PAPERS

Mucosal polyamine metabolism in the columnar lined oesophagus

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Abstract

Mucosal ornithine decarboxylase activity and polyamine content has been proposed as a possible marker for malignant potential in gastrointestinal mucosa. Polyamine content and histological findings were examined in 107 pairs of endoscopic biopsy specimens taken from gastric fundus, fundic and specialised Barrett's oesophagus and Barrett's adenocarcinoma. The content of putrescine (median nmol/mg protein, range) the primary product of ornithine decarboxylase showed a progressive increase from gastric fundus (0.41, 0.15-1.5); fundic (0.45, 0.01-4.08); specialised Barrett's oesophagus (0.54, 0.01-2.0); dysplastic columnar lined oesophagus (0.56, 0.31-3.1) to adenocarcinoma (1.23, 0.29-8.98). Adenocarcinoma putrescine content was significantly greater than gastric fundus ($p < 0.018$) and fundic ($p < 0.03$). Mucosal spermine, spermidine, and total polyamine values were greater in gastric fundus than fundic, specialised Barrett's oesophagus, and dysplastic columnar lined oesophagus (all $p < 0.001$) suggesting failure to further metabolise putrescine to its higher polyamines in the metaplastic epithelium. Although metaplastic columnar lined oesophagus shows significant differences in polyamine metabolic activity from the stomach the important distinction between specialised and dysplastic columnar lined oesophagus cannot be made by measuring the polyamine content.

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amine biosynthesis reduces the tumour yield in animal models of carcinogenesis.¹¹

In Barrett's oesophagus ornithine decarboxylase activity is increased in some cases of dysplasia.¹² In one patient dysplasia was accompanied by a rise in ornithine decarboxylase activity suggesting that ornithine decarboxylase activity and polyamine biosynthesis may have potential as a non-histological marker of dysplasia in CLO.¹³ A previous report has failed to show a direct relation between polyamine content in CLO and concurrent ornithine decarboxylase activity, possibly reflecting the labile nature of ornithine decarboxylase.¹⁴ Polyamines have a longer half life than the ornithine decarboxylase enzyme activity and are less labile. Polyamines can be assayed, after perchloric acid extraction, by high pressure liquid chromatography in a semi-automatic way. For this reason a pilot study was done to assess the possibility of using mucosal polyamine estimations as a non-histological marker of dysplasia in Barrett's oesophagus.

Patients and methods

One hundred and seven pairs of endoscopic biopsy specimens were taken from the upper Barrett's oesophagus (1 cm below the squamocolumnar junction); lower Barrett's oesophagus (4 cm below the squamocolumnar junction), and gastric fundus from 40 patients with simple metaplastic CLO and eight patients with malignant change in CLO. The biopsy specimens were placed immediately in pre-labelled cryotubes and immersed in liquid nitrogen. They were stored at -70°C until perchloric acid extraction. After extraction biopsy protein content was assayed by the Coomassie blue technique and polyamine content was measured by high pressure liquid chromatography. Adjacent biopsy specimens were processed routinely for histological examination.

PERCHLORIC ACID EXTRACTION OF POLYAMINES

Polyamines are acid soluble whereas protein is acid insoluble, hence extraction in perchloric acid allows separation of the two. Biopsy specimens were taken from the liquid nitrogen and placed in 1 ml of ice cold perchloric acid (0.2 M) in a Jencons hand homogeniser. After homogenisation the suspension was transferred to a glass bijou and sonicated for 30 seconds on ice using a

Barrett's columnar lined oesophagus (CLO) predisposes to adenocarcinoma of the oesophagus.¹ Specialised type CLO shows an increased activity in cellular proliferation compared with junctional and fundic types and is recognised as the site of neoplastic change in those patients progressing to adenocarcinoma.^{2,3} To date histological dysplasia and associated ploidy abnormalities are the only known markers of increased risk of malignancy in CLO.^{4,5}

The polyamines - putrescine, spermidine, and spermine - are intracellular polycations synthesised from ornithine in a rate limiting reaction catalysed by the enzyme ornithine decarboxylase.^{6,7} Raised values of polyamine synthetic enzyme activity and polyamine content have been suggested as markers of tumour prognosis in breast carcinoma and pre-neoplastic potential in gastrointestinal mucosa.⁸⁻¹⁰ Inhibition of poly-

Figure 1: Mucosal putrescine.

