

Is transdermal nicotine associated with cardiovascular risk?

ABSTRACT—We had the unique opportunity to study the effects of transdermal nicotine on markers of haemostasis and serum lipids in patients with ulcerative colitis; all were non-smokers and were given transdermal nicotine to assess its value in maintenance therapy for colitis. In a controlled double-blind trial, 45 patients with ulcerative colitis in remission on 5-aminosalicylic acid, were randomly allocated to receive transdermal nicotine (20) or placebo (25) patches. Markers of haemostasis, including platelet activation (platelet volume and surface expression of P selectin), endothelial damage (plasma von Willebrand factor antigen) and plasma fibrinogen were measured at the beginning and after 12 weeks of treatment. The white cell count and serum lipids were also measured. Nicotine significantly lowered plasma fibrinogen but did not affect markers of platelet activation, endothelial damage, white cell count and serum lipids. The possibility that transdermal nicotine may beneficially influence cardiovascular risk factors warrants further exploration.

Debate about the safety of nicotine formulations which are widely available for cessation of smoking is clouded by the historical association of nicotine with smoking. This 'tarnished' image of nicotine may not be justified. We have recently had a unique opportunity to examine the effects of nicotine in a group of patients who were non-smokers, some of them lifelong non-smokers without previous nicotine exposure.

Smoking is associated with an increased risk of cardiovascular events due to thrombosis and atherogenesis [1,2]. Various measures of platelet function, coagulation, endothelial damage and serum lipids may be abnormal in smokers and could account for the increased risk [3–7]. We therefore wondered whether the use of transdermal nicotine patches as treatment for cessation of smoking might increase the risk of cardiovascular damage. In one study, some measurements

returned to normal in subjects who stopped smoking while wearing the patches for a few days [8].

We have recently noted clinical benefit from transdermal nicotine in patients with active colitis, all of whom were non-smokers [9,10]. Should such therapy become established in the future, it would be important to identify both benefit and risk. A series of measurements has therefore been made relating to haemostatic function in patients with ulcerative colitis who were given transdermal nicotine patches or identical placebo in a clinical trial to assess the value of nicotine in maintaining clinical remission in colitis.

Materials and methods

Forty-five patients (mean age 40, range 20–60; 27 women, 18 men) with ulcerative colitis who were in full remission without any disease activity were invited to participate in a randomised controlled trial. All were non-smokers, 18 being ex-smokers. None had a history of cardiovascular disease or had taken aspirin or non-steroidal anti-inflammatory drugs during the previous month.

Blood samples were taken at the start and after 12 weeks' treatment with either transdermal nicotine (20 patients) or identical placebo (25 patients). At the time of the first measurement all patients were taking 5-aminosalicylic acid (5-ASA) as maintenance therapy for their colitis (mesalazine: median dose 1,600 mg, range 400–4,600 mg). The initial dose of nicotine of 2.5 mg was increased every four days over three weeks to a maximum dose of 15 mg. Patches were applied at 7 am and removed at bedtime to establish a nicotine-free period at night. Three to four weeks after starting the transdermal nicotine, the 5-ASA was tailed off over five days, but nicotine was continued daily for the subsequent five months. Placebo and nicotine patches were identical in appearance.

Patients were given a 24-hour telephone number to contact the physician should problems arise, and contact was made regularly by phone at the end of weeks one, two and six to ensure that any difficulties were resolved and that patients were using the patches as intended. Side-effects were recorded by patients on a diary card and by the physician at the time of each phone contact. Blood samples were taken without using a cuff in the afternoon at the same time, usually 8–10 hours after the patch had been applied, the time of maximum nicotine levels. To determine whether any patient had resumed smoking, breath carbon monoxide was measured with a Bedfont MicroSmokerlyzer at each visit.

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Haematological analysis

Haemoglobin, white cell count, platelet count and mean platelet volume were measured in K2EDTA blood using a Technicon H*1 analyser (Bayer Diagnostics, Basingstoke, UK). Samples were kept upright at room temperature prior to testing and were assayed between 30 minutes and four hours after venepuncture. Previous work has demonstrated the stability of mean platelet volume estimations under these conditions [11].

