Pharmacokinetics of nicotine carbomer enemas: A new treatment modality for ulcerative colitis

Background: Ulcerative colitis is largely a disease of nonsmokers, and transdermal nicotine is of therapeutic value in the active disease. Because side effects are common, we developed a topical enema formulation of nicotine.

Objective: To study the pharmacokinetics of nicotine complexed with a polyacrylic carbomer and administered by enema to eight healthy volunteers and to eight patients with active ulcerative colitis, verified sigmoidoscopically.

Patients and methods: All 16 subjects were nonsmokers. The mean age for normal subjects was 33 years; the mean for patients with ulcerative colitis was 60 years. Median stool frequency for patients with ulcerative colitis was four daily. Patients were taking 5-amino salicylic acid compounds and five were taking oral prednisolone (median dose, 12 mg daily). Nicotine, 6 mg, complexed with carbomer 974P, 400 mg, was administered in a 100 ml enema after an overnight fast, with serial blood measurements taken over 8 hours. Serum nicotine and cotinine were measured by gas liquid chromatography. Area under the concentration–time curves were calculated by the trapezoidal method, and the terminal elimination half-life was derived by extrapolation of the log-linear terminal phase.

Results: With the exception of nicotine time to reach peak concentration, which was longer in patients (median of 60 minutes compared with 45 minutes; $p < 0.005$), other comparisons between normal subjects and patients showed no statistically significant difference, although there was considerable inter-subject variation. Maximum concentration of nicotine, 8.1 ± 3.5 ng/ml, in the 16 subjects occurred after a median of 60 minutes (range, 30 to 180 minutes); maximum cotinine concentrations of 60.4 ± 11.5 ng/ml occurred after 4 hours. Side effects in five subjects were mild (four subjects) or moderate (one subject) and included lightheadedness, nausea, and headache; these five subjects were female lifelong nonsmokers of low body weight.

Conclusion: Because most of the active ingredient of nicotine is converted to cotinine on the first pass through the liver, substantial concentrations can be achieved at the site of disease with only modest rises in serum nicotine, which are responsible for side effects; cotinine has low pharmacologic activity. Topical administration of nicotine may be useful treatment for distal ulcerative colitis. (Clin Pharmacol Ther 1997;61:340-8.)

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Ulcerative colitis is largely a disease of nonsmokers; exsmokers usually develop their colitis in the early years after cessation.[1-5] Intermittent smokers often experience improvement in their colitis symptoms while smoking.[6-7] Transdermal nicotine patches added to conventional treatment with 5-aminosalicylic acid (5-ASA) have a beneficial effect on active colitis,[8-10] but were were shown to be of no benefit for maintenance of remission in the only published study.[11] Because 15 mg transdermal nicotine causes substantial side effects in up to two-thirds of patients,[9-11] particularly those who are lifelong nonsmokers, attempts have been made to develop a therapy for colitis with a formulation that is effective and better tolerated; this may be achieved by topical application of nicotine to large bowel mucosa. This makes it possible to administer high doses of nicotine at the site of disease with only moderate rises in the systemic level, which would limit side effects. The reduced systemic concentrations of nicotine partly the result of 60% conversion to cotinine during the first pass through the liver.[12] Venous drainage from the upper two-thirds of the rectum is to the portal circulation; a variable part of the lower third flows to the vena cava.

We have combined nicotine with a polyacrylic carbomer for administration as an enema in ulcerative colitis. The carbomer should both facilitate mucosal adherence[13] and delay release of nicotine before mucosal absorption; as a consequence, side effects should be reduced because the rate of rise of nicotine and the maximum plasma level largely determine their occurrence. Serum levels of both nicotine and cotinine were measured after an enema in healthy volunteers and in patients with active ulcerative colitis with documentation of side effects.

