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Smoking, eicosanoids and ulcerative colitis

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Abstract—In this study, which is the first of its kind using normal tissue samples that are very difficult to obtain, we have investigated the hypothesis that smoking protects against ulcerative colitis by altering the colonic mucosal formation of prostaglandins and related substances. Colonic mucosa biopsied from healthy young men produced prostaglandin E, 6-keto-$PGF_{1\alpha}$ (formed from PGI_2), leukotriene B_4 and leukotriene $C_4/D_4/E_4$ as determined by radioimmunoassay. With each substance, the median yield was lower in the group of smokers who smoked 3 cigarettes in the 2 h before biopsy, than in the non-smokers. However, with each eicosanoid the statistical probability approached only the 10% level, but the fact that the trend was the same for all eicosanoids somewhat strengthens the possibility of a real difference between the groups.

Prostaglandins and leukotrienes are important mediators of inflammation whose synthesis in human gastric mucosa is influenced by smoking (Quimby et al 1986). A similar effect in the colon might be pertinent to the aetiology of ulcerative colitis, and its inverse relationship to smoking (Harries et al 1982). We have investigated this hypothesis by measuring, for the first time, prostaglandin, thromboxane and leukotriene synthesis in biopsies of rectal mucosa from smokers and non-smokers.

Subjects and methods

There were 25 healthy adult males who were either non-smokers (n = 13, mean age 44·5 years, range 22–74) or smokers (n = 12, mean age 44·8, range 23– 67). They were friends or relatives of patients with colitis, and they volunteered for the study which had the approval of the Ethical Committee of the University Hospital of Wales. None of the subjects were taking medication. Those in the test group were accustomed to smoking 10 to 30 cigarettes daily; they smoked three cigarettes in the 2 h before biopsies were taken.

All of the volunteers appeared to have normal rectal and colonic mucosa on sigmoidoscopy performed without enema preparation. Two biopsies were taken from the anterior rectal wall 8 cm from the anal margin, washed immediately in 154 mM NaCl, dropped into liquid nitrogen, and then stored at −80°C for nine months before extraction and analysis at King's College School of Medicine and Dentistry, London. Although the period of storage was long, it is well known that cyclo-oxygenase in stored tissue retains activity over many years, and lipoxygenase activity occurs in tissues that have been stored for several months (J A Salmon, personal communication). If any enzymic deterioration had occurred during storage, we would have expected it to be similar in the test and control samples.

The samples were thawed, carefully weighed (100 ± 5 mg) and pre-incubated in 1 mL phosphate buffer pH 7·4 (PBS) at 0°C for 30 min. The pre-incubation fluid was then discarded and replaced by 1 mL of fresh fluid which, after further incubation at 37°C for 30 min, was removed and stored at −20°C until radioimmunoassay in duplicate.

The antisera, sources and % immunological specificities for radioimmunoassays were:

PGE (ICN Biomedicals Ltd); cross reactions: PGE_2 100; PGE_1 240; $PGF_{1\alpha}$ 0·35; $PGF_{2\alpha}$ 0·5; 15-keto-PGE_2 0·1; PGD_2 0.04; 6,15-diketo-$PGF_{1\alpha}$ < 0·04; 15-keto-$PGF_{1\alpha}$ < 0·04; 13, 14-dihydro-6-keto$PGF_{1\alpha}$ < 0·04; 6-keto-$PGF_{1\alpha}$ 0·6; TXB_2 < 0·04. Since the antibody does not distinguish between PGE_1 and PGE_2, the assay results are expressed as PGE. *6-Keto-$PGF_{1\alpha}$* (Wellcome); cross-reactions: 6-keto-$PGF_{1\alpha}$, 100; PGE_2, 0·01; $PGF_{2\alpha}$, 3·0; TXB_2, 0·02. *TXB_2* (Wellcome); cross-reactions: TXB_2, 100; $PGF_{2\alpha}$, 0·1; PGE_2, 0·1; 6-keto-$PGF_{1\alpha}$, 0·1.

The immunological specificities of the RIA kits used were: *$LTC_4/LTD_4/LTE_4$* (Amersham); cross-reactions: LTC_4, 100; LTD_4, 64; LTE_4, 64; LTB_4, 0·001; prostaglandins and TXB_2 < 0·001. *LTB_4* (Amersham); cross reactions: LTB_4 100; 20-OH-LTB_4 0·4; other arachidonate metabolites < 0·05.

The assays were completed without knowledge of the groups from which the biopsies were taken. Intra-assay coefficients of variation at 50% binding were (%): LTB_4 2·8, $LTC_4/D_4/E_4$ 1·9, TXB_2 3·7, 6-keto-$PGF_{1\alpha}$ 2·2, PGE 6·8. The results were analysed by the Mann–Whitney U-test (2-tailed).

