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Combined Ulcerogenic Effect of Ethanol and Acetylsalicylic Acid on the Gastric Mucosa of the Rat

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Summary. The effect on rat gastric mucosa of ethanol and acetylsalicylic acid given at two doses singly or in combination were studied by administering the drugs into the stomach. 40% ethanol alone caused many ulcerations after 5 h, and the effect was even more marked with simultaneous administration of acetylsalicylic acid (ASA). 10% ethanol and 50 mg ASA/kg b. wt. were tolerated well. The mucosal lesions consisted of coagulation necrosis and hemorrhages, with a disappearance of histamine and serotonin from the mucosal mast cells in and around the lesion.

Key words: Gastric ulcers - Acethylsalicylic acid - Ethanol - Histamine - Serotonin

Zusammenfassung. Die Wirkung von Ethanol, Acetylsalicylsäure (ASS) und deren Kombination auf die Magenschleimhaut wurde bei Ratten nach einer akuten, oralen Zufuhr untersucht. Fünf Stunden nach der Eingabe von 40%igem Ethanol wurden mehrere Schleimhautläsionen beobachtet, und diese Wirkung wurde bei einer gleichzeitigen Zufuhr von 50 und 200 mg ASS/kg verstärkt. Die Kombination von 10%igem Ethanol und 50 mg ASS/kg verursachte keine deutliche Schäden an der Magenschleimhaut. Die Schleimhautläsionen bestanden aus Koagulationsnekrosen und Hämorrhagien. Eine Verminderung von Histamin und Serotonin wurde in den Läsionen und deren Umgebung beobachtet.

Schlüsselwörter: Magengeschwür, durch Ethanol und Acetylsalicylsäure - Histamin - Serotonin

Introduction

We once investigated a case of sudden death where intake of ethanol for 1 week and one single pill (0.5 g) of acetylsalicylic acid (ASA) had caused numerous acute ulcers in the stomach mucosa with ensuing fatal massive hemorrhage of about 2 l. Ethanol had either sensitized the mucosa to ASA or had already caused

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small hemorrhages during the week, since the man had been consuming a strong, sweet aperitif-type wine. In earlier experiments we had found that hyperosmolal ethanol is ulcerogenic in rats (Puurunen et al. 1980) and ASA has similar effects. We decided, therefore, to investigate the combined effect of ethanol and ASA, two drugs that are often taken together or consecutively. One point of interest was whether they have any potentiating effect.

Biogenic monoamines stored in the gastric mucosa seem to play a role in the pathogenesis of acute ulcers, at least in rats (Levine and Senay 1968; Hirvonen and Elfving 1974). In this species histamine is found in the mucosal mast cells and in the enterochromaffin-like cells, and serotonin in the mast cells and enterochromaffin cells (Håkanson 1970; Hirvonen and Penttilä 1970). The mechanism of ulceration may then be associated with vasospasm or capillary leakage caused by these vasoactive amines.

Since these amines seem to be released from the cells in the initial phase of a mucosal lesion, we decided to investigate their fate both histochemically and quantitatively to obtain some data on the mechanism of the lesions caused by the two drugs.

Material and Methods

The experiments were performed on male Wistar rats with a body weight of 250–300 g. They were only given tap water for 20 h before administration of the drugs by oral gavage in a volume of 0.5 ml/100 g. The drugs consisted of either ethanol or acetylsalicylic acid (ASA) or a combination of both. The concentrations of the ethanol solutions were 10% and 40%, giving total doses of 0.5 g/kg and 2.0 g/kg, respectively, and the ASA doses were 50 mg/kg and 200 mg/kg. There were thus eight test groups and one control group, which received 1% carboxymethyl cellulosa as follows:

1. 8 rats, 1% carboxymethyl cellulosa;
2. 8 rats, 10% ethanol, i.e., 0.5 g/kg, an average of 0.13 g into the stomach;
3. 8 rats, 40% ethanol, i.e., 2.0 g/kg, an average of 0.50 g into the stomach;
4. 8 rats, 50 mg/kg ASA, an average of 13 mg into the stomach;
5. 8 rats, 200 mg/kg ASA, an average of 50 mg into the stomach;
6. 8 rats, 10% ethanol (0.5 g/kg) + 50 mg ASA/kg;
7. 8 rats, 10% ethanol (0.5 g/kg) + 200 mg ASA/kg;
8. 8 rats, 40% ethanol (2.0 g/kg) + 50 mg ASA/kg;
9. 8 rats, 40% ethanol (2.0 g/kg) + 200 mg ASA/kg.

