

Effects of smoking on the urine excretion of oral ⁵¹Cr EDTA in ulcerative colitis

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Abstract

Background—Smokers have a reduced risk and ex-smokers an increased risk of ulcerative colitis (UC). Stopping smoking often precedes onset and relapses. Smoking reduces the 24 hour urine excretion of oral chromium-51 labelled EDTA in healthy individuals.

Aims—To estimate the effects of smoking on the urine excretion of oral ⁵¹Cr EDTA in well characterised patients with UC.

Subjects—Sixteen smoking and 16 non-smoking patients with UC in remission were studied. The non-smokers had never smoked. Most were taking 5-aminosalicylic acid. No patient took steroids or immunosuppressants. The control group comprised 25 smoking healthy volunteers and 25 who had never smoked. The median cigarette consumption was equal in the patients and volunteers.

Methods—The 24 hour urine excretion of oral ⁵¹Cr EDTA was measured and the results were correlated with smoking habits, number of cigarettes, and disease extent.

Results—Patients with UC had significantly higher 24 hour urine recoveries than healthy controls ($p=0.04$). This difference was more pronounced when patients who smoked were compared with healthy smokers ($p=0.005$). No significant differences were found when comparing non-smoking patients with non-smoking controls or when comparing smoking and non-smoking patients. Urine recoveries did not correlate with number of cigarettes or disease extent. Smoking was more prevalent in patients with a more limited disease extent ($p=0.033$).

Conclusions—Effects of smoking on the urine excretion of ⁵¹Cr EDTA in health were abolished by the presence of UC. The protective effects of smoking in established UC are not due to a moderating effect of smoking on intestinal permeability.

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Keywords: ulcerative colitis; smoking; intestinal permeability; ⁵¹Cr EDTA

It is now well established that smoking habits are associated with both the onset and progress of inflammatory bowel disease. A number of studies have consistently shown that smoking is negatively associated with ulcerative colitis (UC) and positively with Crohn's disease (CD).¹ Smoking cessation often precedes the

onset or relapse of UC.² So far, no biological explanation of these findings has been presented.

The findings have prompted us to hypothesise that smoking may affect mucosal resistance to foreign, potentially disease provoking substances in the gut lumen. Study of the urine excretion of orally given, inert water soluble test substances such as chromium-51 labelled EDTA and polyethylene glycol 400 (PEG 400) is a way of estimating mucosal integrity. We have previously described that smoking in healthy individuals reduces the urine excretion of orally given ⁵¹Cr EDTA but not of PEG 400.³ With the knowledge of the different ways in which the probes are absorbed,⁴ these findings led us to believe that smoking induces an effect on tight junctions and/or colonic absorption.

Studies in UC on the urine excretion of these substances after an oral load, not considering smoking status, indicate that the PEG absorption is uninfluenced by UC.⁵ Both normal and increased permeability to ⁵¹Cr EDTA have been found.^{6,7}

The aim of the present study was to estimate effects of smoking on the urine excretion of orally given ⁵¹Cr EDTA in well characterised patients with UC and to compare it with effects in healthy individuals.

Materials and methods

Thirty two patients (16 men and 16 women, median age 41 years, range 22-70 years) with UC were studied. All patients were in remission as judged from present clinical history and from laboratory tests. UC was endoscopically confined to the rectum or distal colon in 12 patients and 20 patients had more extensive disease.

Sixteen patients (eight men and eight women) smoked cigarettes (median 12 per day, range 3-30) and 16 had never smoked. No ex-smokers were included. Twenty two patients (11 smokers and 11 non-smokers) took sulphasalazine (1-4 g per day), five took olsalazine (three smokers and two non-smokers), and five took no drugs (two smokers and three non-smokers). No patient was taking steroids or immunosuppressants. The patients had a median disease duration of 10 years (range 0-37).

The extent of the disease was determined by x ray examination of the colon and/or colonoscopy with biopsy examinations. Disease extent had previously been established by the most extensive macroscopic findings of colonic inflammation either in active stage during

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Table 1 Twenty four hour urine recovery of ^{51}Cr EDTA as a percentage of oral dose in patients with ulcerative colitis and healthy controls

	n	^{51}Cr EDTA	
		Median (%)	Range
Patients	32	2.6	0.6–5.9
Controls	50	2.0	0.8–5.8

$p=0.04$, Wilcoxon's rank sum test.

