The effect of nicotine on ethanol-induced gastric ulcers in rats[1]

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Summary. Nicotine, in concentrations of 5 and 25 μg/ml drinking water, given ad libitum for 10 days, dose-dependently increased lesion formation and worsened ethanol-induced ulceration in rat stomachs. Daily fluid intake and b.wt gain were not adversely affected by nicotine pretreatment.
Key words. Nicotine; ethanol; gastric ulcers.

The association between peptic ulcers and cigarette smoking is well known[2,3]. However, the relationship between smoking, ethanol consumption and peptic ulceration remains to be established. Thus, it was decided to investigate the direct influence of chronic nicotine pretreatment, as well as the interaction between nicotine and ethanol, on the gastric mucosa in rats. Some findings are reported.

Method. Male Sprague-Dawley rats (weighing 180–210 g), reared on a balanced laboratory diet (Ralston Purina Co., USA), were randomly assigned to six groups and housed in a room with controlled temperature (22 ± 1 °C) and humidity (65–70%). The daily b.wt and water intake of each rat were recorded. Two groups (total of 19 rats) received tap water to drink, another two (total of 19 rats) were given nicotine bitartrate (BDH) 5 μg/ml in their drinking water and the last two batches (total of 18 rats) drank water containing nicotine bitartrate in a concentration of 25 μg/ml. All the groups were permitted to drink ad libitum. Food, but not drinking fluid, was withdrawn on the 9th day. On the 10th day, one group from each of the duplicated pretreatment batches was given distilled water (10 ml/kg) via a stainless steel intragastric tube; all rats were killed by a sharp blow on the head 5 h later. The remaining groups from the three pairs of pretreatment batches were given 40% ethanol (BDH) v/v in distilled water, in a volume of 10 ml/kg, intragastrically; these animals were also killed 5 h later. Stomachs were removed and opened along the greater curvature. Ulcer size was measured by a grid (each grid square was 1 mm^2) placed on the glandular mucosal surface. In the case of petechiae, five such lesions were taken as the equivalent of 1 mm^2. The sum of the ulcer sizes in each group of rats was divided by the number of animals and expressed as the mean ulcer index. Data were statistically analyzed by Student's t-test or by the X^2 test.

Results. The table shows the findings with nicotine pretreatment on ethanol-induced gastric lesion formation and on daily body weight gain and fluid consumption.

Nicotine administration in drinking water for 10 days dose-dependently increased lesion incidence as well as the ulcer index in rats given distilled water intragastrically. A statistically significant effect was observed with the bigger dose of nicotine. The lesions, found in the gastric glandular mucosa, were in the form of petechiae but small hemorrhagic ulcers were occasionally seen.

Ethanol administration produced extensive hemorrhagic ulceration in the gastric glandular mucosa. This ulceration was markedly potentiated by nicotine pretreatment, reaching significance with the concentration of 25 μg/ml.

Daily b.wt gain and fluid intake were not significantly influenced by treatment with the alkaloid.

Discussion. The present study attempts to examine the gastric effects of a nicotine dose approximating the daily intake of the alkaloid in swallowed saliva and a bigger dose approaching a high level which could possibly be ingested in heavy smoking[4,5]. It clearly shows that there is a dose relationship between nicotine and the severity of gastric lesion formation in rats. The possibility of nicotine interfering with nutrition to weaken mucosal resistance to ulcer formation is unlikely, as reflected by the normal daily b.wt gain and volume of fluid intake. Previous workers[4] found that the same nicotine doses, given over a period of 22 days, did not produce any significant changes in the parietal or chief cell populations. However, these workers did not report the effects of nicotine treatment on gastric lesion formation.