Five hours after application of the drugs the rats were decapitated and the stomach was removed. The ruminal portion was then excised and the glandular portion opened along the greater curvature. After rapid irrigation with saline and gentle wiping, the glandular stomach was pinned flat and photographed.

Determination of Lesion Indices

A macroscopical lesion index was determined for every rat by calculating the sum of the lengths of the individual lesions (mm), both hemorrhages and ulcerations. A median and upper and lower quadriles were calculated for each group and the statistical comparisons between the groups were made using the Mann-Whitney *U*-test. The incidence of the lesions in each group is also given.

A microscopical lesion index for each rat was determined as follows: hemorrhage, necrosis, and ulceration of the mucosa were each assigned one point, the individual indices thus varying from zero to three. The group index was obtained by summing up the individual scores. The indices were compared statistically using the four-field test.

Samples for Histochemistry

a) A piece of the stomach wall, 1 mm wide, was cut from the lesion area, frozen in isopentane, and embedded in paraffin after freeze-drying.

Histamine was demonstrated in the sections by the orthophthalaldehyde vapor method and serotonin by the paraformaldehyde vapor method under a fluorescence microscope (Pearse 1972).

b) A piece 2 mm wide was cut from the stomach wall, frozen in isopentane, and cut in a cryostat at 10 μm for demonstration of succinate dehydrogenase (Pearse 1972).

c) A third piece was fixed in 10% formalin and embedded in paraffin. The sections were stained with hematoxylin-eosin (HE) in the normal way.

Sample for Biochemical Analysis

The rest of the stomach was frozen and stored at −20° C until analyzed for histamine and serotonin. First, the sample was homogenized in 0.4 *M* $HClO_4$.

Histamine was extracted with isoamyl alcohol and $K_2H\,PO_4$ (pH 8.8–8.9), leaving behind most of the spermidine. The intensity of the histamine-orthophthaldialdehyde fluorochrome was maximized in the presence of citric acid (Anton and Sayre 1969).

Serotonin was extracted into butanol with a borate buffer (pH 10.0). The fluorochrome was developed using orthophthaldialdehyde and L-cysteine according to Curzon and Green (1970).

The differences were tested for statistical significance using Student's *t*-test.

Results

Macroscopical Observations

The lesions were mostly situated longitudinally on the wrinkles of the mucosa in the fundus of the stomach (Figs. 1 and 2). The earliest lesion seemed to be an intramucosal punctate hemorrhage. The next step was an extensive hemorrhage and the last phase an ulceration developing in the mucosa. The ulcerations were up to a few millimeters long. The three highest macroscopic lesion indices were in the groups which had received 40% ethanol alone or in combination with ASA. The indices were low in both the 10% ethanol and the 50 mg ASA/kg group, even when the combination had been given. 200 mg ASA/kg caused mild lesions. The statistical significances of the lesion grade differences are given in Table 1.

Microscopical Observations

10% ethanol had no clear microscopic effect on the mucosa. The same result was seen in the 50 mg ASA/kg group, except in one rat which had minor necrosis and ulceration. The effect of 200 mg ASA/kg alone was fairly mild, but the distinct effect of 40% ethanol was clearly demonstrable histologically, with necrosis and/or ulcerations present in all but one rat, and the severe lesions with almost full thickness necrosis and hemorrhage in six (Fig. 3).

The combination of 10% ethanol and 50 mg ASA/kg had caused necrosis in two rats and 10% ethanol with and 200 mg ASA/kg mild necrosis in four rats. When the 40% ethanol was given either with 50 mg ASA/kg or 200 mg ASA/kg all but one of 16 rats developed necrosis and ulcerations. Thus, lesions were significantly more numerous ($P<0.05$ or $P<0.01$) in the groups which had received the strong ethanol solution (Table 1).