Table 2 Twenty four hour urine recovery of ^{51}Cr EDTA as a percentage of oral dose in smoking patients with ulcerative colitis and healthy controls

	n	^{51}Cr -EDTA	
		Median (%)	Range
Smoking patients	16	2.7	0.9–4.6
Smoking controls	25	1.6	0.8–4.2

$p=0.005$, Wilcoxon's rank sum test.

medical treatment, during recovery, or in quiescent stage. The diagnosis was based on clinical history, morphological investigation, and biopsy examinations. No patient had signs of small bowel inflammation as judged by x ray examination of the small bowel.

The control group comprised 50 healthy volunteers (14 men and 36 women, median age 33 years, range 19–57 years). Twenty five subjects (seven men and 18 women) smoked cigarettes (median 12 per day, range 5–30) and 25 had never smoked. The findings have been described earlier.³

No subject ingested alcohol for four days before or during the study, or took non-steroidal anti-inflammatory drugs. Patients and volunteers who smoked were encouraged to smoke as usual during the test. All subjects had normal renal function as judged by plasma urea. Haemoglobin concentration, white blood

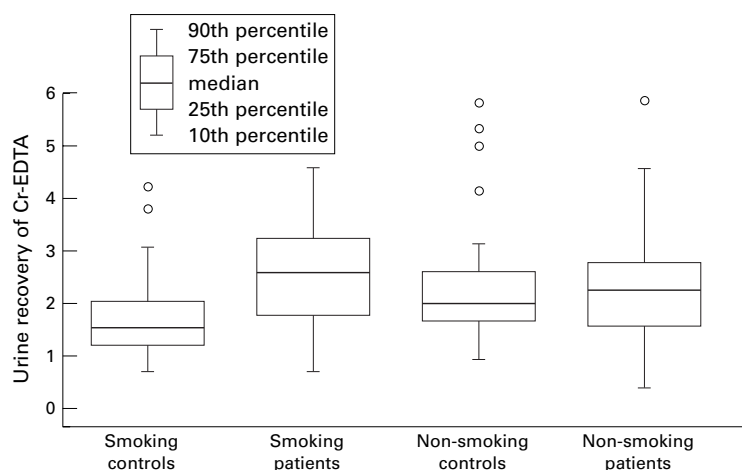


Figure 1 Twenty four hour urine excretion of ^{51}Cr EDTA (percentage of oral dose) in healthy smokers, smoking patients with ulcerative colitis, healthy non-smokers, and non-smoking patients with ulcerative colitis.

Table 3 Serum immunoglobulin values in smoking and non-smoking patients with ulcerative colitis

	Smokers (n=16)	Non-smokers (n=16)	p Value*
Serum IgA	2.0 (0.6)	2.3 (1.0)	NS
Serum IgG	10.2 (2.0)	12.2 (2.5)	0.02
Serum IgM	1.0 (0.6)	1.5 (0.6)	0.03

Results are expressed as mean (SD). *Student's *t* test.

cell count, liver function tests, serum orosomucoid, and serum IgA, IgG, and IgM concentrations were within the normal range.

^{51}Cr EDTA intestinal permeability was measured by the method of Bjarnason and colleagues⁸ as previously described.³ Careful written and oral instructions were given to the patients by the staff.

Student's *t* test, Wilcoxon's rank sum test, Fisher's exact test, and Spearman's *r* were used for statistical analysis.

The study was approved by the local ethics committee of Lund University Hospital. All participants gave written informed consent.

Results

There were significantly more men among the patients than among the controls (50% versus 28%, $p=0.04$, Fisher's exact test). In addition, the patients were significantly older than the controls (41 years versus 33 years, $p=0.0009$, *t* test). However, no significant correlation was found between age and 24 hour urine recoveries of ^{51}Cr EDTA ($p=0.98$, Spearman's $r=0.003$). The 24 hour urine recoveries of ^{51}Cr EDTA were not significantly different in men and women ($p=0.133$, Wilcoxon's rank sum test).

No significant differences in 24 hour urine recoveries of ^{51}Cr EDTA were found when comparing smoking (median 2.7, range 0.9–4.6) and non-smoking (median 2.4, range 0.6–5.9) patients with UC ($p=0.46$, Wilcoxon's rank sum test). Patients with UC had significantly higher 24 hour urine recoveries of ^{51}Cr EDTA than healthy controls ($p=0.04$, Wilcoxon's rank sum test) (table 1). This difference was more pronounced when patients who smoked were compared with healthy smokers ($p=0.005$, Wilcoxon's rank sum test) (table 2). In contrast, no significant differences were found when comparing non-smoking patients (median 2.4, range 0.6–5.9) with non-smoking controls (median 2.1, range 1.1–5.8) ($p=0.94$, Wilcoxon's rank sum test). Figure 1 summarises these results.