METHODS

Subjects. Eight normal healthy volunteers and eight patients with active ulcerative colitis, all nonsmokers were enrolled in this open-label single-dose study; their characteristics are listed in Table I. Six of eight normal subjects were lifelong nonsmokers, compared with two of eight patients with colitis. All subjects gave written informed consent before participation in the study, which was approved by the ethics committee of Bro Taf Health Authority. All had a physical examination and were free from significant abnormal findings on assessment of vital signs; clinical laboratory tests for biochemical and hematologic profiles were also normal. Excluded were subjects with a known sensitivity to nicotine, those with a history or evidence of significant medical disease, subjects who might have been pregnant or lactating, and those with coexisting gastrointestinal disease other than ulcerative colitis or a previous resection of the gastrointestinal tract. Severity of colitis was based on sigmoidoscopic appearance; patients included in this study had visible contact hemorrhages or more severe changes, grade 2 or more according to Dick et al.[14] The median sigmoidoscopic score was 2 (range, 2 to 3), and the median stool frequency was four per day (range, 1 to 12). All patients were taking additional therapy; eight subjects were taking 5-aminosalicylic acid compounds, five were taking oral steroids (with a mean dose of 12 mg prednisolone daily; range, 5 to 20 mg), and four were taking steroid enemas.

Table I. Characteristics of subjects enrolled in the study

Characteristic	*Normal subjects (n = 8)*	*Patients with ulcerative colitis (n = 8)*
Males	3	5
Females	5	3
Age (yr)		
Mean	33	60
Range	21-46	34-82
Height (cm)		
Mean	169	169
Range	160-180	159-180
Weight (kg)		
Mean	66	74
Range	57-76	52-102
Smoking history		
Lifelong nonsmoker	6	2
Exsmoker	2	6

Characteristics of eight normal healthy volunteers and eight patients with active ulcerative colitis given the nicotine carbomer enema.

Formulation of nicotine carbomer enemas. Nicotine was first complexed with a carbomer before its administration as an enema (Fig. 1). Carbomers are synthetic high molecular weight polymers of acrylic acid, cross-linked with allylsucrose and containing 56% to 68% carboxyl groups.[15] The nitrogen on the pyridine ring of nicotine, pKa 6.16, has a lone pair of electrons, and it is this that is expected to be involved in the complex formation with anionic carboxyl groups of the polyacrylate.

Carbopol 974P (Goodrich U.K.) was the chosen carbomer used. The complex was made to ensure muco-adherence to large bowel mucosa, as well as production of both delayed and sustained absorption of free nicotine when released from the com-

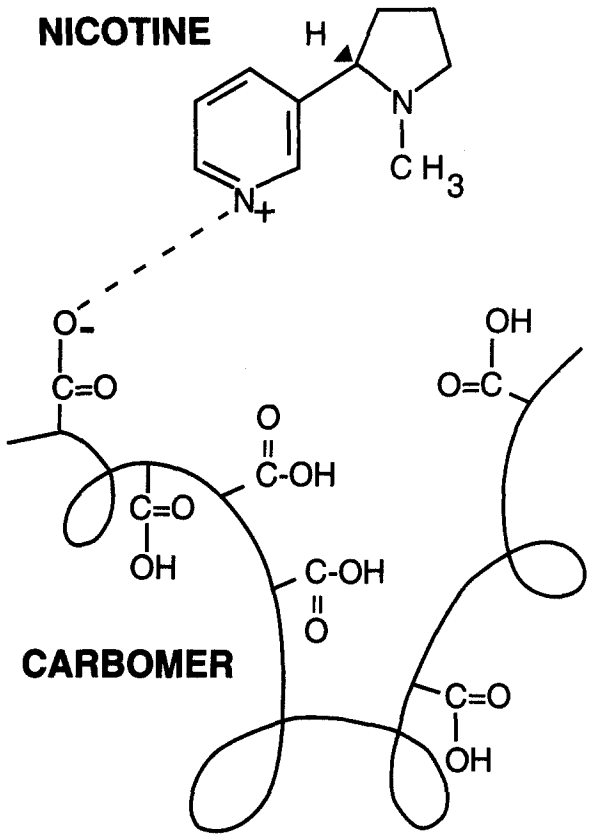

Fig. 1. Diagrammatic representation of the nicotine carbomer complex; the amino group on the pyridine ring is bonded with a carboxyl group on the carbomer.

plex. The neutralized carbomer molecule uncoils into an extended structure that is very hydrophilic and produces a gel with strong muco-adhesive properties.

Fifty grams of Carbopol powder was dispersed in 2500 ml of deionized water and rapidly stirred in a suitable mixer with a blade-type impeller. An homogenizer is unsuitable because at high speed it may shear the carbomer molecule. The powder was slowly sieved into the vortex created by the stirrer, allowing the powder to wet without producing insoluble particles. The colloidal suspension was formed while stirring at a slower speed over 30 minutes.

