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Hereditary aspects of duodenal ulceration: pepsin 1 secretion in relation to ABO blood groups and ABH secretor status

LESLEY WAFT, N. B. ROBERTS, AND W. H. TAYLOR

From the Liverpool Area Health Authority (Teaching), Department of Chemical Pathology, Duncan Building, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XW

SUMMARY Pepsin 1, the ulcer-associated pepsin, occurred significantly more frequently in the gastric juice of those patients with duodenal ulcer who did not secrete A, B, or H antigens into gastric juice than in those secreting these antigens. This observation may explain the increased proportion of such non-secretors among patients with duodenal ulceration. In patients with gastric ulcer and non-ulcer dyspepsia, and in a miscellaneous group of patients, there was no association of pepsin 1 secretion with secretor status, suggesting that the association noted in duodenal ulceration is an indirect rather than a direct one. No increase of pepsin 1 occurred in group O patients with peptic ulcer, so that the increased proportion of such patients in peptic ulcer does not arise from differences in pepsin 1 secretion.

The isolation and preparation of pure human pepsin 1 have recently been described (Roberts and Taylor, 1978). The enzyme is the pepsin which migrates most rapidly to the anode on electrophoresis at pH 5·0 (Etherington and Taylor, 1967) and it is present in increased amounts in the gastric juice of patients with peptic ulcer (Taylor, 1970). Pepsin 1 has subsequently been found in increased amount in other clinical situations associated with acute or chronic peptic ulceration, as in the gastric secretion of patients with postoperative and post-traumatic stress (Walker and Taylor, 1976), and in smokers with peptic ulcer as compared with non-smokers (Walker and Taylor, 1977). Furthermore, the healing action of carbenoxolone in peptic ulcer may be partly explained by the observation that it diminishes the secretion of pepsin 1 in patients who respond, but not in those who do not (Taylor *et al.*, 1978), and that it inactivates intraluminally a proportion of the pepsin 1 that is secreted (Roberts and Taylor, 1973) and of the other pepsins (Berstad, 1972). There is evidence in duodenal ulceration for an increased 'vagal' drive on the gastric mucosa (Baron *et al.*, 1969). Not only does vagal stimulation by insulin hypoglycaemia increase pepsin 1 secretion in man (Walker and Taylor, 1976), but in the cat the homologous enzyme is released only after prolonged intermittent vagal excitation (Wright *et al.*, 1975).

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Among patients with duodenal ulcer there is an increased proportion of subjects who do not secrete blood group (ABH) substances into saliva (24·4 to 42·3% in various series) as compared with controls (21·1 to 25·3%) (for references see McConnell, 1966), and there is an increased proportion of group O patients (53·2 to 59·7% in various series in Britain as compared with 43·5 to 48·9% in controls) (McConnell, 1966). Among patients with gastric ulcer there is an increased preponderance of non-secretors also (25·5 to 31·2% as compared with 21·5 to 24·2% in controls), but a preponderance of blood group O was found in only 21 of 28 series, and not among Liverpool patients (McConnell, 1966).

Blood group O and non-secretor status are clearly genetic factors which play a (statistically) minor role in the pathogenesis of peptic ulcer, but the manner in which they exert their effect at the cellular level is conjectural. Only the 'aggressive' factors, hydrogen ion and pepsin, have been studied in relation to secretor status. Thus Brown *et al.* (1956) and Ventzke and Grossman (1962) found no significant differences in maximum histamine-stimulated acid output in duodenal ulcer among the ABO blood group and salivary ABH secretor phenotypes. Hanley (1964) reported than the mean serum pepsinogen of normal group O subjects was higher than that of group A subjects, thus confirming the work of Sievers (1959) in patients, but not that of Nieder-

man *et al.* (1962) who, in normal subjects, could find no significant difference between the ABO phenotypes. Both Neiderman *et al.* (1962) and Hanley (1964) agree, however, that serum pepsinogen levels do not differ significantly in secretors and non-secretors of salivary ABH antigens. Thus, neither of the two genetic factors affect hydrogen ion secretion; blood group influences serum pepsinogen level and hence, it is implied, peptic-cell mass, but secretor status does not.

Since pepsin 1 is associated with peptic ulcer, the possibility arises that blood group and secretor status influence its secretion. We have, therefore, investigated the relationship between the amounts of pepsin 1 secreted by patients with peptic ulcer and their blood group and secretor status.

