The possibility of dietary protective factors in duodenal ulcer. II.

An investigation into the effect of pre-feeding with different diets and of instillation of foodstuffs into the stomach on the incidence of ulcers in pylorus-ligated rats

* A. PAUL JAYARAJ
M.Phil., M.I.Biol.

† FRANK I. TOVEY
Ch.M., F.R.C.S.

‡ C. G. CLARK
M.D., Ch.M., F.R.C.S.

* Central Food Technological Research Institute, Mysore, South India, † Basingstoke District Hospital, Basingstoke, England, and ‡ Department of Surgery, University College Hospital Medical School, London

Summary

Rats pre-fed on a South Indian diet show a much higher incidence of ulceration of the stomach after pyloric ligation than those on a North Indian diet. Removing vegetables and pulses from the North Indian diet deprives it of its protective effect. Staple foodstuffs with a high buffer content (unmilled rice, unrefined wheat and a millet [ragi]) placed in the stomach after pyloric ligation are also protective, but those with a low buffer content (milled rice, tapioca, sorghum and maize) are not protective. Refined wheat, however, gives protection despite its low buffer content. The significance of these findings in relationship to the distribution of duodenal ulcer in India and Africa is discussed.

Introduction

Duodenal ulcer is seen frequently in areas in India (particularly in the south) where the staple diet is milled polished rice and sometimes tapioca, and infrequently in areas (in the north) where the diet is unrefined wheat. The following experiments were designed to determine whether any possible protective action of diet against peptic ulcer could be ascribed to factors acting systemically on the mucosa to increase its resistance to ulceration, or to a possible local buffering effect as a result of the actual presence of food in the stomach.

The effect of pre-feeding with North Indian diet has been compared with pre-feeding with South Indian diet on the incidence of ulceration in pylorus-ligated rats, and also the effect of removing vegetables and pulses from the North Indian diet.

Address for reprints: Mr F. I. Tovey, 11 Crossborough Hill, Basingstoke, Hampshire.

In addition, the effect on the incidence of ulceration of placing various staple foodstuffs into the stomachs of rats immediately after pyloric ligation was determined. Milled and unmilled rice were compared with unrefined wheat. The experiments were extended to include a millet (ragi or *Eleusine coracana*), *Sorghum vulgare* (cholam or jowar) and maize. This was done having in mind the situation in Africa where duodenal ulcer seems to be uncommon in dry areas where the staple diet is maize, millet or sorghum (Tovey, 1974a, 1975; Tovey and Tunstall, 1975).

Material and methods

It has been accepted that the pylorus-ligated rat (Lee and Bianchi, 1971) is a suitable model for studying the effect of various drugs on the prevention or healing of peptic ulcers and therefore would be applicable for this investigation.

Two groups of experiments were planned as follows:

First group

Female albino rats (Wistar strain) weighing 100-160 g were fed on the required food substances for a period of 2 weeks and then given water only for 24 hr. A pyloric ligation (Shay *et al.*, 1945) was then performed under ether anaesthesia. The stomach was washed out with 4 ml or more of normal saline. Then 2 ml of normal saline were introduced into the stomach and left there until the rats were anaesthetized again 6 hr later. The oesophagus was ligated and the stomach, with contents, was removed. The stomach was then opened and the number of macroscopic ulcers in the rumen and the mucosal part of

the stomach was recorded separately, together with the number of large ulcers (> 2 mm) and the presence of perforation (Shay *et al.*, 1945).

The combination of food substances investigated were as follows:

- (i) North Indian (Punjabi) diet, (a) cooked, (b) uncooked.
- (ii) South Indian diet, (a) cooked, (b) uncooked.
- (iii) North Indian (Punjabi) diet (cooked), (a) minus vegetables, (b) minus pulses, (c) minus vegetables and pulses.

