

CHAPTER

V

DISCUSSION

It is now accepted that the aphorism "no acid no ulcer" is an over simplification of a complex disorder. Also it is now recognised that peptic ulcer is a relapsing disease requiring long term treatment. The basis of the medical treatment of peptic ulcer is to decrease or neutralize the offensive factors or to augment the defensive factors (Table 1). The modern drugs used for the clinical management of the peptic ulcer have not yet been reported to have action on both offensive and defensive factors.

The present investigation on Tamrabhasma (TABH), a traditional preparation of copper advocated in Ayurveda for the treatment of 'amlapitta' i.e. acid pepsin disorder, was undertaken to determine its antiulcerogenic action in various experimental ulcer models in rats. The effectiveness of TABH was also compared with carbenoxolone and cimetidine, both of which have a recognised role in gastroenterological practice for the short term and maintenance treatment of peptic ulcer. Considering the factors such as safety, unwanted effects, cost and acceptability to the patient, TABH has been combined with 50% dose of carbenoxolone or cimetidine and then the effects determined in the various models. Attempts have also been made to study the mode of action and any deleterious effect on long term treatment with TABH.

Many evidences suggest that copper may influence the incidence of the disease. Dietary copper is relatively lower where the disease is more prevalent in India and viceversa (Tovey, 1979). Natural product, the vegetable banana is reported to be antiulcerogenic in different experimental models (Sanyal et al., 1961, 1964, 1965, Elliot & Heward, 1976). Vegetable banana contains significant quantity of copper (Aykroyd, 1963). The ulcerogenicity of nonsteroidal anti-inflammatory drugs is reduced when administered in the form of copper complex (Boyle et al., 1976 ;Rainsford and Whitehouse, 1976), suggesting that copper can influence the disease.

TABH is reported to be antiulcerogenic in pylorus ligated, acetyl salicylic acid and restraint-induced gastric ulcer in rats and histamine-induced duodenal ulcers in guineapig (Sanyal et al., 1982; Pandey et al., 1983). Sanyal et al (1982) reported the minimum effective dose of TABH to range from 1 mg/kg to 5 mg/kg. In the present study the antiulcerogenic action of TABH ranged from 1 mg/kg to 120 mg/kg (Fig.5 a,b). TABH 1 mg/kg produced less effect while 60 mg/kg produced maximum effect. Hence, throughout the study 60 mg/kg of TABH was employed as the antiulcerogenic dose. The following reasons might be responsible for the higher dose of TABH used in this investigation compared to earlier workers.

- (1) The antiulcerogenic action of TABH was initially investigated with dexamethasone-model of gastric ulcers in rats. Compared to ulcers produced in aspirin or histamine or restraint models, those produced in dexamethasone model were more severe and sometimes there was even perforation.

This might have necessitated higher doses of TABH with maximum protection being observed after 4 days of treatment.

- (2) TABH 30 mg/kg and 60 mg/kg produced more or less equal effects 70% and 71% respectively (Fig. 5a,b) suggesting that even 30 mg/kg of TABH could have produced the effect equivalent to 60 mg/kg.
- (3) The preparation of TABH should strictly follow the procedure laid down in the Ayurvedic Pharmacopea (Bhaishaj Samhita., Drug committee Ministry of health, Government of Gujarat, Gujarat State., 1966). The procedures of shodhan & maran play an important role in increasing the biological activity of copper (Pandey et al., 1983). TABH from different sources has been shown to have variable composition, in that the constituents vary quantitatively as well as qualitatively and hence the variation in the dose for its antiulcerogenic action was observed (Pandey et al., 1983).

After confirming the antiulcerogenic action of TABH in dexamethasone induced gastric ulcers TABH was studied for its preventive action if any. Administration of TABH before, simultaneously and after dexamethasone confirms that TABH is effective only when administered after ulceration (Fig.6). TABH administered before or simultaneously shows no significant protection, suggesting that TABH has more curative action than preventive.

The results on ulcer index are in correlation with what has been observed on the effect of TABH administered for 4 or 10 days after dexamethasone. TABH has no significant effect on any of the parameters under study unless ulcer has been produced. It is concluded that TABH produces significant effect after induction of ulcer.

