Role of Taurocholic Acid in Production of Gastric Mucosal Damage after Ingestion of Aspirin

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Summary

The possibility that aspirin-induced gastric mucosal damage may occur more readily in the presence of bile has been studied in man using measurement of transmucosal electrical potential difference as a marker of disruption of the gastric mucosal barrier.

After the introduction of acetylsalicylic acid (600 mg) in suspension to seven subjects the mean electrical potential difference ($\pm$ S.E. of mean) fell significantly from -33.3 ± 2.0 mV to -17.1 ± 2.1 mV, and after the introduction of taurocholic acid (5 mmol/l) to seven other subjects the electrical potential difference fell significantly from -38.1 ± 3.0 mV to -19.1 ± 3.4 mV, the mean duration of these changes being 14.4 and 17.5 minutes respectively. When a combination of acetylsalicylic acid and taurocholic acid was introduced to eight subjects the mean electrical potential difference also fell significantly from -38.6 ± 1.8 mV to -17.9 ± 1.8 mV, but the mean duration of this change (27 minutes) was significantly longer than that found after acetylsalicylic acid or taurocholic acid alone.

These results indicate that the ingestion of aspirin, together with coincidental reflux of bile from duodenum, may be a factor in the pathogenesis of aspirin-induced gastric mucosal damage.

Introduction

One of the important side effects of the administration of aspirin to man is gastric irritation, which may progress to severe gastric haemorrhage and ulceration. The mechanism of this effect is not fully established. One hypothesis is that aspirin alters gastric mucosal permeability, thus allowing back diffusion of H^+ ions. Davenport (1967) has proposed that the back diffusion of H^+ ions into the interstitial spaces causes release of histamine with resultant increase in capillary permeability and subsequent haemorrhage.

Taurine-conjugated bile acids have also been shown to cause back diffusion of H^+ ions across the gastric mucosa (Werther *et al.*, 1970; Ivey *et al.*, 1971). As aspirin and bile acids both break the gastric mucosal barrier it is possible that bile acids in the stomach may be a factor in the pathogenesis of gastric mucosal haemorrhage after the ingestion of aspirin. This has been shown in animals by Semple and Russell (1975), who have shown that simultaneous oral administration of acetylsalicylic acid and taurocholic acid (TCA) to rats causes gastric mucosal bleeding in significantly more animals than acetylsalicylic acid administered alone.

The aim of this study was to determine if the presence of TCA in the stomach, together with aspirin, causes greater damage to the gastric mucosal barrier in man than either alone.

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Patients and Methods

The effect of aspirin and TCA alone and in combination on the gastric mucosal barrier in man was determined by measuring the gastric transmucosal electrical potential difference (E.P.D.).

Transmucosal E.P.D. was measured by the method of Geall *et al.* (1970b), in which probe and reference electrodes filled with saturated KCl in 3% agar were used. The probe electrode consisted of polyethylene tubing (inside diameter 1.64 mm) attached to a radio-opaque stomach tube (Argyle AR-7R). The intravenous electrolyte bridge consisted of a polyethylene tube (inside diameter 0.86 mm). After sterilization the electrode was introduced through a 14-gauge needle. Both the reference and probe electrode led to beakers containing saturated KCl and two balanced calomel half cells (Beckman fibre-junction type) connected to a potentiometric recorder (Watanabe, Servocorder model SR651-2). The recorder gave a continuous write-out for record and analysis (Cochran and Russell, 1973).

The studies were performed on 22 healthy volunteer subjects.

DESIGN OF STUDY

After an overnight fast a nasogastric tube was passed with probe electrode attached. The tube was positioned by fluoroscopy, the tip of the tube being placed in the body of the stomach. During recording the patient lay on the left side, thus encouraging a constant position of the tube on the greater curve of the stomach.

The stomach was emptied of any resting secretion and the gastric E.P.D. measured continuously until a stable recording was obtained; 50 ml of normal saline was instilled initially as a control and the E.P.D. recorded for 15 minutes. The stomach was again emptied and the agent under study introduced.

Aspirin (600 mg) was suspended in 50 ml of normal saline and its effect observed as described above. Similarly, 50 ml of TCA (5 mmol/l) in solution in normal saline was studied. The combination of aspirin and TCA was prepared by suspending 600 mg of aspirin in 50 ml of 5 mmol/l TCA. Recordings were continued until the gastric mucosal E.P.D. had returned to within 10% of the baseline.

