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Secretion of Gastric Hydrochloric Acid and Sialic Acid during Administration of Acetylsalicylic Acid

An Experimental Study in Rats with Chronic Gastric Fistula

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ABSTRACT

It is a well known fact that orally administered acetylsalicylic acid causes a high incidence of gastric side effects (from mild gastritis to ulcer). The mechanism for the development of the side effects is still a matter of dispute. In the present study rats were operated and chronic gastric fistulas were performed. This permitted regular collection of gastric juice. The test compound was administered directly into the stomach as pure acetylsalicylic acid or in enteric coated preparation (Premaspin®) which only permitted liberation of the active principle in the alcaline milieu of the duodenum. In addition, deposition of the drug was also made through a tube into the duodenum. The gastric juice was analysed for its variations in HCl concentration. Similar determination of total and free sialic acid was taken as an indication of changes or destruction of the mucoid component. If the drug was liberated in the duodenum or directly placed there or if a small amount of the pure acetylsalicylic acid was administered in the stomach minimal changes were recorded in the parameters used as compared to controls. A high dose of the drug intragastrically appeared to cause an immediate increase of the mucous content followed by a mucous production with a low content of sialic acid and an increased fraction of free sialic acid. This could be due to an increased break down of the mucus or defect mucous production. The results indicate that reduced side effects of acetylsalicylic acid are to be expected if the gastric mucosa is protected from direct contact with the compound.

INTRODUCTION

The relationship between the substances attacking the gastric mucosa and those protecting it is likely to be of great importance for the development of gastric ulcer. It is a well known fact that treatment with antiphlogistic compounds will not infrequently induce complications such as gastritis or even ulcer. The underlying mechanism probably includes both reduction and destruction of the mucous layer (4).

Gastric mucus is complex and consists of a variety of mucous components. It is generally conceded that the mucus exerting a protecting action against the HCl and enzymes consists of long polypeptide chains with short carbohydrate branches. Evidence that sialic acid is of determinant importance for the biological significance of the mucous substance has been proposed by Dische (3) and Menguy & Masters (5). It thus appears reasonable to study changes in the gastric content of sialic acid as an index of mucus production. By studying total as well as free sialic acid it appears possible to get information both quantitatively and qualitatively on the produced mucus.

The aim of the present study has been to investigate possible interference with the gastric secretion of HCl and mucus in the rat during varied administration of acetylsalicylic acid. A further purpose was to get information whether the effect on the gastric mucosa is a general or local one.

MATERIAL AND METHODS

Male rats (Sprague-Dawley) weighing approximately 250 g were used. At laparotomy a fistula was applied to the rumen of the stomach according to a method developed at our laboratory (1). In two groups of rats (B 5 and 6, see below) a catheter was simultaneously inserted into the duodenum according to the method of Nilsson (1972). Experiments were started 1 week after the operation in the control group (A 1) and after 2 weeks in the remainder of control and experimental groups.

Before onset of collection of gastric juice the animals were fasted for 15 hours. The stopper of the fistula was

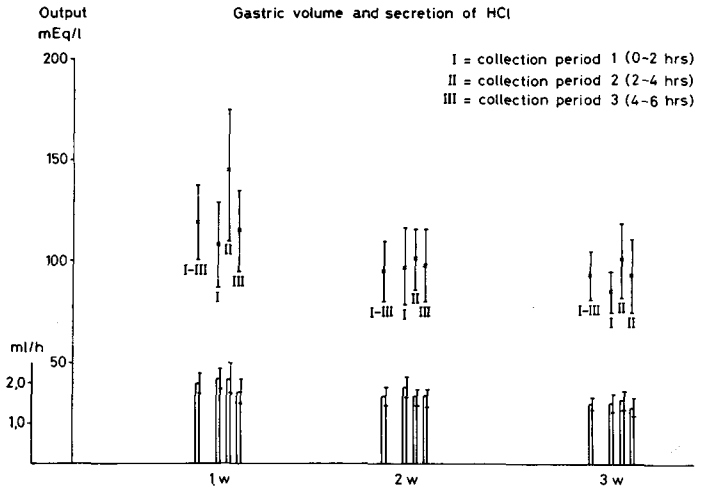

Fig. 1. Gastric secretory volume and acid output in control group (A 1) 1, 2 and 3 weeks after fistula operation. Group means with standard deviation are given.

removed and the stomach was rinsed with luke warm water until clear. The fistula was left open for 45 min. Then collection of gastric juice was begun.

The animals were distributed at random to the following groups:

A. Control groups

1. These groups were u d to study possible variation in the production of ga ric juice with regard to time after fistula operation Collection was performed during three 2-hour per ds at 1, 2 and 3 weeks after the operation. $n = 18$.
2. This group served controls for the respective experimental groups in ction B and were investigated simultaneously. $n = 13$ (C).