Dexamethasone and indomethacin have been selected for inducing gastric ulcers as both the drugs cause severe gastrointestinal damage in man and are widely used therapeutically for many disorders (Logreen and Allander, 1964; 1974; Katz et al., 1965; Jacobson et al., 1969; Beirene et al., 1974 and Green et al., 1981).

Dexamethasone and indomethacin both caused significant increase in the protein content of the gastric juice (Table5, 8). The protein content of the gastric juice indicates the integrity of the mucosa. Gastric juice normally contains

very little serum protein, but once the mucosal damage occurs, the protein content rises (Grossman, 1978). Corticosteroids are known to decrease the cell production whereas an irritant increases the epithelial cell loss. The mucosa remains intact so long as there is an equilibrium between the rate of cell production and the rate of cell loss (Croft, 1977, 1978). DNA estimation of the stomach wash (Table 12) suggests more epithelial cell loss after dexamethasone or indomethacin. This confirms that after dexamethasone or indomethacin treatment the epithelial cell and mucosal integrity is lost leading to gastric lesions (Fig.8. a,b).

Dexamethasone or indomethacin both had no significant effect on gastric juice volume secreted within four hours of pylorus ligation. Total acidity and pepsin activity were significantly increased after indomethacin whereas dexamethasone had no significant action on these parameters.

Dexamethasone or indomethacin both produced no significant decrease in total carbohydrates and of individual carbohydrate (Table 5, 8). This along with increase in the protein content of the gastric juice caused significant decrease in the carbohydrate to protein ratio. A similar decrease in the carbohydrate to protein ratio and of individual carbohydrate had been earlier reported in non dialysable and lyophilised fraction of the mucus in aspirin, indomethacin and phenylbutazone treated dogs (Menguy and Masters, 1965; Menguy and Desbaillets, 1968). Similar results have been reported with alcoholic precipitation of the gastric juice (Sanyal et al., 1982; Pandey et al., 1983). These results tend to confirm that indomethacin and dexamethasone cause ulceration by affecting the mucosal barrier and that the carbohydrate to protein ratio of the gastric juice is a good index of the mucus barrier.

Cysteamine hydrochloride was used for inducing duodenal ulcer in rats. The duodenum of the rat is highly resistant to ulceration; however, cysteamine caused severe lesions in the duodenum of rat (Fig. 8c; Table 9).

In agreement with the report of Robert et al (1974), the present study could not ascertain the gastric secretagogue action of cysteamine. Our results are also in line with those of Fujii and Ishii (1975), except the dose of cysteamine used in the present study is 300 mg/kg, as higher dose proved lethal. Cysteamine caused significant decrease in the gastric juice volume and increase in the total acidity without affecting peptic activity (Table 10). Increase in total acidity was very high compared to dexamethasone and indomethacin. These

results suggest that the presence of gastric juice in the duodenum is a sin qua non for the production of duodenal ulcer. Cysteamine caused a significant increase in the hexosamine content of the gastric juice. Other carbohydrates were not altered, nor was there a significant change in total carbohydrates after cysteamine administration. The protein content of the gastric juice was significantly increased leading to decrease in carbohydrate to protein ratio. Once again carbohydrate to protein ratio was not much changed as observed with dexamethasone and indomethacin. The results of the gastric acid secretion suggest that the increased total acidity of the gastric juice is more responsible for ulceration than the carbohydrate to protein ratio in cysteamine model.

The mean control ulcer index (mm^2) was significantly reduced with TABH (60 mg/kg) administered for 4 days in dexamethasone or indomethacin or cysteamine models; 10 days TABH treatment being more effective than 4 days treatment. The percentage protection ranged from 60% to 80% (Table 3, 6, 9) with corresponding decrease in the number of ulcers.

On gastric acid secretion TABH affected all the parameters. It caused significant decreases in the volume of the gastric juice, total acidity and pepsin activity in dexamethasone or indomethacin or cysteamine models.