Results

Effect of Aspirin on Gastric Mucosal E.P.D.—After a steady baseline recording the introduction of normal saline caused no significant change in gastric mucosal E.P.D. The introduction of aspirin resulted in a fall of E.P.D. in seven patients from a mean ($\pm$ S.E. of mean) of -33.3 ± 2.0 mV to -17.1 ± 2.1 mV ($P < 0.001$; table I). This effect was immediate and reached

TABLE I—Effect of Aspirin (600 mg) on Electrical Potential Difference Recorded at Point of Maximum Change Compared with Effect of Normal Saline

Case No.	Baseline (Saline) (mV)	Point of Maximum Change after Aspirin (600 mg) (mV)
1	-28.6	-15.1
2	-40.4	-27.6
3	-38.7	-10.0
4	-32.8	-18.2
5	-24.4	-12.3
6	-33.2	-18.0
7	-35.1	-20.0
Mean $\pm$ S.E. of mean ..	-33.3 $\pm$ 2.0	-17.1 $\pm$ 2.1

the point of maximum change in six minutes. The E.P.D. returned to within 10% of the baseline 14 minutes after the point of maximum change.

Effect of TCA on Gastric Mucosal E.P.D.—After the introduction of TCA the gastric mucosal E.P.D. fell in seven patients from a mean ($\pm$ S.E. of mean) of -38.1 ± 3.0 mV to -19.1 ± 3.4 mV ($P < 0.005$; table II). The effect was again maximal

TABLE II—Effect of Taurocholic Acid (5 mmol/l) on Electrical Potential Difference recorded at Point of Maximum Change compared with Effect of Normal Saline

Case No.	Baseline (Saline) (mV)	Point of Maximum Change after Taurocholic Acid (5 mmol) (mV)
1	-41.6	-27.3
2	-23.8	-15.0
3	-34.4	-2.0
4	-40.5	-17.0
5	-50.3	-27.0
6	-36.9	-20.1
7	-38.8	-25.0
Mean $\pm$ S.E. of mean ..	-38.1 ± 3.0	-19.1 ± 3.4

in six minutes. Thereafter the gastric mucosal E.P.D. returned to within 10% of the baseline 17.5 minutes after the point of maximum change.

Effect of Combination of Aspirin and TCA on Gastric Mucosal E.P.D.—After the introduction of a combination of aspirin and TCA in eight patients the gastric mucosal E.P.D. fell from a mean ($\pm$ S.E. of mean) of -38.6 ± 1.8 mV to -17.9 ± 1.8 mV ($P < 0.001$; table III). The effect was maximal at nine minutes,

TABLE III—Effect of Combination of Aspirin and Taurocholic Acid on Electrical Potential Difference Recorded at Point of Maximum Change compared with Effect of Normal Saline

Case No.	Baseline (Saline) (mV)	Point of Maximum Change after Combination of Aspirin and Taurocholic Acid (mV)
1	-48.8	-13.5
2	-34.9	-20.5
3	-38.4	-22.5
4	-39.3	-17.0
5	-36.0	-25.0
6	-40.1	-15.0
7	-40.1	-19.5
8	-31.2	-10.0
Mean $\pm$ S.E. of mean ..	-38.6 ± 1.8	-17.9 ± 1.8

Effect of combination of aspirin (600 mg) suspended in taurocholic acid (5 mmol/l) on transmembrane electrical potential difference in man.

and the degree of change was similar to that obtained when aspirin or TCA was used alone. Thereafter the gastric mucosal E.P.D. returned to within 10% of the baseline 27 minutes after the point of maximal change. The time taken for the gastric mucosal E.P.D. to recover after exposure to the combination of aspirin and TCA was significantly longer than that taken when either aspirin ($P < 0.001$) or TCA ($P < 0.005$) were used alone. An example of the effect of a combination of aspirin and TCA on gastric mucosal E.P.D. is shown in the diagram.

Discussion

Two methods are available for measuring disruption of the gastric mucosal barrier—direct determination of ionic flux from the gastric lumen and the measurement of gastric transmucosal E.P.D. With ionic flux determination sodium and hydrogen ion concentrations are measured after exposure of the gastric mucosa to the agent under study, but with this method the recovery of H^+ ions may be falsely low (Murray *et al.*, 1974). The measurement of transmucosal E.P.D. between gastric mucosa and venous blood has been validated by Geall *et al.*, (1970b). Black, *et al.* (1973) have shown a significant correlation between changes in the mean gastric mucosal E.P.D. and back diffusion of H^+ ions after contact with bile. Gastric mucosal E.P.D. measurements can thus be used as an adequate indicator of disruption of the gastric mucosal barrier.