Plasma von Willebrand factor antigen (VIII/vWF) was assayed in citrated plasma by enzyme-linked immunoadsorbent assay, using Dako rabbit anti-human von Willebrand factor (vWF) AO82 (Dako A/S, Glostrup, Denmark), lyophilised normal plasma (Biomerieux, 69280 Marcy l'Etoile, France), reference plasma (National Institutes of Biological Standards and Controls 91/516) and standard methods.

Platelet P selectin (CD62) was estimated in freshly drawn blood in 1:9 citrate as the anticoagulant by flow cytometry using a FACScan (Becton-Dickinson, San Jose, CA, USA). Methods and reagents used were as described [12].

Plasma fibrinogen was assayed by the Clauss method on citrated plasma [13].

Biochemical analysis

Serum cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were measured in clotted blood using a Hitachi 747 analyser (Boehringer Mannheim, UK).

Nicotine and cotinine samples were stored at -20°C until analysis [14].

Statistical analysis

Demographic data for groups were compared using a two-tailed, unpaired t-test for interval data and a chi-squared test for proportions. Haematological and biochemical variables in the nicotine and placebo groups were compared by analysis of covariance with adjustment to the baseline as a covariate. When adjustment to baseline covariate was not predictive, an unpaired t-test was used.

Results

Demographic details were similar for each group (Table 1). The mean levels of serum nicotine and cotinine achieved in those using nicotine patches were similar to smokers of 10 cigarettes per day. The effects of nicotine on haematological variables and serum lipids are shown in Table 2. The level of fibrinogen was significantly lower in those given nicotine than in the

Table 1. Details of 45 patients with ulcerative colitis in remission, all non-smokers, 20 of whom were given active transdermal nicotine patches and 25 placebo patches in a clinical trial (means (SD)).

| Patient details | Transdermal nicotine patch (n = 20) | | Placebo patch (n = 25) | |
|-------------------------|-------------------------------------|--------|------------------------|--------|
| | Mean | (SD) | Mean | (SD) |
| Male/female | 8/12 | | 10/15 | |
| Age (years) | 41.6 | (7.8) | 39.7 | (10.6) |
| Height (cm) | 165 | (8) | 170 | (9) |
| Weight (kg) | 70 | (17) | 75 | (12) |
| Nicotine levels (ng/ml) | 10.3 | (4.6) | 0.21 | (0.2) |
| Cotinine levels (ng/ml) | 101.4 | (42.6) | 0.88 | (0.9) |

placebo group at 12 weeks. Transdermal nicotine had no effect on platelet activation as measured by platelet volume and platelet activation antigen expression of P selectin (CD62), or endothelial damage, as measured by the plasma levels of VIII/vWF. The full blood count, in particular the white cell count, and the lipid profile were unaffected by nicotine.

Most patients were able to tolerate 15 mg of nicotine daily without difficulty; two patients in each group had a lower dose due to side-effects, two in the nicotine and one in the placebo group receiving 10 mg, and one in the placebo a 5-mg patch. Side-effects were more common in the nicotine group but not confined to it, suggesting that the 'blinding' was effective. Eleven in the nicotine group reported side-effects in contrast with seven on placebo. The most common side-effects in the nicotine and placebo groups respectively, were nausea (7, 1), lightheadedness (3, 4) and itch at site of patch (3, 0). There were also some miscellaneous side-effects: in the nicotine group, bad taste in mouth (1), vivid dreams (2), mouth ulcers (1); in the placebo group, single patients complained of vertigo, skin rash or headache.

Discussion

Our observations are the first to be obtained on the effect of prolonged administration of transdermal nicotine in non-smokers. The principal finding was that plasma fibrinogen was lower in those given nicotine than in the placebo group. There was no significant change in the white cell count or in markers of haemostatic function which indicate platelet activation (platelet volume and expression of P selectin, CD62) or endothelial damage (VIII/vWF). Nicotine appeared to have no effect on serum lipids.