Patients and methods

A total of 182 patients who secreted hydrogen ions in response to a standard pentagastrin (6 μg/kg bodyweight subcutaneously) gastric function test were studied. The tests were carried out in hospital before medical or surgical treatment was given and, in particular, none of the patients were taking carbenoxolone at the time of the test or immediately preceding it. The patients were divided into four groups; 102 had duodenal ulcer proved radiologically, or by endoscopy, or by surgery or by all these; 28 had gastric ulcer, proved similarly, including 11 patients who had duodenal ulceration simultaneously or on previous admission; 30 suffered from non-ulcer dyspepsia, ulceration being excluded radiologically or by endoscopy or by both; 22 were classified as miscellaneous and included patients with gall bladder disease, hiatus hernia, and non-specific colonic dysfunction. Patients with iron-deficiency and malabsorption were excluded. Of the 102 patients with duodenal ulcer, blood group and secretor status were determined in 86, secretor status alone in 11, and blood group alone in 5. Of the 30 patients with non-ulcer dyspepsia, secretor status was determined in 23.

There is no satisfactory quantitative method for the determination of pepsin 1, but increased quantities of any of the pepsins can be detected visually on electrophoretograms exactly as plasma proteins, lipoproteins, or isoenzymes of lactic dehydrogenase have been detected for several years. The visualisation can be made semiquantitative by comparing the pepsin 1 band with that of pepsin 3 on the same electrophoretogram and with those of known amounts of porcine pepsin. Agar gel electrophoresis was, therefore, carried out in duplicate by the method of Etherington and Taylor (1969) on the pooled postpentagastrin samples, suitably diluted with 0·001 M HCl. A porcine pepsin standard was included with each plate or pair of plates, consisting respectively of 5 and 10 electrophoretic analyses.

Pepsin 1 was then graded as O, trace, +, ++, or +++; trace represents approximately 10·5 μg pepsin 1/ml (porcine pepsin equivalent) when compared with electrophoretograms of known amounts of porcine pepsin, and +, ++, and +++ represent 22·5, 60, and 250 μg/ml, respectively. Gels were assessed in batches by two observers, one of whom knew nothing of the clinical details of the patient. Neither observer knew the blood group nor secretor status of the patient when the semiquantitative assessment was made. Because of the relatively large differences of concentration of the grades, the assessments agreed in 90 to 100% of samples in the various batches.

Gastric juice, both resting and pooled postpentagastrin samples, were neutralised to pH 7·4 with 4M NaOH and tested for secretor H, A, and B activity by the method described for saliva by Evans *et al.* (1964). Occasionally, a heavily bile-stained juice was encountered which caused lysis of O cells, as the Ulex Europa preparation contains little protein. The addition to the gastric juice of an equal volume of bovine serum albumin (15 g/l) was found to prevent this, presumably by binding the bile salts.

Results

BLOOD GROUP DISTRIBUTION

The proportion of patients with blood group O did not differ significantly in the miscellaneous group (52·4%, n=21) and in gastric ulcer (60·7%, n=28) from the normal population of Merseyside (48·9%) (McConnell, 1966), but was significantly higher in non-ulcer dyspepsia (70·0%, n=30, $P<0·02$) and duodenal ulcer (64·8%, n=91, $P<0·01$).

SECRETOR STATUS

The proportion of patients not secreting blood group substances in gastric juice did not differ significantly in the miscellaneous (36·4%), non-ulcer dyspepsia (26·1%), and gastric ulcer (28·6%) groups from the proportion of the normal population (24·2%) not secreting these substances in saliva, but was significantly higher in duodenal ulcer (42·3%, $P<0·001$).

SECRETION OF PEPSIN 1

Table 1 shows the distribution of the different grades of secretion of pepsin 1 in patients with duodenal ulcer and gastric ulcer, and Table 2 gives similar but less detailed information for the non-ulcer dyspepsia and miscellaneous groups. On

superficial inspection the data suggest that in duodenal ulcer, patients with group B have lower grades of pepsin secretion than in the other groups, and that non-secretors have higher grades. When these differences are expressed as the percentage of patients secreting pepsin 1 in grade + or greater (Table 2), the only statistically significant difference is that between secretors and non-secretors in duodenal ulcer ($P=0\cdot03$). In all four disease categories patients with group A had higher incidences of pepsin 1 secretion than those with groups O or B, but the differences were not of statistical significance either singly or combined.