It is likely that significant haemorrhage after the ingestion of aspirin occurs when a combination of factors exist. Aspirin may adversely affect the production of gastric mucus (Menguy and Masters, 1965), and it may increase the exfoliation of cells from the gastric mucosa (Croft and Wood, 1967). Aspirin may also depress platelet stickiness (Morris, 1967) or prolong bleeding time (Quick, 1966), and in some patients significant bleeding after the ingestion of aspirin may be determined by the presence of subclinical ascorbic acid deficiency (Russell *et al.*, 1968; Russell and Goldberg, 1968). The back diffusion of H^+ ions may, however, be the most important factor and the mucosal damage may be mediated in this way (Davenport, 1967). The presence of acid may be essential for significant damage to occur after exposure to aspirin (Brodie and Chase, 1967; Davenport, 1969). The degree of disruption of the gastric mucosal barrier after aspirin ingestion may be related to the degree of ionization, intragastric pH, and degree of absorption which occurs.

Bile has been shown to be grossly cytolytic to the gastric mucosa (Grant *et al.*, 1951). Reflux of bile into the stomach after meals has been shown in patients with gastric ulcer, and it has been proposed that this may have an aetiological role in gastric ulcer (Du Plessis, 1965; Rhodes, 1972). The concentration of bile salts found in the stomach after a meal in patients with gastric ulcer was found to be 5.3 mmol/l (Rhodes *et al.*, 1969), a concentration similar to that of TCA (5 mmol/l) which we used in this study. Several studies have now been reported in which bile has been shown to break the gastric mucosal barrier (Davenport, 1968; Werther *et al.*, 1970; Ivey *et al.*, 1971). A mixture of taurocholic acid and glycocholic acid has also been shown to cause increased secretion of gastric acid, possibly due to the release of gastrin by bile acids (Bedi *et al.*, 1971).

It has recently been shown that TCA is more readily absorbed from an acid solution in animals, thus producing extensive gastric mucosal injury in the presence of H^+ and haemorrhagic shock. (Safaie Shirazi *et al.*, 1972). The dissociation constant of TCA may also mean a greater degree of ionization in the stomach (Dowling and Small, 1968). TCA in the stomach may thus have similar effects to aspirin on the gastric mucosal barrier in relation to the degree of ionization, intragastric pH, and degree of absorption.

The effect of gall bladder bile on gastric mucosal E.P.D. has been studied in animals by Black *et al.* (1971) and in man by Geall *et al.* (1970a) and a significant alteration in gastric mucosal E.P.D. was found by these workers. The results of these studies

suggest that the other components of gall bladder bile, such as phospholipids, do not exert a protective action as they seem to do when the effect of bile acids on the mucosa of the small intestine is studied (Russell *et al.*, 1973 a, b; Wingate *et al.*, 1973). The effects of individual bile acids thus need to be specifically defined. Ivey *et al.* (1970) showed that TCA alone caused qualitative and quantitative ionic changes in the stomach similar to those induced by a bile acid mixture.

The results of this study confirm the effects of aspirin on the gastric mucosal barrier, as measured by a change in gastric mucosal E.P.D. (Geall *et al.*, 1970; Murray *et al.*, 1974). The results of this study show that the effect of TCA on gastric mucosal E.P.D. is similar to that of aspirin. A combination of aspirin and TCA caused a similar significant alteration in gastric mucosal E.P.D. in our study, but the duration of change was significantly longer than that obtained with aspirin or TCA alone. Semple and Russell (1975) showed that exposure of rats to a combination of TCA and aspirin leads to an increased incidence of gastric haemorrhagic mucosal bleeding as compared to the incidence in rats exposed to aspirin or TCA alone. Our results confirm that the gastric mucosal barrier is significantly broken for a significantly longer time when the combination of aspirin and TCA is used in man. As a longer duration of gastric mucosal barrier breaking will allow a more prolonged back diffusion of H⁺ ions and, subsequently, a longer increase in capillary permeability it may well be that significant gastric mucosal haemorrhage is more likely to occur under such circumstances.

The results of this study thus indicate that the ingestion of aspirin, together with the coincident reflux of bile from the duodenum, may be a factor in the pathogenesis of significant aspirin-induced gastric mucosal damage in some patients.