   Collection was performed during two periods of 2 hours 5, 48 and 120 hours after the beginning of the experiments.

B. After rinsing of the stomach acetylsalicylic acid was administered in single dose to different groups as follows.

1. 250 mg/kg body weight through the gastric fistula. $n = 10$. (SH)
2. 30 mg/kg as above. $n = 12$. (SL)
3. 250 mg/kg as above but in an enteric coated preparation (Premaspin®), which only permits liberation of the active principle in an alcaline medium. $n = 4$. (P–S)
4. 100 mg/kg as in the previous group. $n = 11$. (P)
5. 250 mg/kg through the gastric fistula to rats with duodenal catheter. $n = 4$. (S–S)
6. 250 mg/kg body weight was administered through the duodenal catheter. $n = 4$. (S–D)

   Collection of gastric juice was made as in group A 2.

The collected gastric juice was subjected to determination of volume, concentration of H^+, total and free sialic acid. The acid output and the percentage of free sialic acid of the total fluid could then be calculated.

Chemical methods

The H^+ concentration was determined by titration against 0.1 M NaOH using Töpfer's reagent as indicator. The total content of sialic acid was determined using the Thiobarbituric Acid method of Warren (9) after hydrolysis in 0.1 M HCl for one hour. The content of free sialic acid was determined without previous hydrolysis.

RESULTS

In Figs. 1 to 8 the results are shown diagrammatically. The secretory volume and H^+ output in control group A 1 is depicted in Fig. 1 where the

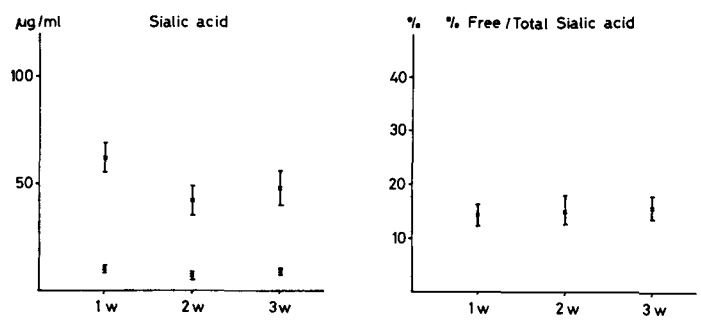

Fig. 2. Left: concentration of total (top) and free (bottom) sialic acid. Right: % free sialic acid of the total. Group means with standard deviation in control group (A 1).

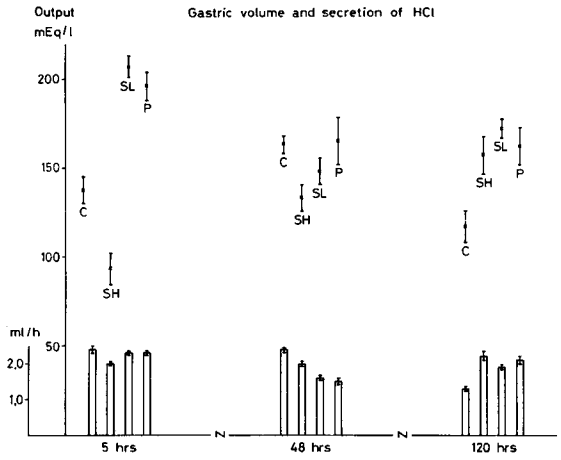

Fig. 3. Gastric secretory volume and acid output after deposition of acetylsalicylic acid in the stomach (groups B 1, 2 and 4). Group means with standard error of the mean are given. Acetylsalicylic acid was deposited as follows: SH 250 mg, SL 30 mg, P 100 mg of the enteric coated substance. *C*, control group.

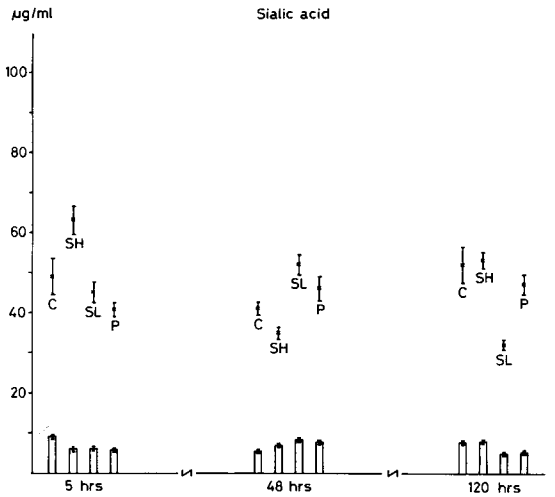

Fig. 5. Concentration of free (bottom) and total (top) sialic acid in gastric secretion (groups B 1, 2 and 4). Group means with standard error of the mean are given.

fractionated determinations are demonstrated of the different collection periods 1, 2 and 3 weeks after fistula operation respectively. After 1 week these parameters revealed a greater tendency of variation but appeared generally to be stabilized after 2 and 3 weeks. Fig. 2 shows the results of the determinations of free and total sialic acid and the ratio free/total sialic acid. These results also appeared to indicate that the experimental conditions were not constant until the 2nd week.