Results and discussion

Synthesis of eicosanoids by the rectal mucosa varied greatly between biopsies (Fig. 1). The measured products formed (overall range pg mg^{-1}/30 min) were: PGE 16·3–175; thromboxane B_2 29·4–548; 6-keto-$PGF_{1\alpha}$ 29·4–124; $LTC_4/D_4/E_4$ 7·1–97·2; LTB_4 0·8–4·5.

The median yield of each eicosanoid was lower in smokers, compared with non-smokers, but in each case the statistical probabilities approached only the 10% level. However, the fact that the median was lower in every case, and each set of results is an independent measurement of eicosanoid synthesis, somewhat strengthens the possibility of a real difference. Since both the cyclo-oxygenase and lipoxygenase products showed a tendency for reduction, smoking might affect both these enzyme pathways and/or arachidonate release. Studies on more subjects might

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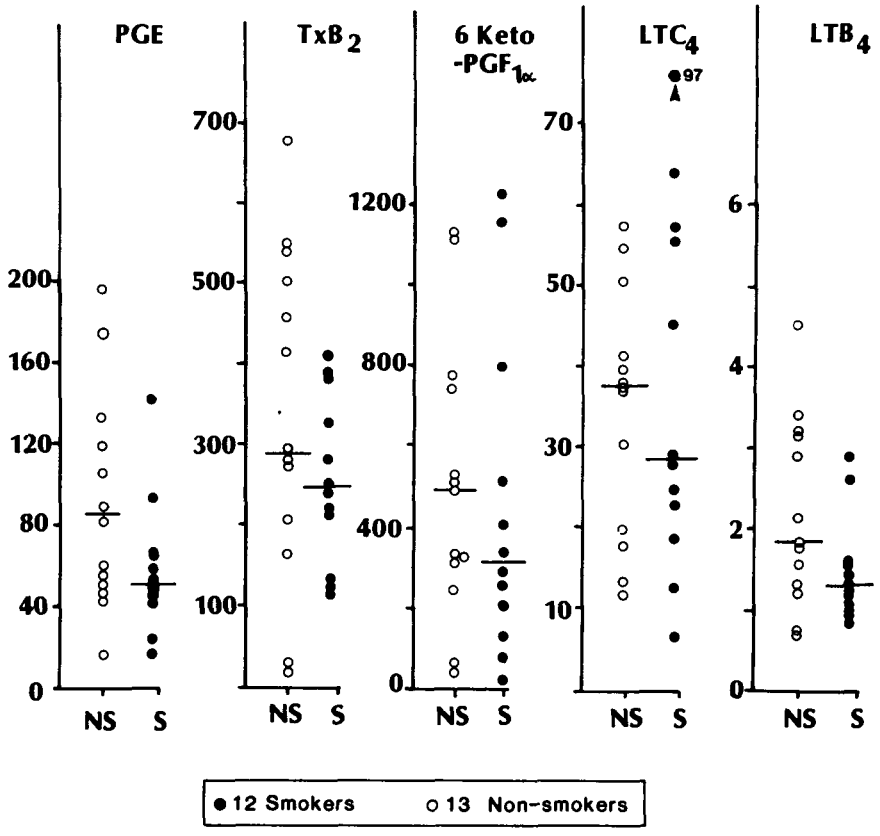

FIG. 1. Eicosanoid metabolites in incubates of rectal biopsies from 13 non-smokers (○) and 12 smokers (●). Values are expressed in pg mg^{-1}/30 min for prostaglandin E (PGE), thromboxane B_2 (TXB_2), 6-keto-$PGF_{1\alpha}$, and the leukotrienes LTB_4 and $LTC_4/D_4/E_4$. Median values are represented by horizontal bars.

clarify the relationship between smoking and rectal eicosanoid synthesis, but it is very difficult to obtain mucosal biopsies from normal subjects. The trend in the effect of smoking on eicosanoid formation by the rectal mucosa is the same as the reduced prostaglandin synthesis in gastric mucosa (and the associated reduction of mucus and bicarbonate secretion; Quimby et al 1986). Gastric mucosal thromboxanes and leukotrienes have not been studied with respect to smoking.

The effects of smoking on the stomach have been linked with gastritis and peptic ulcer, but, in contrast, ulcerative colitis is a disease of non-smokers (Harries et al 1982). Perhaps smoking protects the rectal mucosa by reducing the formation of leukotrienes, which may be the most important eicosanoids in inflammatory bowel disease (Lauritsen et al 1986). Increased amounts of colonic mucosal prostaglandins are produced during active ulcerative colitis (Sharon et al 1978), and they too may act, at least partly, as inflammatory mediators. However, the adverse clinical effect of non-steroidal anti-inflammatory inhibitors of prostaglandin synthesis makes it difficult to draw any simple conclusions about the relationship of eicosanoids to ulcerative colitis or the protective effect of smoking on this disease.

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