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Nicotine and ascorbic acid effects on cold-restraint ulcers in rats[1]

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Summary. Rats were orally administered 1-ascorbic acid, nicotine, 1-ascorbic acid and nicotine, or distilled water for 10 days. Following this treatment they were fasted for 24 h and then restrained in a cold environment for 2 h. Nicotine alone produced significantly more gastric ulcers than any other treatment. 1-Ascorbic acid increased ulceration relative to controls. The combined effects of 1-ascorbic acid and nicotine resulted in reduced ulcer incidence and severity. It appears that 1-ascorbic acid and nicotine do not act synergistically to augment stress-induced gastric ulcer.

The effect of nicotine on gastric and secretion and gastric ulcer formation is unclear. Some investigators suggested that nicotine augments gastric secretion[3,4], while others report an inhibitory effect of nicotine on gastric function[5,6]. Pare[7] observed that nicotine augmented the formation of activity-stress ulcers in rats, but not significantly. It has been suggested[8,9] that nicotine 'sensitizes' or predisposes the gut to the ulcerogenic effects of gastric secretion or even to the effects of other substances. A similar sensitizing effect was reported to occur with 1-ascorbic acid administration. It was reported that rats pre-treated with 1-ascorbic acid displayed increased gastric damage produced by restraint-cold[10]. It is of interest to examine the synergistic effects of commonly used combinations of substances. Aspirin and large doses of 1-ascorbic acid have been shown to act synergistically to potentiate ulcer formation[11], however, the combination of nicotine and ascorbic acid has received little attention in the literature. This study focussed on the effects of pre-treatment with nicotine alone, 1-ascorbic acid alone or combined nicotine and 1-ascorbic acid on the subsequent development of restraint-cold-induced gastric lesions in rats.

Methods. 40 male Wistar rats (200± 10 g at the start of the study) were used. Rats were randomly divided into 4 equally sized groups. One group of 10 rats was treated for 10 days with 1-ascorbic acid (Fisher Chemical Co.) in their drinking water (30 g/l; pH = 3.2) available ad libitum. A 2nd group of 10 rats was treated with nicotine (BDH Chemical Ltd) in their drinking water (500 µg%; pH = 9.9) available ad libitum. A 3rd group of 10 rats was treated for 10 days with a combination of 1-ascorbic acid (30 g/l) and nicotine (500 µg%; pH = 4.9) in their drinking water available ad libitum. A 4th group of 10 rats was untreated and given distilled water (pH = 5.4) ad libitum. Liquid intake was recorded daily. Following the 10 day treatment period, all rats were starved for 24 h and then restrained in the supine position for 2 h in a cold (4–6 °C) environment[12]. Previous data have shown that 12–24 h of pre-restraint starvation and 2 h of restraint are optimal durations for producing a reliable degree of gastric ulcers in rats[13]. Following the period of restraint, all rats were sacrificed with chloroform, their stomachs excised and examined for ulcer disease with a dissecting microscope. The location (rumenal or glandular), number, and cumulative length in millimetres of the ulcers were recorded.

Results. The table shows ulcer incidence, frequency and severity (cumulative length of ulcers) for the 4 treatment groups. Nicotine-treated rats developed significantly more frequent ($F(3,36) = 16.02$; $p < 0.01$; Tukey HSD-test) and significantly more severe ($F(3,36) = 4.18$; $p < 0.05$; Tukey HSD-test) gastric ulcers than rats in the other groups. 1-Ascorbic acid treatment increased ulceration relative to that seen in control animals (Tukey HSD-test; $p < 0.05$). Control rats (given only distilled water) exhibited a small number of glandular ulcers in response to the restraint procedure. This is a typical observation. Regardless of other treatment, animals subjected to restraint will exhibit some gastric damage. The combination of nicotine and 1-ascorbic acid resulted in a level of ulceration not significantly different from that seen in control rats. Correlations between amount of solution consumed and ulcer severity were: $r = 0.98$ for 1-ascorbic acid alone; $r = 0.29$ for nicotine alone; and $r = 0.10$ for nicotine and 1-ascorbic acid.

Summary of stomach pathology* for the 4 treatment groups

Group	No. of tested rats	No. of rats with ulcers	Mean No. of ulcers (± SE)	Mean cumulative lenght of ulcers in mm (± SE)
Nicotine	10	10	5.70±0.81	13.80±4.31
1-Ascorbic acid	10	10	2.70±1.30	3.70±1.20
Nicotine+ 1-ascorbic acid	10	10	1.20±0.43	2.00±0.73
Distilled water	10	10	1.57±0.31	2.50±1.31

*Glandular ulcers only.