Any observations which seek to relate abnormal

Table 2. Comparison of changes in patients with ulcerative colitis given transdermal nicotine or placebo. Analysis of covariance compares changes with treatment using baseline changes as covariate.

| Variable | Initial | | | | At 12 weeks | | | |
|--|----------|-------------|----------|-------------|-------------|-------------|----------|-------------|
| | <i>n</i> | Nicotine | <i>n</i> | Placebo | <i>n</i> | Nicotine | <i>n</i> | Placebo |
| Haemoglobin (g/dl) | 20 | 13.7 (0.8) | 25 | 13.9 (1.9) | 20 | 13.7 (0.9) | 25 | 13.7 (1.3) |
| White cell count (10 ⁹ /l) | 20 | 7.3 (1.8) | 25 | 7.3 (1.7) | 20 | 7.7 (1.8) | 25 | 7.8 (2.3) |
| Platelet count (10 ⁹ /l) | 20 | 283 (59) | 25 | 296 (77) | 20 | 286 (58) | 25 | 299 (84) |
| Mean platelet volume (fl) | 19 | 9.0 (0.6) | 24 | 8.9 (0.6) | 16 | 9.0 (0.7) | 25 | 8.9 (0.6) |
| P selectin# (% of cells positive for CD62) | 16 | 0.58 (0.43) | 20 | 0.49 (0.31) | 20 | 0.52 (0.32) | 24 | 0.56 (0.33) |
| von Willebrand Ag (IU/dl)# | 20 | 132 (72) | 24 | 119 (49) | 17 | 105 (40) | 25 | 116 (46) |
| Fibrinogen (g/l) | 20 | 2.53 (0.75) | 24 | 2.44 (0.82) | 17 | 2.14 (0.48) | 25 | 2.58 (0.79) |
| Triglycerides (mmol/l) | 20 | 1.6 (0.7) | 24 | 1.6 (1.2) | 19 | 1.7 (1.1) | 21 | 2.2 (1.3) |
| Cholesterol (mmol/l) | 20 | 5.2 (0.8) | 24 | 5.3 (1.0) | 19 | 5.2 (0.8) | 21 | 5.1 (0.9) |
| HDL cholesterol (mmol/l) | 20 | 1.3 (0.4) | 24 | 1.4 (0.5) | 19 | 1.2 (0.4) | 21 | 1.3 (0.5) |

#when the baseline covariate was not predictive, an unpaired t-test was used.

*significance set at $p = 0.050$.

†Non-adjusted difference.

HDL = high-density lipoprotein.

measurements of coagulation or haemostatic function to the increased risk of clinical thrombotic events are fraught with difficulties. With the exception of fibrinogen [15,16], few haemostatic measurements have been related to the subsequent onset of cardiovascular disease in prospective studies, so that the absence of differences in the other factors measured may or may not be of biological significance. Epidemiological evidence shows that fibrinogen is not only increased in smokers [5], but it is also an independent risk factor for cardiovascular disease [17]. The possible mechanisms involved in the atherogenic process are unclear but fibrinogen strongly affects blood coagulation, blood rheology and platelet aggregation and has direct actions on the vessel walls [18]. There are reports of abnormalities in platelet activation and endothelial damage in smokers [3–5] and other groups with an increased risk of thrombosis [19–21], but it is unclear whether these changes are directly responsible for the higher risk of thrombosis.

Our aim was to identify whether transdermal nicotine produced changes in some of the values in patients with colitis who were in full remission. It cannot be assumed that the basal values would be normal, but in the absence of any evidence of disease activity abnormalities would not be expected. We recognise the limitations of our measurements against the background of less than definitive evidence of consistent change in values from measurements on smokers and clinical groups; there is considerable variation in

results from different studies comparing smokers and non-smokers which may be partly accounted for by differences in the time at which blood was taken. However, measurements of platelets and their function which have been associated with 'platelet activation', include increased platelet volume or size, measures of eicosanoids [3], changes in plasma alpha-granule constituents [8] and the expression of surface markers such as P selectin (CD62). The surface marker, P selectin, is one of the most recently described measurements to indicate platelet activation [21]. Elevated levels of plasma fibrinogen and serum lipids are well documented in smokers [5,6] and in those with an increased cardiovascular risk [17], which is why we measured these factors. VIII/vWF antigen levels are elevated in patients with endothelial damage, particularly those with Raynaud's disease [22]. Raised levels have also been documented in smokers [5] and in those with cardiovascular disease [23].