Ethanol in a concentration of 40% is often consumed. This potency does indeed induce hemorrhagic gastric ulcers in rats. Also, as found in this investigation, ethanol ulceration is worsened by nicotine pretreatment. Although the volume (10 ml/kg) of this concentration of ethanol, given acutely in the present study, could be considered excessive when extrapolated to man, the findings nevertheless suggest the possibility that lower concentrations of the alcohol could produce gastric lesions more easily in smokers. Augmentation of aspirin- or reserpine-induced gastric ulcers by nicotine has also been reported in rats[6]. Nicotine administration in drinking water does not wholly reflect the effects of smoking on gastric lesion formation, because other factors, such as tar, which are also swallowed must be considered. None the less, the current findings do indeed indicate that nicotine itself has ulcerogenic potentials.

The mechanisms involved in lesion formation by nicotine itself and in its potentiation of ethanol-induced ulceration are still unclear. Further studies are in progress.

Effects of 10-day nicotine pretreatment (5 or 25 μg/ml drinking water) on ethanol-induced gastric glandular ulceration (animals killed 5 h after ethanol) and on daily b.wt gain and fluid intake

Pretreatment	No. of rats with petechiae and/or hemorrhagic ulcers	Ulcer index (mm^2)	Body weight gain (g/day) (mean ± SEM)	Fluid intake (ml/day)
A) Rats given distilled water (10 ml/kg) by intragastric tube				
Tap water only	2 (10)	0.03 ± 0.03	6.55 ± 0.58	37.80 ± 3.10
Nicotine 5 μg/ml tap water	4 (10)	0.24 ± 0.16	7.09 ± 0.44	35.51 ± 1.71
Nicotine 25 μg/ml tap water	7 (10)*	0.42 ± 0.10**	7.55 ± 0.33	39.34 ± 2.48
B) Rats given 40% ethanol (10 ml/kg) by intragastric tube				
Tap water only	8 (9)$^{+}$	25.96 ± 10.40^{++}	7.92 ± 0.27	33.87 ± 2.29
Nicotine 5 μg/ml tap water	9 (9)$^{+}$	50.44 ± 13.80^{++}	7.18 ± 0.35	34.31 ± 1.95
Nicotine 25 μg/ml tap water	8 (8)	76.75 ± 18.00*$^{++}$	6.34 ± 0.34	35.46 ± 2.54

The number of rats used in each group is shown in parentheses. * $p < 0.05$, ** $p < 0.01$ when compared with the its own control pretreated with tap water only. $^{+}$ $p < 0.01$, $^{++}$ $p < 0.001$ when compared with the corresponding group given distilled water intragastrically (A).

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Age-dependent changes of rat liver plasma membrane composition

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Summary. The chemical composition of liver plasma membrane was studied in Wistar rats aged between 3 and 24 months. Results obtained indicate a significant age-dependent positive correlation of both the protein:phospholipid and cholesterol:phospholipid ratios, whereas the protein:cholesterol ratio seems to remain unaffected. Phospholipid analysis of liver plasma membrane reveals that only the phosphatidylcholine content has a significant negative correlation with age; all other phospholipid species remain basically unchanged.
Key words. Liver plasma membrane; aging; phospholipids; cholesterol.

A number of cellular functions which involve, at least partially, the participation of the plasma membrane show age-related modifications[2,3]. Whether aging affects the gross plasma membrane composition, leading in turn to the observed modifications, is still under debate since the characteristics of many membrane-bound proteins, including enzymes and receptors, are largely influenced by the physicochemical properties of the membrane microenvironment[4].

Data to be reported here deal with some major modifications occurring in rat liver plasma membrane composition over the post-maturation period of life, spanning from 3 to 24 months. As it is well known, the hepatocyte plasma membrane is a complex organelle consisting of three functionally and morphologically distinct domains: i.e. the blood sinusoidal front, the bile canalicular front and the lateral surfaces, accounting respectively for about 72%, 13% and 15% of the total surface area[5]. As a first approach, and in order to obtain membrane preparations representative of all three domains, we chose to follow the isolation procedure recently developed by Hubbard and coworkers[5] which gives a yield of about 50% sinusoidal front membranes, whereas previous methods seem to favor an enrichment of bile canalicular and lateral membranes[5]. The present work indicates that liver plasma membrane composition is indeed affected by aging, in a fashion which is suggestive of a possible decrease of membrane lipid fluidity.