The volume of the gastric secretion and H^+ output for the different experimental groups in relation to their respective control groups are shown in Figs. 3 and 4. The immediate effect of a high dose of acetylsalicylic acid into the stomach (SH, S–S) was revealed by a diminished

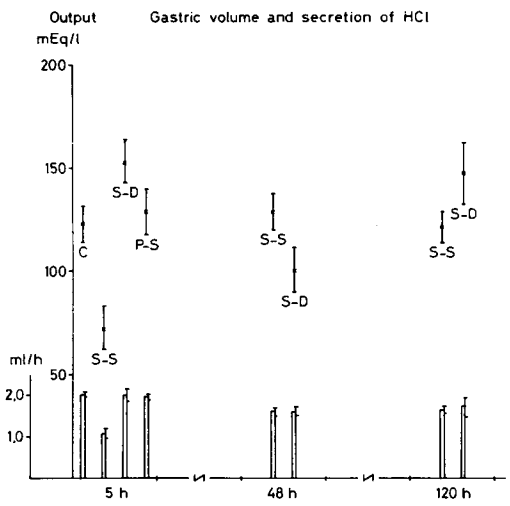

Fig. 4. Gastric secretory volume and acid output after deposition of acetylsalicylic acid in the stomach and into duodenum (groups B 3, 5 and 6). Group means with standard error of the mean are given. Acetylsalicylic acid was deposited as follows: 250 mg into stomach = S–S, into duodenum = S–D, as enteric coated substance into stomach = P–S. *C*, control group.

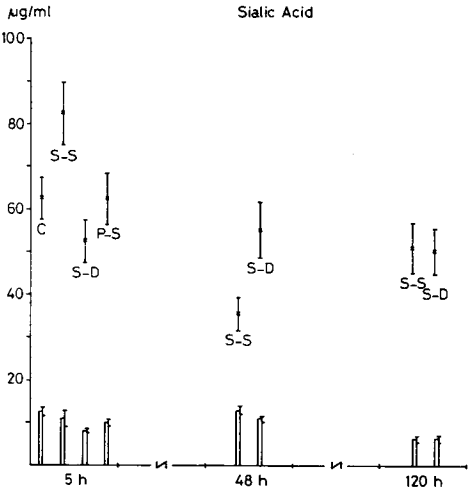

Fig. 6. Concentration of free (bottom) and total (top) sialic acid in gastric secretion (groups B 3, 5 and 6). Group means with standard error of the mean are given.

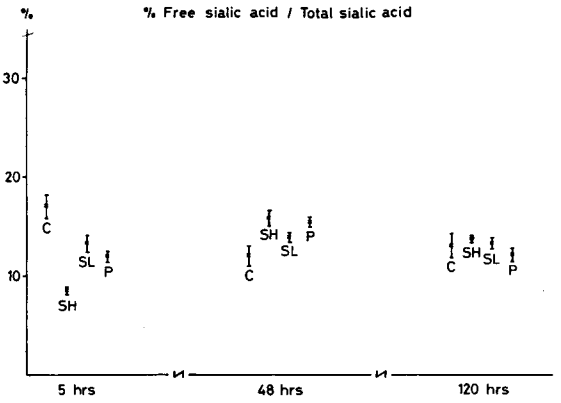

Fig. 7. Ratio of free/total sialic acid (groups B 1, 2 and 4). Group means with standard error of the mean are given.

secretory volume and H^+ output, while administration of a low dose of acetylsalicylic acid (SL) or a high dose of enteric coated substance (P–S, P) or duodenal application (not enteric coated—S–D) resulted in an unchanged secretory volume but increased H^+ output. After 48 hours the secretory volume was decreased in all groups. An apparent decrease in H^+ output was now recorded only in those animals previously being administered with a high dose of acetylsalicylic acid intragastrically (SH, Fig. 3). After 120 hours an increased secretory volume (Fig. 3) and H^+ output was recorded (Figs. 3, 4).