Table 1. Macroscopic quantitative lesion index (hemorrhages and ulcerations) and severity of necrosis (hemorrhagia, necrosis, ulceration, each assigned one point in the microscopic samples) and number of rats with observed lesions in each group

	Macroscopical		Microscopical	
	Index (median ± range)	Rats with lesions	Necrosis (sum)	Rats with lesions
1) CMC	0.2 (0.0- 5.4)	4	3	2
2) 10% ethanol	0.3 (0.0-20.5)	4	0	0
3) 40% ethanol	23.0 (1.8-57.4)	8	19	7*
4) 50 mg ASA/kg	4.2 (0.0-22.9)	7	2	1
5) 200 mg ASA/kg	15.3 (0.0-32.6)	6	9	5
6) 10% eth. + 50 mg ASA/kg	5.4 (0.5-20.4)	8	4	2
7) 10% eth. + 200 mg ASA/kg	18.8 (1.2-55.6)	8	7	4
8) 40% eth. + 50 mg ASA/kg	34.5 (4.3-59.3)	8	16	7*
9) 40% eth. + 200 mg ASA/kg	32.8 (16.2-93.4)	8	18	8**
	Mann-Whitney *U*-test		Four field test	* $P<0.05$ ** $P<0.01$
	1-2 ns	2-3 **		
	1-3 **	4-5 ns		
	1-4 *	6-7 ns		
	1-5 *	8-9 ns		
	1-6 **	6-8 **		
	1-7 **	7-9 ns		
	1-8 ***			
	1-9 ***			

ASA = acetylsalicylic acid

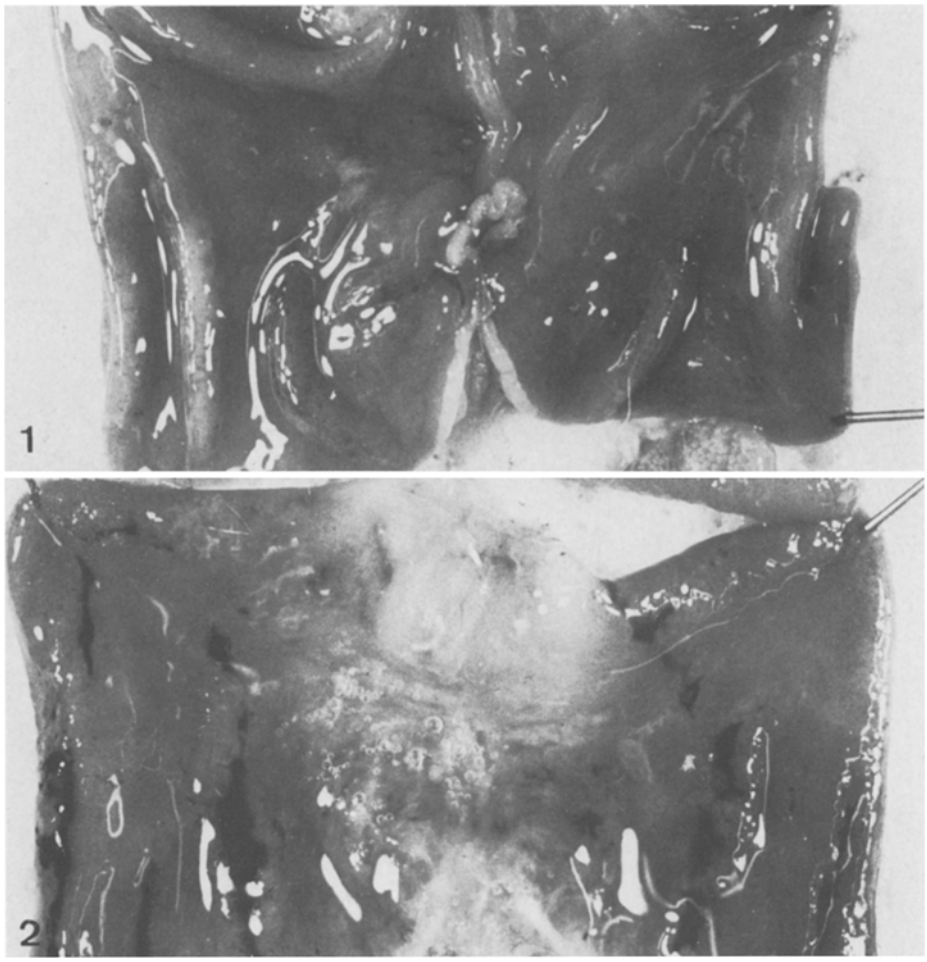

Fig. 1. Stomach from a rat given 10% ethanol and 50 mg ASA/kg. Only some petechiae have developed but no real necrosis

Fig. 2. Stomach from a rat given 40% ethanol and 200 mg ASA/kg. Extensive necrosis and ulcerations mainly on the mucosal ridges

The *succinate dehydrogenase reaction* in the parietal cells had disappeared in the necrotic areas but remained intact elsewhere. Thus, the application of the fluid as such was not harmful to the mucosa.