Material and methods. Male Wistar rats, aged between 3 and 24 months, fed with a standard diet, were starved 16 h before experiments. Before sacrifice, animals were anesthesized by i.p. injection of sodium pentobarbital (12 mg/kg b.wt). The average b.wts of animals employed were as follows (mean ± SD): 140 ± 35 g (3 months); 440 ± 65 g (12 months); 465 ± 40 g (18 months); 505 ± 45 g (24 months). Each age group consisted of six animals.

Livers were perfused in situ with 300 ml of cold 0.15 M NaCl before starting the plasma membrane isolation procedure according to Hubbard and coworkers[5]. Isolated liver plasma membranes were first washed with 0.015 M EDTA (pH 7.4), then resuspended and washed with 0.05 M Tris-HCl buffer (pH 7.5) containing 0.1 M NaCl, and finally resuspended in twice distilled water at a final concentration of 1.5 mg protein/ml. The purity of our plasma membrane preparations was checked by the distribution of marker enzymes (Na^+–K^+)-ATPase and 5'-nucleotidase, assayed as previously reported[6]. The average enrichment factor of specific activity in the plasma membrane fraction with respect to homogenate was 24 and 19 for (Na^+–K^+)-ATPase and 5'-nucleotidase respectively. In order to rule out a possible contamination of plasma membrane preparations by endocellular membranes, routinary assays of cytochrome oxidase, acid phosphatase and glucose-6-phosphatase were carried out following already reported procedures[7]. The average enrichment factors were 0.3, 1.0 and 0.9 for cytochrome oxidase, acid phosphatase and glucose-6-phosphatase respectively. Proteins were estimated by the method of Lowry[8] using bovine serum albumin as a standard.

Total phospholipids were extracted according to Bligh and Dyer[9] all steps being performed under nitrogen. Individual phospholipid species were separated by thin layer chromatography using silica gel G plates (Merck, Darmstadt, FRG) activated for 1 h at 120°C as previously reported in detail[10]. The average recovery was 85%, a result which remains constant with aging. Total cholesterol was determined by the cholesterol oxidase method using a high performance 'Monotest' kit (Boehringer, Mannheim, FRG).

Results and discussion. Figure 1 shows three major ratios of plasma membrane components. Protein:cholesterol ratio (fig. 1, A) remains fairly constant with aging, whereas protein:phospholipid (fig. 1, B) as well as cholesterol:phospholipid ratio (fig. 1, C) show a positive correlation with age, increasing significantly between 3 and 24 months; it is worth mentioning that an increase of the latter ratio with age has been reported so far only in human erythrocyte plasma membrane[4,11]. In particular a t-test for means obtained from data reported in figure 1, B, gave the following results: 3 months vs 12 months, n.s.; 3 months vs 18 as well as 24 months, $p < 0.001$; 12 months vs 18 months, n.s.; 12 months vs 24 months, $p < 0.01$; 18 months vs 24 months, $p < 0.01$. For data reported in figure 1, C, only the comparison between age groups 3 and 24 months gave a significant ($p < 0.001$) t-test. The phospholipid pattern of liver plasma membranes in aging Wistar rats is reported in figure 2. The phosphatidylcholine level (fig. 2, A) shows a significant negative correlation with age, whereas phosphatidylserine + phosphatidylinositol (fig. 2, B), phosphatidylethanolamine (fig. 2, C) and sphingomyelin (fig. 2, D) levels do not seem to be significantly related to age. The comparison between age groups for means obtained from data reported in figure 2, A gave the following results as for a t-test evaluation: 3 months vs 12, 18 and 24 months, $p < 0.001$; all other differences being not significant.

Our results represent, to our knowledge, the first report of the