secretion and is consistent with other reports.^{4 5} This could be an autonomic effect mediated by changes in blood flow. Smoking also caused a significant reduction in the amount of PGE₂ found in the gastric lumen. That smoking reduced luminal PGE₂ in habitual smokers makes our finding even more striking as non-smokers might be expected to be more sensitive to the effects of smoking. Fung *et al*³⁰ found no change in the concentration of 6-keto prostaglandin F_{1α} (the stable metabolite of prostacyclin) in the gastric juice of volunteers who smoked three cigarettes over a one hour period. In that study there was enormous variation in the 6-keto PGF_{1α} values in the basal collections and throughout the control experiment. This would have concealed all

Table Results obtained in the control experiment

	Baseline	Sham-smoking	Post-smoking
PGE ₂ (ng/15 Min)	19.2±5.9	24.5±7.4	23.4±7.8
Vol (ml/15 min)	75.6±6.2	68.7±3.3	64.2±5.3
H ⁺ (mmol/15 min)	9.0±1.1	9.4±1.0	9.2±1.1
Glucose (mmol/l)	4.96±0.09	4.96±0.07	5.14±0.12
Co Hb	0.41±0.07	0.41±0.06	0.40±0.06

but the most profound inhibition of prostacyclin release. We chose to study PGE₂ because it is the predominant prostanoid found in human gastric juice³¹ and we used a background of maximal gastric secretion in an attempt to stabilise PGE₂ output. This was successful as demonstrated by the remarkably stable PGE₂ concentrations in the sham-smoking experiment.

The precise mechanism by which PGE₂ appears in the gastric juice is not known. We do not know whether smoking inhibited the cyclooxygenase system, inhibited the active secretion of PGE₂, reduced passive transport of PGE₂ into the lumen, or enhanced PGE₂ degradation. The lack of effect on gastric acid output and the stability of the luminal pH, makes the latter explanation less likely. Whatever the mechanism, the end result was that smoking reduced the absolute amount of PGE₂ in contact with the surface mucosal cells. Admittedly the amount of PGE₂ detected by us in the gastric juice was very small and substantially smaller than the smallest amount of exogenous PGE₂ (40 µg) found by us to protect against aspirin injury.³² If, however, as seems likely, endogenous PGE₂ plays a role in the complex regulation of mucosal integrity⁶ then an approximately 50% reduction in luminal PGE₂ may be sufficient to impair resistance to injury, facilitate the development of ulceration, and prevent the healing of an ulcer once formed. Measurement of PGE₂ in the gastric juice may not be the optimum way of examining this problem. It may be preferable to examine the prostaglandin synthetic capacity of mucosal biopsies.⁸ We are now conducting endoscopic studies in normal subjects and patients with peptic ulcer to explore the effect of smoking on the major prostanoids of the gastric mucosa.

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