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Polyamines in colorectal cancer—a clinical and experimental approach

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SUMMARY The urinary polyamines putrescine, spermidine and spermine were measured prior to operation in 10 patients with colorectal cancer and 10 control subjects. Carcinoembryonic antigen assays were also performed in an attempt to correlate these values with polyamine excretion. The total polyamine rates in patients with colorectal cancer were 3·2 ± 1·5 (SD) mg/24 h and 2·6 ± 1·2 (SD) mg/24 h in the controls. The difference between the group with colorectal cancer and the controls was not statistically significant.

Urinary polyamines were also measured in an experimental animal model for colorectal cancer in which tumour cell mass could be assessed. Only marginal differences occurred in polyamine rates between animals with extensive tumours and controls. These findings suggest that urinary polyamine measurement is unlikely to be a useful procedure to assess tumour cell mass in patients with colorectal cancer.

The group of polycationic compounds called polyamines include spermine, spermidine and putrescine. Marked increases in polyamines in 24-hour urine collections from patients with several forms of cancer have been reported.[1–3] These observations and subsequent work with improved analytical systems led to the postulate that polyamines could be predictors of success or failure in cancer chemotherapy: spermidine could serve as a marker of tumour cell kill and putrescine reflected the proliferative behaviour of the tumour.[4] The aim of the work reported here was to evaluate measurements of urinary polyamine rates in the detection and assessment of colorectal cancer. Despite the prevalence of colorectal cancer only isolated reports of polyamine rates associated with this disease have been made.[3] We report the polyamine rates of a group of patients with advanced colorectal cancer. Analyses were also performed on experimental animals in which colonic polyps and neoplasia were induced.

Material and methods

CLINICAL

Twenty-four hour urine collections were made from 10 control patients. Patients with urinary tract infection as well as those with cellular proliferative disorders were excluded. Of 10 patients with colorectal cancer, 5 had clinical evidence of metastatic disease. In all cases the disease was confirmed at operation and on histological examination of the tumour.

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EXPERIMENTAL

Seven male Sprague-Dawley rats (150 g) received 1:2 dimethylhydrazine dihydrochloride (DMH) subcutaneously for induction of polypoidal neoplasia in the colon.[5] Four rats acted as controls and received subcutaneous injections of carrier substance (EDTA). The rats were placed in metabolic cages and 24-hour urine collections were made at 26 wk. Urine samples were pooled for groups of three or four rats. The experimental animals were sacrificed at 30 wk to determine the number of tumours induced as well as the total tumour weight.

POLYAMINE ASSAY

Urine samples were hydrolysed in 6 M HCl for 18 h at 110°C in evacuated sealed tubes. After removal of acid the samples were analysed on a Locarte amino acid analyser using a column (0·9 cm × 3 cm) packed with Locarte High Grade ion exchange resin, maintained at 55°C. Elution was at a flow rate of 30 ml/h with a buffer programme in which three citrate buffers pH 5·28, 0·35 M, 1·35 M and 2·35 M

(with respect to sodium) were pumped in turn for 20 min, 60 min, 170 min.

Results

CLINICAL PATIENTS

The amount of polyamines excreted in 24-hour urine samples from patients with colorectal cancer are shown in Table 1. The spermine rates in all patients were close to the limit of detection by the analytical procedure employed. Five patients had carcinoembryonic antigen levels which were grossly raised, corresponding with the clinical disease (Table 1).

In 10 control patients the amounts of polyamines excreted (mg/24 h) were: total polyamines 2·58 ± 1·18 (SD), putrescine 1·37 ± 0·71 (SD), and spermidine 0·98 ± 0·48 (SD). The rate of spermine was barely detected, being less than 0·1 mg/24 h.

Although the results showed a marginal increase in the total polyamines excreted in 24 h in patients with colorectal cancer, this was not a statistically significant change (Student's *t* test).

ANIMAL EXPERIMENTS

A total of 34 colonic polypoidal tumours were induced which were well differentiated adenocarcinoma. The total tumour weight was 5·26 g. The controls were perfectly healthy and had no colonic tumours induced spontaneously.

Discussion

Oncofetal antigens have not lived up to initial expectations of their suitability as screening agents for malignant disease. In particular, carcinoembryonic antigen is not always a reliable marker in relation to colorectal cancer. Of the 10 patients examined in the present study, only five had significantly raised concentrations of CEA.

The aim of this study was to examine urinary polyamine rates as potential markers for colorectal cancer. It was found that the group of patients with advanced colorectal cancer had mean rates of urinary polyamines only marginally higher than those of the control group (Table 1). The mean values for the two groups were not statistically different and therefore it appears that urinary polyamine rates are unlikely to be useful markers for human colorectal cancer. Similarly, animals in which colorectal tumours of polyps had been induced by injection of dimethylhydrazine showed little or no increase in mean polyamine rates compared to the control group.

In addition to their association with cellular proliferation polyamines may be influenced by several other factors. These include variation in food intake, simultaneous infection and bacterial contamination of urine.[6] The latter could be eliminated if polyamines are measured in the blood. Experimental work in this area may yet prove them to have

Table 1 *Polyamine excretion (mg/24 h) in colorectal cancer*

Subject	*Urine vol (ml)*	*Putrescine*	*Spermidine*	*Spermine*	*Total*	*CEA* (μg/l)*	*Diagnosis*
1	1140	2·0	1·2	0·3	3·5	100	Ca colon
2	1110	2·5	1·4	0·5	4·4	30	Ca colon
3	1140	3·9	2·6	<0·1	6·6	25	Ca colon
4	915	1·4	1·1	<0·1	2·6	23	Ca colon
5	1102	0·9	1·0	<0·1	2·0	0	Ca colon
6	1035	1·5	1·2	0·3	3·0	58	Ca colon
7	1700	0·7	0·9	0·3	1·9	>100	Ca colon
8	2120	1·2	1·2	0·5	2·9	50	Ca rectum
9	3145	0·9	0·3	<0·1	1·3	15	Ca rectum
10	2085	1·8	1·7	0·1	3·6	>100	Ca rectum
Mean ± SD		1·68 ± 0·96	1·37 ± 0·72	<0·2	3·18 ± 1·51		

*Normal value ≏25 μg/l.

Table 2 *Polyamine excretion (μg/24 h/animal) in experimental animals*

Group	*No of rats*	*Putrescine*	*Spermidine*	*Spermine*	*Mean tumour no/rat*	*Mean tumour wt/rat*
Experimental 1	4	295	281	106	7·25	1·27
Experimental 2	3	213	195	51	1·7	0·05
Control	4	241	271	110	0	0

some value as diagnostic or prognostic tools in oncology.[7]

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