One gram of *l*-nicotine base as the oil (Sigma Chemical Co.) was diluted with 1 ml of absolute alcohol, which was then added drop-wise into the vortex of the suspension, with continuous stirring for 1 hour. Some of the complex was then freeze dried for later analysis.

Enemas were formulated which contained 2, 6, and 12 mg nicotine, 400 mg Carbopol, 100 mg xanthan gum (Keltrol) to increase viscosity, 150 mg methyl hydroxybenzoate, and 15 mg propyl hydroxybenzoate and deionized water to make up to 100 ml; phosphate buffer was added to produce a final pH of 5.5, with the effect of increasing the stability of nicotine. The nicotine content of sample enemas was first confirmed by diluting a small volume of the contents in dilute hydrochloric acid to produce an approximate concentration of 30 ng/ml, which could then be accurately measured by our assay.

Chemical composition of nicotine carbomer. Fourier transform infrared (FTIR) spectroscopy was performed to analyze freeze-dried nicotine carbomer complex and the starting materials, *l*-nicotine base and Carbopol 974P. The absorbencies of these materials were consistent with the presence of a new compound, not merely a mixture of the starting materials. Thin layer chromatography with spots of the free materials and the complex showed the *l*-nicotine moved freely and the polymer was immobile, whereas nicotine in the carbomer complex remained largely confined to the baseline spot.

^{1}H-Nuclear magnetic resonance (NMR) was of only limited value because of the large amount of water present, the relatively small proportion of nicotine, and the high viscosity. Nevertheless, the analysis showed considerable differences in ^{1}H resonances arising from nicotine in the carbomer, compared with the ^{1}H-NMR spectrum of free nicotine alone. The most noticeable chemical shift differences were with the aromatic protons associated with the pyridine ring. In the free nicotine they account for the signals at 8.18 δ, 7.50 δ, and 7.15 δ. However, in the complexed nicotine these shifted to 8.85 δ, 8.60 δ, and 8.05 δ, respectively—a change best accounted for by ring-current changes in the pyridine ring associated with protonation of the nitrogen.

Drug administration and blood sampling protocol. During preliminary dose-ranging observations with two subjects, no side effects were observed with 2 and 6 mg nicotine, but marked symptoms of nausea and lightheadedness occurred after 15 minutes with 12 mg. On this basis the 6 mg dose was chosen for subsequent observations in all 16 subjects. It was administered after a 10-hour fast at 9 AM after it was first warmed to body temperature; it was given slowly over 4 minutes while the subject was in the left lateral decubitus position. Blood was taken from an indwelling venous cannula at times 0, 5, 15, 30, 45, 60, 120, 180, 240, 300, 360, and 480 minutes; serum was obtained by centrifugation was stored at

−20° C before analysis. Subjects remained horizontal for 2 hours, after which they mobilized. The serum nicotine and cotinine levels were measured by gas liquid chromatography.[16]

Pharmacodynamic assessment. Side effects experienced by the subjects were recorded as absent, mild, moderate, or severe. Subjects were asked to report the time, nature, and severity of any symptoms at the beginning of the study and at each hour through the study, and they were questioned particularly about nausea, vomiting, lightheadedness, tremor, palpitations, and headache. Blood pressure and pulse rate were also recorded each hour and when any symptoms occurred.

Pharmacokinetic and statistical analysis. Time concentration curves were generated from the data with use of the arithmetic means of the serum concentration at each time point. The peak plasma concentration (C_{max}) and concentration peak times (t_{max}) were derived directly from the original measured variables. The area under the concentration-time curves (in ng · min/ml) from 0 to 480 minutes [AUC(0-480)] was calculated by the linear trapezoidal method. The terminal elimination half-life ($t_{1/2}$) was derived from the slope of the log-linear terminal phase. For the nicotine data, the area under the curve from zero to infinity [AUC(0-∞)] was calculated by the trapezoidal rule and extension of the linear terminal slope. The data was also fitted to a one-compartmental model [AUC(1 co)] to compare the results that would be obtained from a model-dependent method. The pharmacokinetic analysis was performed by the Siphar computer program (Simed SA, Créteil, France).