The risks of cardiovascular thrombosis associated with smoking may not necessarily apply to transdermal nicotine. Contrary to expectation, we demonstrated that fibrinogen decreased in those given nicotine. This is particularly interesting in view of the strong association of hyperfibrinogenaemia with cardiovascular risk and smoking, but the mechanism by which nicotine decreases fibrinogen is unclear and warrants further investigation. Our study and others [8] demonstrate that transdermal nicotine does not produce platelet activation, endothelial damage or the changes in lipid

| Adjusted difference (active-placebo) | 95% confidence interval (for the difference) | t-ratio | p value |
|---|--|---------|---------|
| +0.14 | -0.32 to +0.56 | +0.59 | 0.55 |
| -0.03 | -1.0 to +0.97 | -0.07 | 0.95 |
| -1.8 | -27.4 to +23.6 | -0.15 | 0.88 |
| -0.017 | -0.32 to +0.28 | -0.12 | 0.91 |
| †-0.04 | -0.16 to +0.24 | - | 0.69 |
| †+11.3 | -16.1 to +38.8 | - | 0.41 |
| -0.45 | -0.81 to -0.09 | -2.51 | 0.016* |
| -0.38 | -0.92 to +0.16 | -1.36 | 0.18 |
| +0.27 | -0.09 to +0.63 | +1.47 | 0.15 |
| -0.056 | -0.42 to +0.30 | -0.48 | 0.63 |

profile which are commonly seen in smokers. Benowitz and colleagues [8] measured changes in eicosanoid and platelet function over five days, during which subjects smoked 22 cigarettes daily. All the abnormalities other than an elevated white cell count returned to normal in the subsequent five days when the subjects did not smoke but wore nicotine patches, showing that transdermal nicotine did not maintain the changes induced by smoking.

Whatever limitations may be attributed to such a study, the practical difficulties of studying the effect of giving up smoking in any group must be taken into account. A role for nicotine cannot be fully excluded because of pharmacodynamic considerations. The mode of delivery may be important: smoking results in bolus release of nicotine with high peak plasma levels which are associated with catecholamine release [8]. Slow-release transdermal nicotine does not show the same effect. In humans, intravenous infusion of adrenaline increases plasma beta-globulin levels indicating *in vivo* platelet activation [24]. High peaks of nicotine achieved through smoking may exert their effect through catecholamine release. Although nicotine may be responsible for some of the changes observed in smokers, there is little supporting evidence. Nicotine is rarely administered to non-smokers, which makes it difficult to study its effects other than during smoking cessation—which is itself associated with changes in serum lipids and haematological measurements which may confound results [25,26]. *In vitro*

work has shown that platelet aggregation may be altered by high concentrations of nicotine but not at the concentrations normally seen in smokers [27]. However, enhanced platelet aggregation in smokers does correlate with plasma nicotine concentrations [28], and there were more circulating 'platelet aggregates' after smoking conventional cigarettes than cigarettes that did not contain tobacco [29]. Nicotine consumed in the form of chewing gum [30] or smokeless tobacco [31] has no effect on platelet aggregation or activation. Our results support these findings.

Smoking alters the lipid profile to one associated with a greater risk of atherosclerosis. This effect might be mediated by nicotine releasing free fatty acids, thus increasing the synthesis of triglycerides and very low density lipoproteins by the liver, which in turn decrease HDL production [7]. Our study showed no important changes in the lipid profile.

In conclusion, transdermal nicotine lowered plasma fibrinogen levels and had no adverse effect on markers of platelet activation, endothelial damage and serum lipids. Thus, the possibility that transdermal nicotine may be used to reduce one of the cardiovascular risk factors warrants further investigation.

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