Karlfmann in 1908 first suggested the idea of investigating the qualities of gastric mucus in relation to the aetiology of peptic ulcer. Glass (1953) defined visible mucus as a complex gel. Recent development in the molecular pathology of ulcer have included studies on the effects of ulcerogenic and ulcer healing drugs on the composition and the rate of synthesis of gastrointestinal mucus. TABH caused qualitative and quantitative change in the dissolved mucin content of the gastric juice. TABH caused 2 to 3-fold increase in the formation of the fucose and sialic acid compared to dexamethasone or indomethacin or cysteamine models (Table 5, 8, 11). The sialic acid and fucose content were increased, suggesting a qualitative change in the mucus. TABH also affected total hexoses without significant change in hexosamine.

The mucinous fraction consists of polymers of glycoprotein molecules which, by their high molecular weight, impart high viscosity to gastric mucus. The viscosity of gastric mucus is important in the concept of the 'mucus barrier' in that the more viscous the mucus is, more it clings to the mucosa. The increased fucose and sialic acid in the gastric juice of TABH treated rats may contribute

towards its antiulcerogenic effect by increasing the viscosity of the gastric mucus (Curt and Pringle, 1967; Baume et al., 1967).

TABH caused significant decrease in the protein content of the gastric juice and significant increase in the total carbohydrate leading to increase in the carbohydrate to protein ratio. TABH administered for 10 days did not modify carbohydrate to protein ratio and increased levels of sialic acid and fucose were maintained. This result suggests that TABH caused a quantitative increase in the dissolved content of the gastric juice and improved the quality of mucus. Thus it is concluded that TABH increased the efficiency of the mucus barrier which appears to have contributed to antiulcerogenic effect.

Carbenoxolone 30 mg/kg administered for 4 days after dexamethasone or indomethacin or cysteamine caused maximum decrease in the ulcer index and percentage protection observed ranged from 70 to 80% (Table 3, 6, 9). The effects were equivalent to those with 10 days TABH treatment. Carbenoxolone had no effect on volume and total acidity of the gastric juice but reduced the pepsin activity (Table 4, 7, 10). The results are in line with those reported by May and Baker Laboratory, England (Sircus, 1972). On mucus, carbenoxolone produced maximum changes causing 3 to 4-fold increase in sialic acid and fucose, the content of total hexoses and hexosamine were also increased. Maximum decrease in protein observed in all these experimental models lead to increase in the carbohydrate to protein ratio.

Carbenoxolone generally affects only defensive factors, it modifies the quality and quantity of mucus and increases the life span of the epithelial cell so that they become more mature in producing more mucus (Croft, 1978). Carbenoxolone has recently been shown to increase the release of PGE₂ from gastric mucosa of rat and man (Martin et al., 1982; Rask-Madsen, 1982).

Investigations into the pathogenesis of peptic ulceration and intestinal mucosal injury produced by non-steroidal anti-inflammatory drugs and other damaging agents such as alcohol suggest that the prostaglandin maintain integrity and accelerate ulcer healing (Robert et al., 1976; Robert, 1980; Johansson et al., 1980; Peskar et al., 1984).

The synthesis of eicosanoids in the samples of mucosa from opposite the ulcer site are similarly reduced when compared with mucosa close to the ulcer. It would seem, therefore, that the prostanoïd synthesising defect is not just associated with the immediate ulcer disease and not secondary to the formation of the ulcer. It is suggested that reduced PGI_2 and PGF_2 may predispose the duodenal mucosa to injury and ulceration. Recently it has been reported that there is a defect in the ability of the human duodenal mucosa in duodenal ulcer disease to synthesize PGF_2 and PGI_2 (Hiller et al., 1985). Mucosa close to the ulcer site synthesized significantly less PGF_2 and PGI_2 than healthy mucosa from non ulcerated patients without affecting the synthesis of PGE and TXA_2 suggesting that reduced PGF_2 and PGI_2 may predispose the duodenal mucosa to injury and ulceration (Hiller et al., 1985).