Most comparisons were with the independent *t* test. Because the t_{max} data was not of normal distribution, differences in changes between the groups were compared by the Mann-Whitney test. A two-tailed *p* value was used with a statistical significance level of $p < 0.05$.

RESULTS

Pharmacokinetics. The mean concentration-time curves for the nicotine and cotinine levels are in Fig. 2. Both nicotine and cotinine profiles were largely similar in the normal and patient groups. The pharmacokinetic parameters are given in Table II. There were no statistically significant differences between normal subjects and patients with ulcerative colitis in any of the parameters except for the t_{max}, which gave median values of 45 and 60 minutes, respectively ($p = 0.0047$; Mann-Whitney).

Maximum mean concentrations of nicotine of 8.1 ng/ml were achieved after a median of 60 minutes in the total group of subjects. The mean $t_{1/2}$ of nicotine was 175 ± 48 minutes. Mean concentrations of cotinine, the principal metabolite of nicotine, were achieved after 4 hours. There was considerable individual variation in the profiles for both nicotine and cotinine (Fig. 3). The one outlying nicotine profile in Fig. 3, *A,* shows consistently higher concentrations. This was a 59-year-old lifelong nonsmoking woman with active ulcerative colitis and a low body weight of 55 kg.

Multiple regression analysis was not performed because of the small numbers in each group. It might be anticipated that many of the variables would be interrelated, such as weight, sex, and side effects. The higher C_{max} values for nicotine occurred in female lifelong nonsmokers with lower body weights, but no relationship was observed between this value and nicotine t_{max} or cotinine C_{max}.

Pharmacodynamics. Five of the subjects, four normal subjects and one patient reported side effects. These occurred in five of eight women and in five of the eight lifelong nonsmokers. Those with higher C_{max} values for nicotine and lower body weights were more likely to report side effects. The average onset of symptoms was about 20 minutes after administration of the enema (range, 15 to 30 minutes), and symptoms lasted for a mean of 58 minutes (range, 45 to 70 minutes). All five subjects felt lightheaded; two subjects also had nausea and one had a headache. All symptoms were mild (with the exception of one subject with moderate nausea); all symptoms were self-limiting and not associated with changes in the pulse rate or blood pressure.

DISCUSSION

In the search for a topical form of nicotine to treat ulcerative colitis, our observations on the nicotine carbomer enema are of interest. They constitute some of the first detailed pharmacokinetic data of nicotine administered by enema. Mean maximum nicotine concentrations about 8 ng/ml were achieved after 60 minutes, with an extended $t_{1/2}$ of nearly 3 hours. Mean maximum concentrations of cotinine, the principal metabolite of nicotine, were achieved at 4 hours and remained relatively constant thereafter. There was considerable individual variation