In the smokers, no significant correlation was found between the number of cigarettes smoked per day and 24 hour urine recoveries of ^{51}Cr EDTA ($p=0.22$, Spearman's $r=-0.14$). No differences were found when comparing ^{51}Cr EDTA and disease extent in UC ($p=0.48$, Wilcoxon's rank sum test).

Smoking was more prevalent in patients with UC with limited disease. Nine of the 12 patients with proctosigmoiditis and seven of the 20 patients with more extensive disease were smokers ($p=0.033$, Fisher's exact test). The number of cigarettes smoked per day was not significantly different in patients with proctosigmoiditis and patients with more extensive disease.

There were no differences in laboratory test results in non-smoking patients and non-smoking controls. Smoking patients with UC had significantly lower serum levels of IgG and IgM but not IgA compared with non-smoking patients ($p=0.02$ and 0.03 respectively, *t* test) (table 3). Smoking controls had significantly

lower levels of IgG and IgA but not IgM compared with non-smoking controls ($p=0.03$ and 0.02 respectively, t test).

Discussion

The present study showed that the urine excretion of ^{51}Cr EDTA after an oral load was not significantly different in smoking and non-smoking patients with clinically inactive UC. Patients with UC had slightly higher urine recoveries of the probe than healthy controls. Smoking patients with UC had highly significantly increased urine recoveries of ^{51}Cr EDTA compared with smoking controls. No such difference was observed when comparing non-smoking patients with UC with non-smoking controls, however.

In contrast to the present findings in patients with UC, we previously showed lower urine recoveries of ^{51}Cr EDTA in healthy smokers than in healthy non-smokers. However, smoking habits did not seem to influence urine recoveries of another probe, PEG 400, also used in this study in volunteers.³ Smoking accelerates gastrointestinal transit in healthy persons. If the effect of smoking was due to change in transit time, urine recoveries of the two probes should have been similarly influenced. Therefore, we have interpreted the effects of smoking observed in healthy volunteers as due to reduced intestinal permeability to ^{51}Cr EDTA. The difference between urine recoveries of the probe in smoking patients with UC and in healthy smokers is suggestive of a true effect of the disease itself on the permeation of the probe. This is contradicted, however, by the finding that the urine excretion of ^{51}Cr EDTA in non-smoking patients with UC did not differ from that of healthy non-smokers.

^{51}Cr EDTA is one of the few permeability probes to be absorbed in the colon after oral administration.⁹ The method demands meticulous 24 hour urine collection. We informed and applied routines in the same way as in our previous study, which showed no correlation between urine volumes and ^{51}Cr EDTA excretion.³

Few studies have been performed in UC using the ^{51}Cr EDTA intestinal permeability test; one showed increased excretion of the probe⁷ and the other did not.⁶ Both colonic inflammation and steroid therapy could theoretically increase intestinal permeability to ^{51}Cr EDTA. Any effect of such factors was excluded in our study as the patients were studied when in clinical remission and none were taking steroids.

Disease extent could possibly influence intestinal permeability to ^{51}Cr EDTA in UC. The patients with UC who smoked had a more limited disease extent than the non-smokers, as previously reported by Samuelsson *et al.*¹⁰ Furthermore, ^{51}Cr EDTA recoveries in urine were similar in patients with proctosigmoiditis and in those with more extensive disease.

Active inflammation in the colon could facilitate permeation of ^{51}Cr EDTA and thus increase urine recovery. Our patients were asymptomatic and had normal laboratory markers of inflammation but they were not routinely examined by endoscopy at the time of the test. Therefore, patients with subclinical inflammation may have been included and this might possibly explain the slightly increased urine recoveries of ^{51}Cr EDTA in the patients with UC compared with the healthy controls. Differences in cigarette exposure, age, and sex in patients and controls were also ruled out as an explanation for the different effects of smoking on urine recovery of the probe.

Further biological effects of smoking in UC were found in this study. Smoking patients with UC had significantly lower levels of circulating immunoglobulins than non-smokers with UC. This difference was also present in the healthy controls. These findings are most likely due to the immune modulating effects of smoking as reviewed by Holt.¹¹

In conclusion, we have not been able to show that the protective effects of smoking in UC are due to a moderating effect of smoking on intestinal permeability. The tightening effects of smoking in health were abolished by the presence of the disease. One might speculate that smoking could prevent the onset of UC in a predisposed individual through a tightening effect on the colonic mucosa and this effect could be important in the pathogenesis of the disease. In established UC, smoking exerts protective effects against relapses through other modes of action. Such potentially protective mechanisms in established UC induced by smoking—for example, immunosuppression, should be studied.

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