Histamine and *serotonin* fluorescence had disappeared in and at the edge of the necrotic areas. This indicated release of the amines from the mast cells even in the vicinity of the necrosis proper. The cells in the intact areas of the mucosa had retained their monoamines (Fig. 4).

Biochemical Results

In all test groups *histamine* values were lower than in the control group, but the differences were not statistically significant due to great individual variation. The serotonin values showed no discernible trend (Table 2).

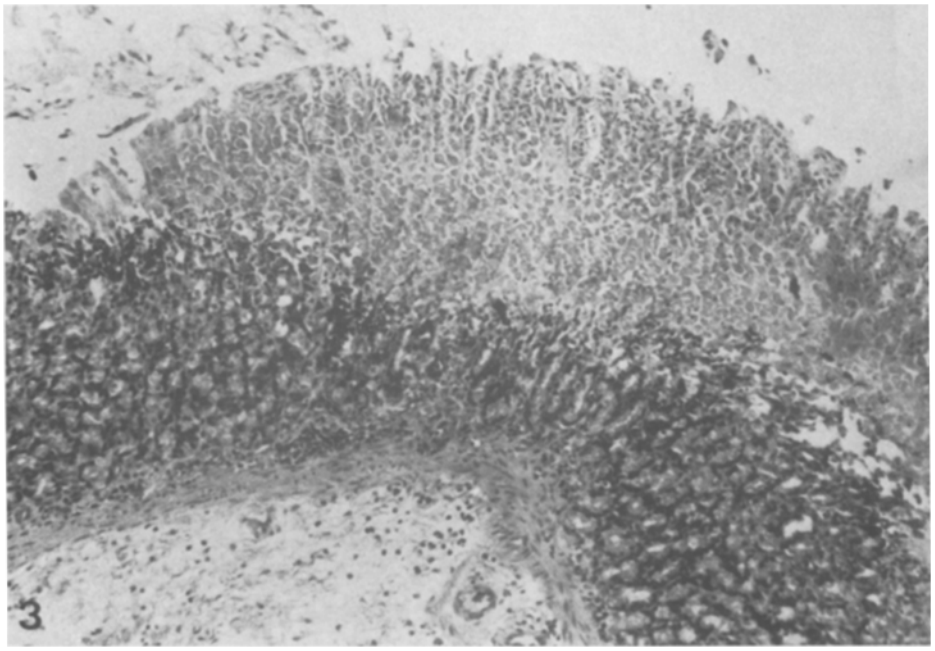

Fig. 3. Extensive coagulation necrosis of the stomach mucosa after 40% ethanol and 200 mg ASA/kg. Incipient inflammation in the submucosa. HE, ×60

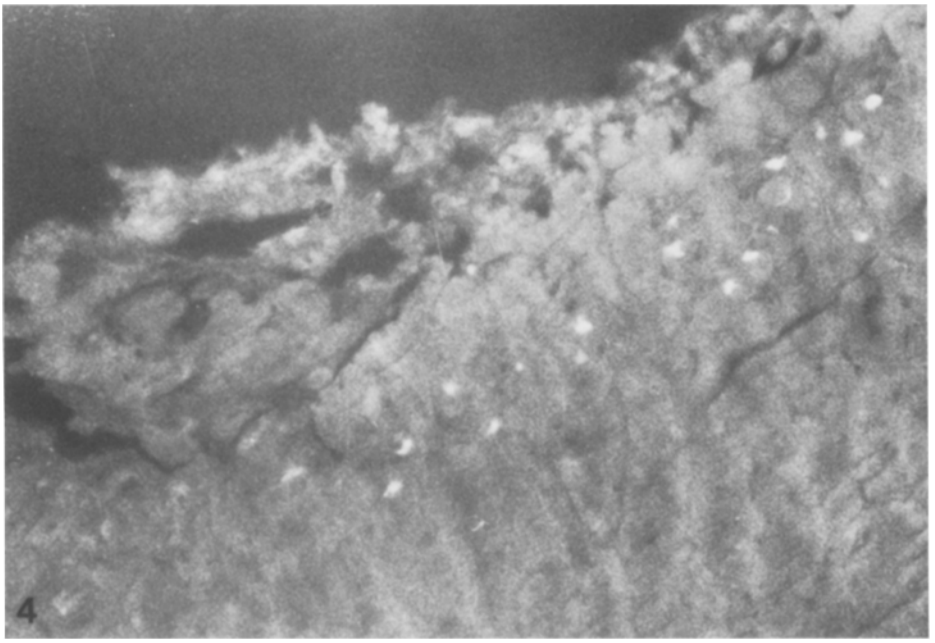

Fig. 4. Mucosal mast cells depleted of histamine at the ulceration *(left)*. A similar phenomenon was observed also with mast cell serotonin. Orthophthalaldehyde fluorescence method, ×250