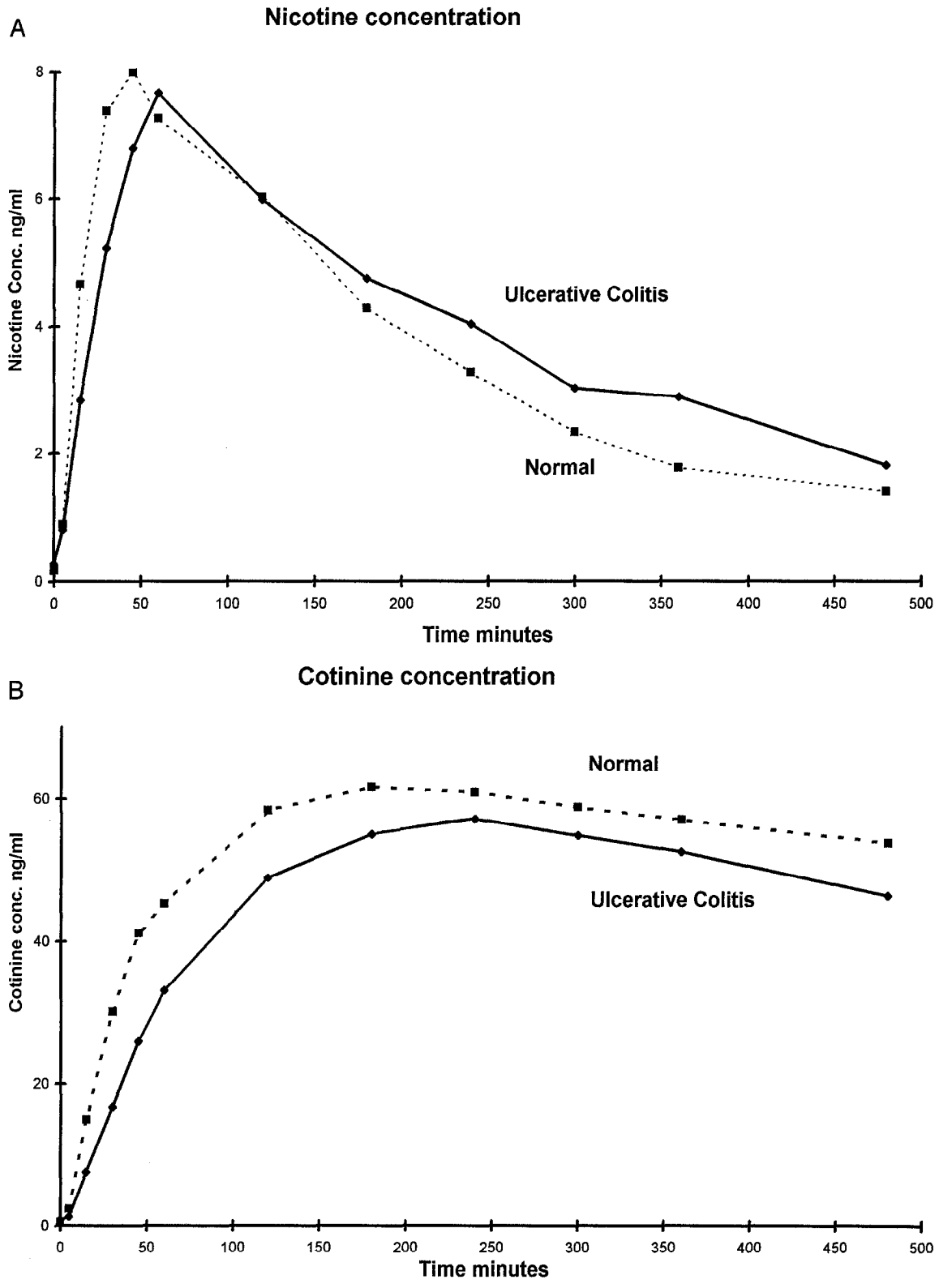

Fig. 2. Mean serum concentrations of nicotine **(A)** and cotinine **(B)**, in nanograms per milliliter, in eight healthy normal volunteers and in eight patients with active ulcerative colitis during 8 hours after administration of an enema containing 6 mg nicotine.

Table II. Pharmacokinetic results

	Normal subjects	*Patients with ulcerative colitis*	*All subjects*
Nicotine			
C_{max} (ng/ml)	8.3 ± 2.7	7.8 ± 4.3	8.1 ± 3.5
t_{max} (min)			
Median	45	60	60
Range	30-60	60-180	30-180
AUC(0-480) (ng · min/ml)	1,770 ± 635	1,902 ± 1,144	1,836 ± 897
AUC(0-∞) (ng · min/ml)	2,120 ± 819	2,444 ± 1,375	2,282 ± 1,106
AUC(1 co) (ng · min/ml)	2,059 ± 827	2,382 ± 1,404	2,221 ± 1,125
$t_{1/2}$ (min)	154 ± 42	197 ± 47	175 ± 48
Cotinine			
C_{max} (ng/ml)	62.7 ± 10.4	58.2 ± 12.9	60.4 ± 11.5
t_{max} (min)			
Median	240	240	240
Range	180-360	120-300	120-360
AUC(0-480) (ng · min/ml)	25,707 ± 4,184	21,598 ± 4,519	23,652 ± 4,712

Pharmacokinetic variables after administration of a single enema that contained 6 mg nicotine carbomer in eight healthy normal volunteers, eight patients with active ulcerative colitis, and the total group of 16 subjects.

All results expressed as mean ± SD except for t_{max}, which are given as median and range.