Table 1 *Pepsin 1 secretion of patients with duodenal and gastric ulcer, according to blood group and secretor status*

		No	Pepsin 1 secretion			
			O	Trace +	++	+++
Duodenal ulcer	Blood group O	59	6	12	22	17	2
	A	24	1	5	12	6	0
	B	6	1	3	1	1	0
	AB	2	0	0	2	0	0
	Secretor	56	5	16	20	13	2
	Non-secretor	41	3	4	21	13	0
Gastric ulcer	Blood group O	17	0	5	8	3	1
	A	9	0	2	5	2	0
	B	2	0	1	0	1	0
	AB	0					
	Secretor	20	0	6	8	6	0
	Non-secretor	8	0	2	5	0	1

Table 2 *Percentage of patients secreting pepsin 1 in pooled post-pentagastrin gastric juice in amounts of grade + or greater according to blood group and secretor status*

	DU	GU	Non-ulcer dyspepsia	Miscellaneous
Blood group O	69·5 (59)	70·7 (17)	62·0 (21)	27·3 (11)
A	75·0 (24)	77·8 (9)	77·8 (9)	55·6 (9)
B	33·3 (6)	50·0 (2)	—	0 (1)
AB	100 (2)	—	—	—
Secretor status				
Secretors	62·5 (56)	70·0 (20)	64·8 (17)	42·8 (14)
Non-secretors	82·9*(41)	75·0 (8)	50·0 (6)	50·0 (8)

Grade + = approx. 22·5 μg/ml. Numbers in parentheses are those of the total patients in each group; *indicates a significant difference between secretors and non-secretors.

Table 3 expresses the pepsin 1 secretion in duodenal and gastric ulcer in relation to both blood group and secretor status. Inspection shows the expected increased incidence of pepsin 1 secretion in the non-secretors of each blood group where there are six or more patients in each group. The highest incidence is found in A non-secretors, although the value does not differ significantly from that in O non-secretors.

Table 3 *Percentage of patients secreting pepsin 1 in pooled post-pentagastrin gastric juice in amounts of grade + or greater*

	DU		GU	
O secretor	64·1	(39)	69·2	(13)
O non-secretor	87·5	(16)	75·0	(4)
A secretor	69·2	(13)	66·7	(6)
A non-secretor	90·0	(10)	100	(3)
B secretor	25·0	(4)	100	(1)
B non-secretor	50·0	(2)	0	(1)
AB secretor		(0)		(0)
AB non-secretor	100	(2)		(0)

Grade + = approx. 22·5 μg/ml. Numbers in parentheses are those of the total patients in each group.

PEAK ACID OUTPUT, BLOOD GROUP, AND SECRETOR STATUS

The mean peak acid output did not differ significantly with blood group or secretor status in any of the groups of patients. Thus, data are available for 58 of the patients with duodenal ulcer; in the remainder the 60 min secretion was aggregated before analysis, or the periods of collection did not allow the peak acid output to be calculated. The mean peak acid output (mmol/h), the standard deviation, and the number of patients (n) for each category were: O secretor, $33\cdot8 \pm 13\cdot2$, n=24; O non-secretor, $29\cdot1 \pm 10\cdot4$, n=13; A secretor, $26\cdot9 \pm 12\cdot7$, n=8; A non-secretor, $30\cdot8 \pm 14\cdot3$, n=6; B secretor, $27\cdot0 \pm 12\cdot1$, n=4. The differences in pepsin 1 secretion between secretors and non-secretors are not, therefore, secondary to differences of acid output between the various phenotypic groups.

Discussion

The duodenal ulcer patients of this study showed the expected excess proportions of subjects with blood group O and with inability to secrete A, B, or H substances into gastric juice. Comparison had necessarily to be made with the saliva of normal subjects, but since saliva contains only 82% of the concentration of blood group substances of gastric juice (Hartmann, 1941), the high proportion of non-secretors is unlikely to have been introduced erroneously.

The pepsin 1 concentration of the combined peptic ulcer patients was grade + or higher in 90 of 130 patients, a proportion significantly higher than in the miscellaneous group (9 of 22 patients; $0\cdot72>P>0\cdot01$) confirming the similar observations of Taylor (1970) and Walker (1976). The results show that the excess proportion of group O patients with duodenal ulcer is not associated with an excess secretion of pepsin 1 in this group, so that the only known association of group O with the aggressive factors in duodenal ulceration remains that with the serum total pepsinogen (? equivalent to peptic cell

mass) (Hanley, 1964). The data of group B patients are of interest. Of the patients, nine were in group B and only three secreted pepsin 1 in grade + or above. If this low proportion were to be confirmed in a larger number of subjects, it would raise the interesting speculation that group B may have a protective effect in peptic ulceration, mediated via a lower pepsin 1 secretion. McConnell (1966) has listed the blood group distribution of many series of patients with peptic ulcer. In duodenal ulcer, 32 of 41 series had a lower proportion of group B subjects than normal controls; for gastric ulcer, 25 of 27 series behaved similarly. It is usually considered that this lower proportion, together with that of group A (41 of 41 series for duodenal ulcer, 21 of 28 series for gastric ulcer), is secondary to the increased proportion of group O subjects, but the primary effect could be that there is a lower proportion of group A and B subjects in these diseases. The present data indicate that in the case of group A such an effect would not be attributable to a lowered secretion of pepsin 1.