Table 2. Histamine and serotonin concentrations (mean ± SD) in the gastric mucosa. The decrease in the histamine values was not significant (Student's *t*-test)

	Histamine (μg/g)	Serotonin (μg/kg)
CMC	9.39 ± 5.59	0.82 ± 0.46
10% ethanol	6.85 ± 5.98	0.92 ± 0.17
40% ethanol	8.04 ± 5.53	0.69 ± 0.36
50 mg ASA/kg	6.37 ± 2.20	0.98 ± 0.34
200 mg ASA/kg	8.56 ± 5.50	0.82 ± 0.31
10% eth. + 50 mg ASA/kg	6.32 ± 3.39	0.90 ± 0.43
10% eth. + 200 mg ASA/kg	5.68 ± 3.44	0.92 ± 0.49
40% eth. + 50 mg ASA/kg	5.32 ± 3.86	0.72 ± 0.34
40% eth. + 200 mg ASA/kg	5.34 ± 2.78	0.72 ± 0.37

Discussion

The present results confirmed our earlier observation that a strong ethanol solution (40%) is ulcerogenic in the rat (Puurunen et al. 1980). In fact, its damaging effect increased when ASA was given simultaneously. 10% ethanol and 50 mg ASA/kg were tolerated fairly well, causing minor lesions only in individual rats, even when administered in combination. The sensitivity of the rat's stomach to ulcerogenic noxas is probably greater than of the human stomach, but the present ulceration model explains to some extent the fatal gastric hemorrhage of the man who had consumed strong wine for 1 week followed by 0.5 g ASA.

According to Davenport (1975) the initial event in the process resulting in EtOH or ASA-induced injury is an increase in mucosal permeability, enabling back-diffusion of hydrogen ions into the mucosa. This triggers off a complex process involving the release of histamine, an increase in ions in the gastric mucosa, the stimulation of pepsinogen secretion and changes in mucosal blood flow, which finally lead to tissue damage.

Some evidence could be found for this mechanism in the drug ulcerations, and the necrosis might even have been caused by HCl directly, which digests the cells either in the capillary walls or in the mucosal glands in guinea pigs (Watt 1959). One phase in the pathogenesis of ulcerations is ischemia via the constriction of arterioles. Diminished blood flow certainly increases the incidence of ulcers, but the factors affecting the arterioles are under dispute. Histamine and 5-HT are vasoactive and can cause constriction. They might thus play an important role in the ulceration process. Histochemical methods showed that histamine had been released from the mast cells and the histamine-containing glandular cells in and around the lesion and 5-HT depleted from the mast cells. The amount of histamine was slightly, but not significantly, decreased in the damaged mucosa, although the fluorescence had disappeared in and around the ulcers. The samples for the quantitative analyses had to be taken from that part of the

stomach which was left after histological sampling, i.e., from near the pyloric region, which did not have many lesions.

In a few rats there was discrepancy between the macroscopic and microscopic lesion scores, the histological investigation failing to show the lesions seen in the macroscopic scrutiny. Of course, this is due to the sampling method, and although attempts were made to include an area of lesion in the histological sample, the sections did not always reveal the ulceration.

The ulcers were mainly located on the ridges of the mucosa. The reasons for this selectivity could be many. The ridges are better exposed to the fluid in the ventricle containing the drugs, and the rats had fasted, so that the wall of the empty stomach was folded. The musculature of the mucosa might also determine the site of the lesion, perhaps by interfering with the blood circulation.

The present observations indicate that both ethanol and ASA are capable of liberating histamine from the gastric mucosa of the rat. Whether the human stomach reacts the same way is not known. The human gastric mucosa may well be less sensitive since it contains less histamine, and in only one cell type, the mast cells (Penttilä et al. 1969).

EtOH and ASA have a synergistic effect on lesion formation in the rat gastric mucosa.

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