C_{max}, Peak plasma concentration; t_{max}, time to reach C_{max}; AUC(0-480), area under the concentration-time curve from 0 to 480 minutes; AUC(0-∞), AUC from zero to infinity; AUC(1 co), AUC fitted to a one-compartment model; $t_{1/2}$, half-life.

for values obtained with both nicotine and cotinine. Higher maximum concentrations of nicotine with side effects were more common in women, lifelong nonsmokers, and in those with low body weights. Other contributory factors may be variation in the distribution of the enema in the colon and in the proportion of rectal venous drainage, which was systemic or portal. A comparison of normal subjects and patients with active ulcerative colitis showed no statistically significant differences, although those with colitis were significantly older. Patients had an overall longer time to C_{max}, which could have been attributable to the disease process or age difference.

The findings are valid because these 16 sets of observations, with frequent blood measurements particularly in the first hour, are sufficient to identify the rapid changes in serum nicotine concentration that occur during the early rapid phase of absorption. Nicotine at pH 5.5 is stable at both room and body temperature. All measurements were made in a laboratory that has international recognition for measuring these compounds accurately and precisely for more than 20 years. Stringent precautions are taken to avoid contamination, which include a nonsmoking environment with samples handled by nonsmokers. The lower limit of detection for their analytical method is 0.1 ng/ml for both nicotine and cotinine, with an average coefficient of variation over the range from 1 to 100 ng/ml for nicotine and 1 to 1000 ng/ml for cotinine of 3.9% and 2.1%, respectively.[16]

The only comparable data available for serum levels of nicotine and cotinine after administration by enema are from the Mayo clinic.[17] In 24 healthy volunteers, 45 μg/kg nicotine tartrate, equivalent to approximately 3 mg nicotine, gave a C_{max} about 3 ng/ml. Rises in blood nicotine levels after a single cigarette ranged from 5 to 30 ng/ml, depending on how it was smoked. These concentrations rose steeply and peaked at the completion of the cigarette.[18] When 4 mg nicotine gum was chewed, peak levels of about 9 ng/ml were observed after approximately 30 minutes[18]; blood levels of nicotine in the systemic circulation were lower than might be expected because some was swallowed and only about of 72% was extracted.[19]

Orally ingested capsules that contained 4 mg nicotine base as its bitartrate salt gave peak serum concentrations of about 7.5 ng/ml after 1 hour.[20] In the trials of transdermal nicotine in ulcerative colitis, mean serum nicotine levels of 8 ng/ml were observed with 15 mg patches[11] and 13 ng/ml with doses up to 22 mg.[10] In a review of 30 current smokers with ulcerative colitis, their mean cotinine level was 220 ng/ml (range, 55 to 452 ng/ml).[21] The mean $t_{1/2}$ of nicotine is 120 minutes, but with considerable individual variation (range, 1 to 4 hours).[22] Cotinine, the main metabolite of nicotine, has low