Figs. 5 and 6 display the results from the determinations of free and total sialic acid, and the ratio between these parameters is shown in Figs. 7 and 8. Local administration of a high dose of acetylsalicylic acid into the stomach (SH, S–S) promoted a rather prompt increase in the content of total sialic acid and a minimal decrease of the free sialic acid. The conditions were reversed after 48 hours and after 120 hours the data were close to the pretreatment values. Administration of a low dose of acetylsalicylic acid in the stomach (SL), or a high dose given intraduodenally (S–D) or in enteric coated tablets (P–S) did not alter the composition of the gastric juice except for a tendency of lowered sialic acid after 120 hours (SL, P, S–D).

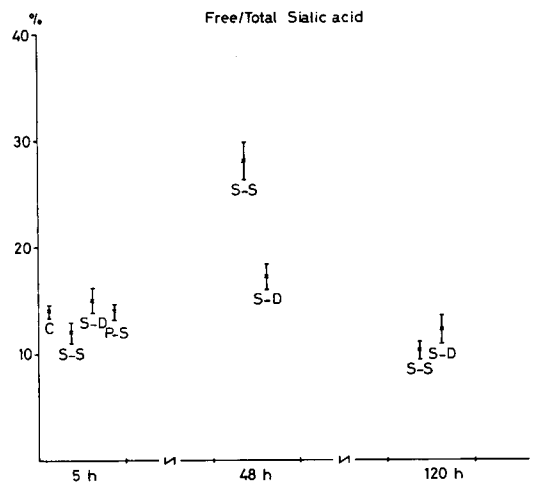

Fig. 8. Ratio of free/total sialic acid (groups B 3, 5 and 6). Group means and standard error of the mean are given.

DISCUSSION

Clinical and experimental experience offer an overwhelming evidence of gastrointestinal side effects upon administration of acetylsalicylic acid. In recent years there is an accumulating support for the concept that the interference involves the mucous secretion rather than the acid secretion (4, 5). There is still a matter of dispute if the gastric side effects are initiated by local action or systemically. In an earlier report we suggested the probability of a combination of the two mechanisms (4). In previous experiments we used rats operated according to Shay. This method may, however, be regarded upon as unphysiological. For this reason the present study was performed using gastric fistula as well as duodenal fistula as indicated in the various experiments. The fistulas permitted the administration of the drug in the desired preparation. The technique of "enteric coating" does not permit the release of the drug in acid medium, but this occurs in the alcaline milieu in the duodenum (8). We are not aware of any earlier study with a similar design. Not until two weeks after the fistula operation constant biological conditions were reached with small fluctuations between the different experimental periods and between animals.

Local administration in the stomach of a high dose of acetylsalicylic acid decreased initially the H^+ output. This might be in accordance with Davenport's (2) observation, that the permeability of the mucosa increased probably because of capillary damage permitting an increased diffu-

sion of H^+. Similar administration of a low dose, or if the compound was given in enteric coated preparation or into the duodenum promoted an increased H^+ output, which was also shown earlier by Roth et al. (7). This was in contrast to previous studies in the Shay operated rat (4), which supports the concept that the Shay method is of questionable suitability.

The gastric mucous secretion was estimated through determinations of total and free sialic acid. If the sialic acid is removed from the mucous molecule by sialidase the remainder is easily broken down by the digestive enzymes (5). An increased fraction of free sialic acid may indicate an increased break down of the mucus or the production of defect mucous molecules. The administration of a high dose of acetylsalicylic acid into the stomach resulted in an initially increased output of total sialic acid and a simultaneous reduction of the fraction of free sialic acid. But after 48 hours the total content of sialic acid had decreased while the fraction of free sialic acid had increased. These findings might be interpreted so that the acetylsalicylic acid initially stimulates the mucous production which later decreases possibly due to exhaustion of the mucus cells. The raised fraction of free sialic acid after 48 hours might be an expression of an increased mucus destruction. The reduction of mucus was in accordance with comparable studies (5) and in Shay experiments (4). The mucous reduction appeared to be dependent upon the time and the dose. A small dose of acetylsalicylic acid caused minute changes in the gastric reaction initially but a reduction could be observed 120 hours later. It is also of interest that liberation of the drug in the duodenum or deposition of it there did not interfere with the composition of gastric juice as compared to the controls. The results are in agreement with earlier reports from our laboratory when using the less specific PAS stain to detect mucopolysaccharides (4).

The present investigation supports our previous concept that the side effects of acetylsalicylic acid on the gastric mucosa are mainly exerted by local action. The same conclusion was put forth by Roth et al. (7). The local corrosion on the gastric mucosa will most likely be avoided by enteric coating of acetylsalicylic acid. The systemic side effects of the compound appear to be less pronounced than the local ones. In conclusion, preparations of acetylsalicylic acid, which do not permit the liberation of the compound in the stomach but in the duodenum, are likely to reduce the incidence of side effects.

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