Discussion. These data suggest that nicotine administration augments restraint-cold-induced gastric ulcers. Similar results have been reported using other ulcerogenic techniques[7]. In addition, l-ascorbic acid was shown to increase restraint-cold-induced ulcers relative to control animals, confirming earlier results from this laboratory[10]. Especially interesting is the relationship between l-ascorbic acid intake and glandular ulcer severity. Perhaps l-ascorbic acid has more than a sensitizing effect on the stomach. Establishing a dose-response relation-ship between ascorbic acid administration and gastric damage is clearly indicated. Preliminary observations in this laboratory indicate that increasing doses of l-ascorbic acid are associated with increasing degrees of gastric damage in both rats and guinea-pigs. Finally, it appears that nicotine and l-ascorbic acid do not act synergistically to potentiate stress-induced gastric ulceration. Rats treated with the combination of both substances exhibited a level of gastric damage similar to that of control animals. It appears that l-ascorbic acid exerts a protective effect against nicotine-induced gastic ulcer. The combination of nicotine and l-ascorbic acid resulted in less frequent and less severe gastric damage than nicotine administered alone. Following Robert's[8] data concerning nicotine and intestinal damage, it is evident that several doses of nicotine and several doses of l-ascorbic acid must be explored in the context of their effects upon restraint-induced gastric ulcer.

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Effect of topically applied phenobarbital on O-dealkylase activity in mouse skin

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Summary. Topical application of phenobarbital to mice produces an increase in cutaneous microsomal proteins and apparently also of cytochrome P450, but results in a kinetically noncompetitive inhibition of p-nitrophenethole O-dealkylase activity in preparations of 10,000 × g supernatant from skin.

The significance of the skin as a drug-metabolizing organ is gaining increasing recognition and has been recently reviewed[2]. We have previously examined the ability of skin 10,000 × g supernatant preparations from untreated mice to O-dealkylate p-nitroanisole and p-nitrophenetole[3]. Detectable and quantifiable O-dealkylase activity was shown to exist in these preparations, the reaction being NADPH-dependent and mediated by cytochrome P450. In the present study, we examine the effect of topically applied phenobarbital, chosen as an inducer of cytochrome P450 (as opposed to cytochrome P448). It is known that classical inducers of hepatic enzymes do not necessarily have the same effects in extrahepatic tissues[4,5], and drug-drug or drug-excipient interactions are conceivable at the cutaneous monooxygenase level following topical medication.

Methods. White Swiss E mice aged 8–10 weeks were used. Batches of 10 animals (5 males and 5 females) were carefully shaved on the back and abdomen, and after 24 h an acetone solution of phenobarbital was sprayed on a dorsal and on an abdominal area, during 4 days at 24-h intervals. The animals were killed by cervical dislocation 24 h after the last treatment. The preparation of skin homogenates and of fractions from it (10,000 × g supernatant and microsomes), the incubation conditions and the GC analytical method were as previously described[3]. The microsomal protein concentration was determined according to Lowry et al.[6]. The K_m- and V_{max}-values were calculated by the method of Lineweaver and Burk[7]. The SD of these values were calculated by the method of error propagation[3,8]. For the spectral determination of cytochrome P450, microsomes isolated at 106,000 × g were resuspended in 6 ml of a 0.05 M PO_4 buffer pH 7.6 containing EDTA 10^{-3} M. A Hewlett-Packard 8450A spectrophotometer was used.

Results. Phenobarbital causes a clear and statistically significant increase in microsomal protein content (table). Under the conditions of this study however, the increase is not dose-dependent, the 2 extreme doses of PB causing practically identical increases; 33 and 32%, respectively.

The determination of cytochrome P450 in mouse cutaneous microsomes is rendered difficult by large quantitites of

The effect of toppically applied phenobarbital on protein content of mouse skin microsomes and p-nitrophenetole O-dealkylation by cutaneous 10,000 × g supernatant preparations

Animals	Proteins[c] (mg/g tissue)	Number[e]	K_m (μM)	V_{max} (nmole/mg protein/min)
Untreated	1.45 ± 0.03	6	1.21 ± 0.30[f]	0.239 ± 0.046[f]
Acetone[a]	ND[d]	8	1.20 ± 0.21	0.231 ± 0.036
Phenobarbital 0.75[b])	1.93 ± 0.001	12	1.26 ± 0.10	0.194 ± 0.013[g]
Phenobarbital 7.5[b])	ND[d]	6	1.19 ± 0.15	0.172 ± 0.020[g]
Phenobarbital 75[b]	1.91 ± 0.08	6	1.22 ± 0.13	0.173 ± 0.015[g]

[a]Animals treated with acetone only; [b]animals receiving 0.75, 7.5, or 75 mg phenobarbital/kg/day during 4 days, respectively; [c]average values (± SD) for 3 batches (3 animals per batch); [d]not determined; [e]No. of assays (2 assays for each concentration of substrate and phenobarbital); [f]values from Pannatier et al.[3]. [g]$p < 0.01$ from control.