The mechanism underlying the beneficial actions of prostaglandins are uncertain but studies (mainly with PGE_2) have shown that it can increase mucus (Bolton et al., 1976; Johansson and Kollberg, 1979) and bicarbonate secretion (Ross and turnberg, 1983). PGE_2 can also inhibit acid secretion (Robert et al., 1976)

increase gastric mucosal blood flow (Ber and Nies, 1982) and prevent ion flux and potential difference changes produced by noxious stimuli (Chaudhury and Jacobson, 1978; Whittle, 1977; Tepperman et al., 1978).

PGE and F series have been unequivocally shown to inhibit not only gastric secretion but also to augment the defensive factors and have been shown to possess cytoprotective action (Robert, 1976; Whittle, 1977). The concept of cytoprotection suggests protection against mucosal damage by a variety of noxious agents but this property is unrelated to the presence of gastric acid. Cytoprotective drugs such as sucralfate and misprostol (an orally effective synthetic derivative of prostaglandin E_1) and other prostaglandin synthetic derivatives have emerged as valuable antiulcer agents especially in the treatment of gastric ulcer.

Since in the present experiments TABH was found to act similar to carbenoxolone, it may be suggested that antiulcerogenic effect may be due to its action on endogenous prostaglandin. Even in minute quantity copper is known to enhance the synthesis of prostaglandin (Winter et al., 1962; Maddox, 1973). Epithelial cell loss is significantly reduced with TABH administration for 10 days after dexamethasone or indomethacin. Similar decrease in the epithelial cell is reported in human beings after carbenoxolone (Croft, 1978).

Evidence and results suggest that TABH may have cytoprotective action by increasing synthesis of endogenous prostaglandin although the efficacy is less compared to modern drugs available for the treatment of peptic ulcer.

Cimetidine (20 mg/kg p.o.) treatment for 4 days after dexamethasone or indomethacin or cysteamine significantly reduced ulcer index and produced 60 to 70% protection (Table 3, 6, 9). Cimetidine (20 mg/kg) produced less effect compared to carbenoxolone (30 mg/kg); however it produced effect equivalent to TABH 60 mg/kg for 4 days. Cimetidine is found to be equally effective in gastric as well as duodenal ulcer models of the present study.

On offensive factors like total acidity, pepsin activity and volume of gastric juice, cimetidine produced maximum effect, which was more than TABH 60 mg/kg administered for 4 days or 10 days or carbenoxolone (Table 7, 10). Cimetidine produced more effect after cysteamine than after dexamethasone or indomethacin suggesting that cimetidine is preferably more helpful in duodenal ulcers than in gastric ulcers. Cimetidine did not produce any significant change in total or individual carbohydrate contents of the gastric juice, however it caused significant increase in carbohydrate to protein ratio. This increase was because of significant decrease in the protein content of the gastric juice. The effect observed on carbohydrate to protein ratio with cimetidine was more or less equivalent to that observed with 4 days TABH treatment and significantly less than that with carbenoxolone.

Although a large number of duodenal ulcer patients are normosecretor of the gastric acid, the histamine H_2 -receptor antagonists continue to play an important role even in this group. Acid reduces the healing rate and therefore lowered acidity is beneficial. The ability of H_2 -receptor blockers to increase gastric ulcer healing has led to research into the other mechanisms of these drugs, because acid appears not to contribute so much to gastric ulcer formation as it does to duodenal ulceration. Even in the present study cimetidine was found to be equally effective in both duodenal and gastric ulcers. Significant decrease in the protein content of the gastric juice suggests that cimetidine might have some effect on mucosal integrity.

Experiments carried out with combinations of TABH 60 mg/kg with 50% reduced dose of carbenoxolone (15 mg/kg) or cimetidine (10 mg/kg) suggest that TABH affects both offensive and defensive factors involved in ulceration. Addition

of TABH to cimetidine produced better effect than that produced by cimetidine alone or by TABH 4 days treatment. Considering the results of combination of TABH with cimetidine on ulcer index, total acidity and various carbohydrate fraction and protein, it is concluded that TABH acts similar to cimetidine, but at the site other than cimetidine. Copper has been reported to interfere with histamine synthesis (Ostar and Salgo, 1977). The observed antiulcerogenic action of TABH might be due to interference with synthesis of histamine rather than specific H_2 -receptor blockade.