References

- Bedi, B. S., *et al.* (1971). *Gastroenterology*, **60**, 256.
 Black, R. B., Hole, D., and Rhodes, J. (1971). *Gastroenterology*, **61**, 178.
 Black, R. B., Rhodes, J., and Hole, D. (1973). *Digestive Diseases*, **18**, 411.
 Brodie, D. A., and Chase, B. J. (1967). *Gastroenterology*, **53**, 604.
 Cochran, K. M., and Russell, R. I. (1973). *Gut*, **14**, 903.
 Croft, D. N., and Wood, P. H. N. (1967). *British Medical Journal*, **1**, 137.
 Davenport, H. W. (1967). *New England Journal of Medicine*, **276**, 1307.
 Davenport, H. W. (1968). *Gastroenterology*, **54**, 175.
 Davenport, H. W. (1969). *Gastroenterology*, **56**, 439.
 Dowling, H. R., and Small, D. M. (1968). *Gastroenterology*, **54**, 1291.
 Du Plessis, D. J. (1965). *Lancet*, **1**, 974.
 Geall, M. G., Phillips, S. F., and Summerskill, W. H. J. (1970 a). *Gastroenterology*, **58**, 437.
 Geall, M. G., *et al.* (1970 b). *Gut*, **11**, 34.
 Grant, R., *et al.* (1951). *Journal of Cellular and Comparative Physiology*, **37**, 137.
 Ivey, K. J., Denbesten, L., and Clifton, J. A. (1970). *Gastroenterology*, **59**, 683.
 Ivey, K. J., Denbesten, L., and Clifton, J. A. (1971). *Gut*, **12**, 257.
 Menguy, R., and Masters, Y. F. (1965). *Surgery, Gynecology and Obstetrics*, **120**, 92.
 Morris, C. D. W., (1967). *Lancet*, **1**, 279.
 Murray, H. S., Strotzman, M. P., and Cooke, A. R. (1974). *British Medical Journal*, **1**, 19.
 Nahrwold, D. L. (1972). *Surgery*, **71**, 157.
 Quick, A. J. (1966). *American Journal of Medical Science*, **252**, 265.
 Rhodes, J., *et al.* (1969). *Gastroenterology*, **57**, 241.
 Rhodes, J. (1972). *Gastroenterology*, **63**, 171.
 Russell, R. I., *et al.* (1968). *Lancet*, **2**, 603.
 Russell, R. I., and Goldberg, A. (1968). *Lancet*, **2**, 606.
 Russell, R. I., *et al.* (1973 a). *Clinical Science and Molecular Medicine*, **45**, 301.
 Russell, R. I., Gerskowitch, V. P., and Cochran, K. M. (1973 b). *Personal observation*.
 Safaie Shirazi, S., Denbesten, L., and Hamza, K. N. (1972). *Proceedings of the Society for Experimental Medicine and Biology*, **140**, 924.
 Semple, P. F., and Russell, R. I. (1975). *Gastroenterology*. In press.
 Werther, J. L., *et al.* (1970). *Gastroenterology*, **59**, 691.
 Wingate, D. J., Phillips, S. F., and Hofmann, A. F. (1973). *Journal of Clinical Investigation*, **52**, 1230.

Oestrogen Receptors in Breast Cancer: A Changing Concept

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Summary

Oestrogen receptors were found in 156 (73%) out of 214 primary breast cancers and in 98 (58%) out of 168 metastatic deposits. These proportions reached 82% and 70% respectively in the second half of the study. Receptors were not found in samples of normal breast tissue but small amounts were present in tissue from some hyperplastic lesions and in male gynaecomastia. Receptor concentrations in the malignant samples were evenly distributed over a wide range of values, suggesting that even "negative" tumours might contain trace amounts undetectable by the method used.

Each tumour was characterized by a given level of receptor concentration. In most cases the amounts found in the invaded axillary nodes and their corresponding primary tumours were the same. We suggest that quantitative rather than qualitative assessment should provide an appropriate criterion for studies of biochemical and clinical correlations.

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Introduction

Some mammary tumours contain oestrogen receptors (Wittliff 1974). Jensen *et al.* (1971) found a striking correlation between the presence of receptors in advanced breast cancers and the response to adrenalectomy. They are extending their studies to other therapeutic procedures and the potential clinical applications are being investigated in several centres (Braunsberg *et al.*, 1973; Engelsman *et al.*, 1973; Jensen *et al.*, 1973; Leung *et al.*, 1973 a; Trams and Maass, 1973; Savlov *et al.*, 1974; Heuson *et al.*, 1975). Sensitive tests for the presence of oestrogen receptors have been developed. Of these the measurement of the oestradiol-binding capacity of cytosol preparations has been found to be the most convenient. Nevertheless, concentrations of receptor measured by this method in both primary and metastatic cancers have varied greatly and it is not known whether these are true variations or whether they reflect an inherent fault in the technique. The present paper reports an investigation of this question.

Materials and Methods

Tissues.—A total of 214 primary tumours and 168 metastatic deposits were studied. They were carcinomas of various histological types in about the same proportions as in a previous study (Leclercq *et al.*, 1973). Forty-five invaded axillary lymph nodes were analysed in 23 mastectomy specimens together with the primary cancer. The metastatic tumours were from skin (124), lymph nodes (32), liver (5), bone (5), muscle (1), and ovary (1). The specimens were obtained at operation and within minutes or,