Rapidis 150 ultrasonicator (Ultrasonics Ltd, Simpley, Yorkshire). Cell disruption was checked by microscopy. The suspension was then allowed to stand on ice for 15 minutes. Samples were then centrifuged at 13 000 rpm at 4°C in an Eppendorf microcentrifuge. The supernatant and the protein pellets were transferred to a cryotube and frozen at -20°C. Protein and polyamines were assayed separately in batches.

PROTEIN ASSAY

Pellets were suspended again in 1 ml 0.3 M sodium hydroxide that required incubation in a 40°C water bath overnight. Protein was assayed by the Coomassie blue technique.¹⁵ Triple serial dilutions of protein were prepared. 0.1 ml of

protein suspension was used and a standard curve was constructed for each assay performed using a bovine serum albumin standard (Sigma Diagnostics) suspended in 0.3 M sodium hydroxide between 0 and 1400 mg protein/ml.

HIGH PRESSURE LIQUID CHROMATOGRAPHY FOR POLYAMINES

Polyamines were quantified by high pressure liquid chromatography after the method of Wallace *et al.*¹⁶ Briefly, individual polyamines were separated using reverse phase high pressure liquid chromatography with the ion pair reagent, octane sulphonic acid, used to aid separation. The column effluent was mixed 1:1 with o-phthalaldehyde reagents to produce derivatives which were quantified by fluorescence spectrometry.

Results

The results of the mucosal polyamines are expressed in nmol/mg protein. The results are grouped by histological type into gastric fundus (n=32), fundic type CLO (n=34), specialised type CLO (n=19), dysplastic CLO taken from patients with adenocarcinoma (n=11) and Barrett's adenocarcinoma (n=10).

The mucosal putrescine content showed a progressive increase from gastric fundus (median 0.41 nmol/mg protein; range 0.15–1.5 nmol/mg) to fundic type CLO (median 0.45 nmol/mg; range 0.01–4.08 nmol/mg) to specialised CLO (median 0.54 nmol/mg; range 0.01–2.0 nmol/mg), dysplastic CLO (median 0.56 nmol/mg; range 0.3–3.1 nmol/mg), and adenocarcinoma (median 1.23 nmol/mg; range 0.29–8.98 nmol/mg). Only adenocarcinoma, however, showed a significant difference from gastric fundus ($p<0.018$) or fundic type CLO ($p<0.03$). No significant difference was seen between dysplastic and non-dysplastic CLO. (Fig 1).

Spermidine values in gastric fundus (median 9.77 nmol/mg; range 1.69–33.82 nmol/mg) were significantly higher than in fundic type CLO (median 4.38; range 0.64–20.45 nmol/mg) ($p<0.005$), specialised type CLO (median 4.89; range 0.94–29.47 nmol/mg) ($p<0.002$), and dysplastic CLO (median 5.98; range 0.62–8.05 nmol/mg) ($p<0.03$). Adenocarcinoma (median 6.97; range 0.7–45.68 nmol/mg) showed no significant difference. (Fig 2).

Spermine values in gastric fundus (median 17.57 nmol/mg; range 0.73–54.31 nmol/mg) exceeded those of fundic CLO (median 8.93; range 1.39–33.95 nmol/mg) ($p<0.005$), specialised CLO (median 5.11, range 0.94–34.44 nmol/mg), and dysplastic CLO (median 7.75; range 2.48–11.36 nmol/mg) ($p<0.006$). Adenocarcinoma (median 8.89; range 3.20–94.02 nmol/mg) was not significantly different as a group. No difference was seen between intestinal and dysplastic CLO. (Fig 3).

Total polyamines in gastric fundus (median 29.04 nmol/mg; range 3.08–98.63 nmol/mg) exceeded those in fundic CLO (median 14.09 nmol/mg; range 0.73–55.44 nmol/mg), specialised CLO (median 10.63; range 0.34–51.08 nmol/mg) ($p<0.006$), and dysplastic CLO

Figure 2: Mucosal spermidine.

Figure 3: Mucosal spermine.

(median 14.49 nmol/mg; range 2.48–30.54 nmol/mg) ($p < 0.003$). Again adenocarcinoma as a group were similar to gastric fundus (median 18.7 nmol/mg; range 5.07–148.68 nmol/mg) and no difference could be detected between specialised and dysplastic CLO. (Fig 4.)

The spermine to spermidine ratio in gastric fundus (median 2.065; range 0.88–3.62) was similar to fundic CLO (1.85; range 0.32–3.74). Gastric fundus ratio exceeded both specialised CLO (median 1.16; range 0.18–2.80) ($p < 0.002$) and dysplastic CLO (median 1.34; range 0.65–3.65) ($p < 0.036$) but not adenocarcinoma (median 1.43; range 0.73–37.9). Again there was no difference between specialised and dysplastic CLO. (Fig 5.)