Prostaglandin E_2 is liberated from gastric mucosa during gastric acid secretion by cAMP. PGE_2 inhibits gastric acid secretion in response to histamine or pentagastrin, this action may be due to inhibition of formation of cAMP. Thus the cAMP and prostaglandin system of parietal cell may interact to form an inhibitory feed-back loop (Fig.2). Aspirin and other anti-inflammatory drugs having an inhibitory effect on prostaglandin synthetase would disrupt the inhibitory loop and increase gastric acid production (Bowman and Rand, 1975).

Copper is known to be concentrated several folds (relative to other metals) in sympathetic nerve endings, and in the synaptic vesicle of brain and other nerve tissue. There it seems to assist in storing norepinephrine in a ternary complex with ATP (Colburn and Mass, 1965) and 5-hydroxytryptamine is similarly stored (Roberts, 1966). Cupric ions, adenosine triphosphate and norepinephrine form of tightly bound ternary complex.

Similarly TABH may affect this inhibitory loop directly by increasing PG synthesis or indirectly by forming a stable complex with ATP in the parietal cell, so that sufficient amount of ATP will not be freely available for the formation of cAMP and hence decrease in the formation of gastric acid.

The results of similar combination of TABH with carbenoxolone suggest that TABH acts similar to carbenoxolone affecting individual carbohydrate fractions especially fucose and sialic acid. The combination produced less effect than that produced by carbenoxolone alone, suggesting that both have similar site of action with carbenoxolone having more efficacy than TABH.

Combined therapy of TABH with cimetidine appears to be more helpful in duodenal ulcer whereas similar combination with carbenoxolone may be more helpful in gastric ulcer.

TABH has been reported to have longer lasting effect (Pandey et al., 1983). It seems that copper, being a heavy metal, gets bound for prolonged period to certain metallo-enzymes and produces its long lasting action. Hence it is of great interest that TABH requires less frequent administration in clinical practice. Pandey et al. (1983) also reported significant increase in the mucosal thickenss with TABH treatment after aspirin induced ulceration.

TABH, while antagonising anti-ulcer effect of aspirin, did not antagonise anti-inflammatory effect of aspirin. So combination of aspirin and TABH may reduce toxicity of aspirin when used in high doses as an anti-inflammatory agent. (Pandey et al., 1983).

TABH contains very high concentration of copper (Table 14). Copper is also reported to inhibit proteolytic enzyme activity and carbonic anhydrase enzyme (Robert, 1979; Kowalski et al., 1956). How far this is important in antiulcerogenic action of TABH require investigations.

The ulcerogenic action of dexamethasone or indomethacin or cysteamine (Fig 9,13,18) and antiulcerogenic action of TABH was also confirmed by histopathological study. Healing of the ulcers after TABH in the form of fibrosis, congested blood vessels, presence of inflammatory cells and regeneration can be seen in most of the sections (Fig.10,11,15,20). Sections stained by PAS show increased PAS activity, suggesting more mucus formation after TABH treatment.

Acute and subacute toxicity study of TABH suggest significant increase in the haemoglobin content and total WBC content (Sanyal et al., 1982). Chronic toxicity carried out in the present investigation revealed no observed haematological or biochemical side effect. TABH (120 mg/kg) administered for 30 days produced significant increase in haemoglobin and total RBC count due to the presence of iron in this preparation. TABH caused insignificant rise in total WBC count. After TABH administration microscopic and gross examination of the liver, kidney and heart did not show any deleterious effect.

It may be concluded that TABH possesses distinct antiulcerogenic action and acts like carbenoxolone and cimetidine. It affects both offensive and defensive factors, although the site and mode of actions are different from cimetidine.

TABH seems to have cytoprotective action similar to prostaglandins. The exact mechanism of cytoprotection is still unclear, the mucosal cell protecting property is now recognised as a reflection of the important function of mucosal cell resistance in the aetiology of gastric ulcers in particular. The results of the present investigation are significant; however, extrapolation from the experimental model to human ulceration has obvious limitations.