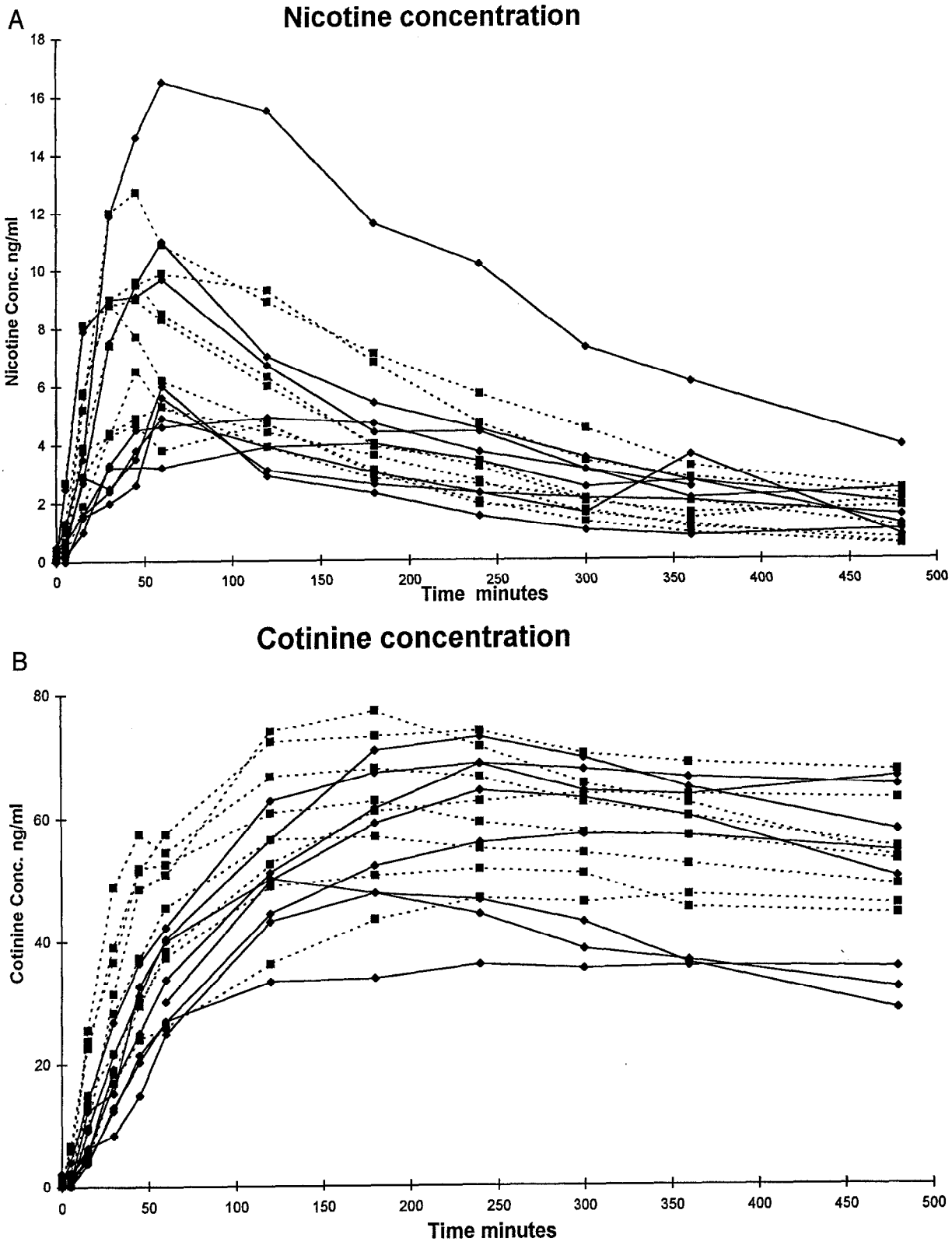

Fig. 3. Serum concentrations of nicotine **(A)** and cotinine **(B)**, in nanograms per milliliter, in eight healthy normal volunteers and in eight patients with active ulcerative colitis during 8 hours after administration of an enema containing 6 mg nicotine. *Solid lines,* Ulcerative colitis; *broken lines,* normal subjects.

pharmacologic activity and a much longer $t_{1/2}$ of 16 to 20 hours[23]; this gives some indication of overall exposure to nicotine.

Our preliminary observations with this form of topical nicotine given over 4 weeks have shown some clinical benefit in patients with active disease (Green JT, et al. Unpublished data, 1996). Despite extensive efforts to identify how nicotine may affect the course of this disease, the underlying mechanism responsible for the relationship between smoking and ulcerative colitis remains unresolved. Topical nicotine is an attractive therapeutic approach because large concentrations of the drug can be delivered to the disease site, while serum levels are only modestly elevated and most of the nicotine is converted to cotinine on first pass through the liver. The carbomer interacts with the adherent mucus layer on the surface of large bowel mucosa and therefore acts as a carrier for the nicotine molecule, bringing it into immediate contact with the inflamed mucosa. The relatively small rise in serum nicotine and extended $t_{1/2}$ may be partly caused by slower absorption from the nicotine carbomer complex over an extended period. Nicotine as a weak base in an acid environment will be more ionized; this in turn will reduce the initial bioavailability and cause delayed absorption during neutralization. Considerable individual variation in the handling of nicotine is well recognized, which makes it desirable to use the same subjects in any comparative studies with different formulations of nicotine.

The nicotine carbomer enema was well tolerated by all subjects, side effects were limited to women who were lifelong nonsmokers. The enema was also retained without difficulty in patients with active disease and may prove to be a useful therapy in this group. Because the patients with lower body weights achieved higher maximum concentrations of nicotine and then side effects; a smaller dose of nicotine calculated according to body weight would overcome the initial problems with administration. Further modifications to the enema formulation, which would delay the rate of absorption and reduce the maximum nicotine concentration, may lower the incidence of side effects. We would then propose to evaluate the efficacy of the enema formulation in randomized controlled trials.

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