By contrast with the blood group data, the proportion of patients with duodenal ulcer who produce grade + or more of pepsin 1 is greater in non-secretors than in secretors and this difference persists, though not at a statistically significant level, within each of the blood groups O, A, and B. The data for gastric ulcer, although not achieving statistical significance, show the same features, except for group B. The possibility is therefore raised that the *se* genes exert their influence in predisposing to peptic ulceration by influencing the chief cells to secrete more pepsin 1. An alternative hypothesis would be that the H antigen, which is present in the gastric juice of all secretors, and possibly the A and B antigens which are also present in A and B secretors, respectively, inhibit pepsin 1 to some extent. The present data do not enable a satisfactory distinction between these two hypotheses to be made.

It might be argued that if there is a direct association between secretor status and pepsin 1 secretion it should hold whether the patients had ulcer, other disease, or no disease at all. The numbers in our groups, other than duodenal ulcer, are perhaps too small to enable a conclusion to be reached. However, if the data from the groups other than duodenal ulcer are combined, the proportion of secretors (60·7%) and non-secretors (59·1%) secreting + or more of pepsin 1 is very much the same. It would appear, therefore, that the association of pepsin 1 and non-secretion of blood group substances is not a general or direct one but is contingent upon some other factor peculiar to duodenal ulcer The experimental evidence of Wright *et al.* (1975) that prolonged direct vagal stimulation eventually releases pepsin 1, coupled with the accumulated evidence that there is excess vagal 'drive' in duodenal ulcer, leads to the hypothesis that, in non-secretors, excess vagal 'drive' causes secretion and release of pepsin 1 more readily than in secretors. It is hoped that investigation of this hypothesis may prove fruitful. If correct, the hypothesis would imply that in gastric ulcer the increased secretion of pepsin 1 arises other than by vagal 'drive' and that the influence of non-secretion in the aetiology of gastric ulcer is exerted other than via pepsin 1.

The increased proportion of group A patients secreting pepsin 1 in concentrations of grade + or more, when compared with group O, is explained, at least in part, by the fact that the proportion of non-secretors within group A is higher than within group O (Table 3) in all four patient groups. The proportion (43·5%) of non-secretors in blood group A in the patients with duodenal ulcer is significantly higher than in Merseyside controls (23·1%) (McConnell, quoted by Race and Sanger, 1975), but this is not the case for non-secretors in blood group O (29·1%).

In disease of multifactorial origin it is often difficult to explain how factors which exert a slight but statistically significant influence, such as age, sex, race, blood group, and cigarette smoking, impinge on pathogenic processes at a cellular or molecular level. It would be expected, furthermore, that any cellular or molecular differences that do emerge would have a relatively low statistical significance, as in the present study and in the study of serum pepsinogen levels by Hanley (1964). So far as the accepted 'aggressive' factors, the hydrogen ion and pepsin content of gastric juice, are concerned, the present position in the aetiology of peptic ulcer would appear to be as follows.

(1) The difference between a normal gastric juice and that of a patient with gastric ulcer, so far as these two aggressive factors are concerned, lies in the increased proportion of pepsin 1 in the secreted pepsins in gastric ulcer (Walker and Taylor, 1976) rather than in any differences in total H^+ or total pepsin output.

(2) In duodenal ulcer this difference is found too, but a proportion of patients also have increased hydrogen ion and total pepsin secretion.

(3) Cigarette smoking is associated with an increased incidence of duodenal ulceration; hydrogen ion secretion and total pepsin secretion are unaffected by smoking, but pepsin 1 secretion in smokers with duodenal ulcer is increased (Walker and Taylor, 1977).

(4) Secretor status does not affect hydrogen ion secretion and total pepsin, but does influence pepsin 1 secretion in duodenal ulcer, thus adding to the body of evidence implicating this enzyme in the pathogenesis of duodenal ulcer.

(5) Blood group status does not influence pepsin 1 secretion, except possibly in the case of group B, but may exert its effect via increasing total pepsin secretion, as evidenced by a raised serum pepsinogen level (Hanley, 1964).

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Requests for reprints to Dr W. H. Taylor, Department of Chemical Pathology, Duncan Building, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XW.