The composition of the diets was as follows, expressed as percentage by weight:

<i>North Indian diet</i>		
Unrefined wheat flour		41.2
Rice flour		5.7
Maize flour		8.6
Pulses		
Bengal gram	1.7	5.6
Green gram	1.7	
Black gram	1.7	
Lentil	0.5	
Green vegetables (leafy)		
Amaranthus		4.0
Other vegetables		
Potato		6.2
'Ladies fingers'		6.2
Banana powder		0.6
Vegetable fat (dalda)		4.4
Sugar		9.5
Corn starch		5.0
Common salt		0.2
Skimmed milk powder		2.9
<i>South Indian diet</i>		
Rice flour		78.5
Turdhal flour		5.0
Groundnut oil		5.0
Leafy vegetables		2.1
Non-leafy vegetables		8.2
Common salt		0.3
Skimmed milk powder		0.9

The constituents of the diets were evenly mixed together and the rats showed no tendency to be selective in their eating.

Initially there were eight rats in each group. The experiment was repeated in the case of cooked Punjabi and South Indian diet and in Punjabi diet minus vegetables and pulse with reproducible results.

Second group

Similar female rats fed on stock diet were taken and fasted for 24 hr. A pyloric ligation was performed and the stomach was washed out with 4 ml

or more of normal saline. Half a gram of the food substance in 2 ml of normal saline were then instilled into the stomach. After 6 hr the rats were again anaesthetized, the oesophagus ligated and the stomach removed. The stomachs were examined as before for mucosal and rumenal ulcers.

The following food substances were used:

- (i) Polished rice, (a) cooked, (b) uncooked.
- (ii) Unpolished rice, (a) cooked, (b) uncooked.
- (iii) *E. coracana* (ragi), (a) cooked, (b) uncooked.
- (iv) Unrefined wheat, (a) cooked, (b) uncooked.
- (v) White wheat flour (cooked).
- (vi) Tapioca, (a) cooked, (b) uncooked.
- (vii) Maize, (a) cooked, (b) uncooked.
- (viii) Jowar (cholam) *S. vulgare*. (a) cooked, (b) uncooked.

Results

Group 1

The results are shown in Table 1.

Group 2

The results were as shown in Table 2.

Figures 1 and 2 (a and b) show the mean ulcer scores. These are calculated by dividing the number of macroscopic ulcers recorded by the number of rats in each dietary group.

The results are discussed below:

Group 1. Prefeeding experiments

- (1) Punjabi diet offers a marked protection in comparison with South Indian diet.
- (2) Cooking the food made no difference except in the appearance of mucosal ulcers in the group on cooked South Indian food.
- (3) Removal of either the vegetables or the pulses deprives the Punjabi diet of its protective effect. When both are removed the number of perforations is high. (Note: When perforation occurs the loss of gastric juice from the stomach may result in a smaller number of actual ulcers.)

Group 2. Instillation experiments

- (1) Unpolished rice gave more protection than polished rice.
- (2) Ragi, wheat (refined and unrefined) afforded marked protection.
- (3) Tapioca, maize and jowar offered little protection.
- (4) Cooking the food had no effect on its local protective action.

The above differences are all significant using the χ^2 test ($P = < 0.01$).

TABLE 1. Pre-feeding experiments. Incidence of mucosal and rumenal ulcers

Diet	No. of rats	Mucosal ulcers		Rumenal ulcers			
		No. of rats with ulcers	Total no. of ulcers	No. of rats with ulcers	Total no. of ulcers	Large ulcers	Perforated
Punjabi							
Cooked	16	0	0	9	14	0	0
Uncooked	8	0	0	2	2	0	0
South Indian							
Cooked	16	16	45	16	68	19	9
Uncooked	8	0	0	8	37	14	4
Punjabi							
Minus vegetables	8	0	0	8	25	3	0
Minus pulses	8	0	0	7	32	10	2
Minus vegetables and pulses	16	0	0	13	40	8	10

TABLE 2. Post-pyloric ligation instillation experiments. Incidence of mucosal and rumenal ulcers