Discussion

The finding that total mucosal polyamine and

Figure 4: Total mucosal polyamines.

individual higher polyamine (spermidine and spermine) content in the gastric fundus exceeded that of the Barrett's mucosa was unexpected. The implication of this finding is that mucosal polyamines are not useful as markers of dysplasia in CLO because biopsy specimens taken from normal fundus have a higher polyamine content than metaplastic or dysplastic CLO. Further, although CLO showed significantly lower values than gastric fundus, polyamine content did not differ significantly between dysplastic and specialised CLO.

An important feature of the polyamines is their ionic interactions with the DNA double helix where they play a part in helping DNA replication and RNA transcription.^{17,18} Polyamines are absolute requirements for progression through the cell cycle and depletion can lead to failure of recruitment of cells into G_1 from G_0 , lack of progression through the S phase, and interference with mitosis causing the formation of binucleate cells.¹⁹ Ornithine decarboxylase is rapidly inducible and has a short half life (10–15 minutes). A rise in ornithine decarboxylase activity and a consequent increase in the polyamine content to tissues is characteristic of gastrointestinal proliferation seen in response to weaning, mucosal injury, or resection.^{19–24} An increase in the spermine to spermidine ratio is seen in proliferating cells in culture.²⁴ A ratio of 1.5–2.0 is indicative of rapid proliferation and a fall in the ratio is seen in confluent cell culture.²⁵ Thus there is an established link between polyamine content and physiological gastrointestinal epithelial proliferation.

Tissue putrescine values are related most closely to ornithine decarboxylase activity and in this study of patients with columnar lined oesophagus mucosal putrescine content increased in a graded fashion from gastric fundus to adenocarcinoma. These small increments did not reach statistical significance other than to distinguish gastric fundus from adenocarcinoma; this is not the clinical problem faced. Distinction between specialised and dysplastic CLO cannot be made on this basis.

Our findings agree with a small study on ornithine decarboxylase activity and polyamine values in CLO in which mucosal polyamine content was unrelated to ornithine decarboxylase activity and higher values of total polyamines were found in the gastric and small intestinal mucosa compared with the metaplastic columnar mucosa.¹⁴ Possible explanations for the lack of correlation between polyamine content and ornithine decarboxylase activity include the known fluctuations of ornithine decarboxylase activity in response to a large number of influences including diurnal rhythm and feeding, whereas polyamine content fluctuates less rapidly.^{19–24} In addition proliferating gastrointestinal epithelium can take up polyamines derived from the circulation and also by the transportation of polyamines from the intestinal lumen; polyamines may be ingested in food or taken from bacteria. Animal models of proliferation associated polyamine metabolism in gastrointestinal mucosa using germ free animals and difluoromethyl-ornithine inhibition of ornithine decarboxylase activity, suggest that uptake of circulating polyamines

Figure 5: Mucosal spermine/spermidine ratio.

across the basolateral membrane is the dominant extramucosal source of higher polyamines.²⁶

Another possible reason for the unexpected finding of a lower polyamine content in CLO compared with gastric fundic mucosa might be related to the gastrointestinal hormone state of these patients. All the patients studied took either H_2 antagonists or omeprazole as part of their treatment for anti-reflux. A sustained rise in serum gastrin is seen in patients taking omeprazole and post-prandial gastrin is increased in patients taking H_2 antagonists.^{27,28} Gastric fundic mucosal polyamine synthesis may be stimulated by increased gastrin release as a result of therapeutic acid suppression.²⁹ Alternatively, gastrin might act by promoting further metabolism of putrescine to the higher derivatives.

A trial of short term low dose difluoromethylornithine (specific ODC inhibitor) has shown decreased mucosal polyamine values in metaplastic CLO as well as in all other upper gastrointestinal mucosae.³⁰ This suggests that polyamine biosynthesis can be manipulated in CLO with possible treatment implications for patients with dysplastic CLO; although the suppression of gastric adenocarcinoma cell growth in vitro has not found a useful clinical role in vivo.^{31,32} Depression of mucosal polyamine content was not specific to the metaplastic mucosa in the CLO patients but dysplastic mucosa may be more vulnerable to ornithine decarboxylase inhibition because of the higher rate of cell turnover and more reliant on ornithine decarboxylase for the production of the higher polyamines. This hypothesis remains to be tested.

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