Food substance instilled	No. of rats	Mucosal ulcers		Rumenal ulcers			
		No. of rats with ulcers	Total no. of ulcers	No. of rats with ulcers	Total no. of ulcers	Large ulcers	Perforated
Polished rice							
Cooked	8	3	3	5	9	3	0
Uncooked	8	0	0	8	13	7	0
Unpolished rice							
Cooked	8	1	1	1	2	0	0
Uncooked	8	0	0	2	2	0	0
Ragi							
Cooked	8	0	0	2	4	0	0
Uncooked	8	0	0	2	3	0	0
Unrefined wheat							
Cooked	8	0	0	1	1	0	0
Uncooked	8	0	0	1	1	0	0
White flour, cooked	8	0	0	0	0	0	0
Tapioca							
Cooked	8	2	2	5	16	2	0
Uncooked	8	0	0	8	9	3	1
Maize							
Cooked	8	4	4	6	18	3	0
Uncooked	8	0	0	5	8	2	0
Jowar							
Cooked	8	2	4	6	9	2	0
Uncooked	8	0	0	4	6	1	0

FIG. 1. Mean ulcer scores. South Indian *versus* Punjabi diet.

Pyloric ligation instillation experiments (0.5 g in 2 ml saline)

FIG. 2. Mean ulcer scores (Group (a), low scores, Group (b), high scores).

Discussion

It has been suggested that a factor in the aetiology of peptic ulcer may be the absence of protective factors in certain diets. Cheney (1940, 1948, 1949, 1950a, b, 1952), Singh, Zaidi and Bajpal (1962), Adami (1955, 1964) and Wissmer and Adami (1965), in experiments on chicks, guinea-pigs and rats, using several methods of producing experimental ulcers, repeatedly showed that pre-feeding with certain foodstuffs—egg yolk, milk, butter, green vegetables, lettuce and, in particular, cabbage—would protect the animals against ulceration. The protective factor was thermolabile and was destroyed to a variable extent by heating and pasteurization.

These present experiments suggest that North Indian (Punjabi) diet has a marked protective effect compared with South Indian diet. Cooking has no effect on the protective action of North Indian diet, but is accompanied by the appearance of mucosal ulcers in South Indian diet. The appearance of mucosal ulcers is taken as an indication of a more severe degree of ulceration than rumenal ulceration alone (Tovey, Jayaraj and Clark, 1975).

Haaf (1972) has suggested that the protective action of a diet may be related to its protein content, and thinks that this may account for the situation in Ghana where duodenal ulcer is uncommon in the northern savannah regions and common in the southern rain forest and coastal zone. The same difference in protein intake exists between North and South India. This suggestion is supported by the experiments of Shay *et al.* (1948), who showed that pre-feeding rats with protein-hydrolysate conferred protection against ulceration after pyloric ligation. Tovey *et al.* (1975) found that skimmed milk powder gave similar protection and that raw cabbage, brinjal and dhal were also protective. In these experiments the exclusion of green vegetables or of

pulses, which have relatively little effect on the protein content, from the North Indian (Punjabi) diet seems to deprive it considerably of its protective effect.

In the earlier experiments quoted above it was found that there was no difference in the incidence of experimental ulceration between pre-feeding with unrefined carbohydrate or with refined carbohydrate foods. Cleave (1962, 1974), however, suggested that an important factor in the aetiology of peptic ulcer is the loss of protective buffer to gastric acid resulting from the refinement of staple carbohydrate foods, and that this could account for the different incidence of duodenal ulcer in North and South India. Lennard-Jones, Fletcher and Shaw (1968) showed that refined carbohydrates produced lower intra-gastric pH levels than unrefined carbohydrates. Tovey (1974b) found that wheat and rice bran and certain unrefined grains had a significant buffering effect *in vitro*, but that *in vivo* this was offset by their stimulatory effect on the gastric antrum resulting in higher acid outputs. The present experiments suggest that unrefined rice, the millet ragi, and unrefined wheat, which have a high buffer content, protect rats against experimental ulceration when present in the stomach, and that polished rice, tapioca and sorghum, all with a low buffer content, do not give any protection. These findings fit in with Cleave's hypothesis, except for the anomaly that refined wheat with a low buffer content also gave protection. It is difficult, however, to account for the findings in respect of maize, which is the main constituent of the diet in all the low incidence areas of Africa. In the earlier series of experiments (Tovey *et al.*, 1975) it was found that pre-feeding with a supplement of maize alone offered no protection. It seems to have no protective effect also in the instillation experiments, and its buffer content is low. It looks as if there must

be some other factor besides maize in the diet to account for the low incidence of peptic ulceration in the maize-eating areas of Africa.

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