Increased Urinary Polyamine Excretion in Unselected Cancer Patients Related to Host Factors

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Unselected patients with solid malignant tumours were investigated in order to determine whether they displayed elevated urinary excretion of polyamines; and if so, whether polyamine excretion in such patients predicts disease progression, or is secondary to host and systemic factors. Thirty-eight male and female patients with generalized solid, mainly gastrointestinal, malignant tumours were investigated. Ten male patients operated on for infrarenal aortic aneurysms and 15 otherwise healthy male and female patients hospitalized for minor surgical procedures served as reference patients, representing individuals with and without metabolic stress. Urine samples were collected from all patients during 24 h for measurement of both total and individual excretion of polyamines during three consecutive days. Polyamine excretion was not significantly increased in cancer patients when compared by analysis of variance among the three patient groups. However, polyamine excretion was significantly elevated in both cancer and stressed, non-cancer patients compared with patients without stress ($p < 0.05$). A multivariate analysis indicated that plasma protein and albumin concentrations, abnormal liver function tests and liver metastasis predicted variation in polyamine excretion in cancer patients ($p < 0.01$), but this was unrelated to survival. Our results demonstrate that increased polyamine excretion in cancer patients is related more to host factors than to tumour growth itself.

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Polyamines, i.e. putrescine spermidine and spermine, are normal cellular constituents which play a pivotal role in regulation of cell proliferation, differentiation and growth (1–3). Polyamines are excreted via the kidneys, and urinary concentrations have been used as a reflection of tissue polyamine production (2). Increased urinary polyamine excretion has been reported in patients with solid tumours, suggesting activation of the immune system, tumour growth, increased acute-phase reaction and malnutrition in concert with elevated proliferation of various host cell populations (4–8). Theoretically, urinary excretion of polyamines may therefore represent a quantitative measure of tumour growth in vivo, and possibly constitutes a prognostic factor in human cancer as supported by studies in experimental cancer (9). However, other non-malignant clinical conditions such as infection, liver cirrhosis, sepsis and trauma are also associated with similar metabolic alterations including increased urinary excretion of polyamines (9–12). Polyamine excretion in cancer patients may therefore reflect host activation including immune cell proliferation rather than being a direct tumour growth marker (13). The aim of the present study was to re-evaluate whether unselected patients with progressive solid tumours display increased urinary excretion of polyamines, as reported earlier; and if so, whether polyamine excretion in cancer patients is related to host factors rather than being a direct tumour growth indicator.

MATERIAL AND METHODS

Thirty-eight patients with disseminated solid cancer served as study patients. Reference patients with and without metabolic stress were used; 10 male patients who had surgery for infrarenal aortic aneurysm with postoperative metabolic stress and 15 otherwise healthy patients without metabolic stress were hospitalized for minor surgical procedures. All patients were allocated to study and reference groups consecutively. Cancer and reference patients were thus recruited from the same large population. None of the patients had undergone any treatment of known importance for the study in the 6 months preceding enrolment.

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Table 1

Age, nutritional variables and biochemical tests. Mean ± SEM

	Cancer	Reference patients		ANOVA
		with stress	without stress	
Sex (M:F)	20:18	10:0	8:7	
Age (yr)	69 ± 2[b]	73 ± 2[c]	59 ± 4	0.05
Height (cm)	172 ± 1[a]	180 ± 2[c]	171 ± 2	0.05
Body weight (kg)	64 ± 2[a]	78 ± 2	70 ± 3	0.05
Weight loss (%)	14 ± 2[a,b]	2 ± 1	1 ± 2	0.05
AMC (cm)	22 ± 1[a]	28 ± 1	24 ± 1	0.05
TSF (mm)	11 ± 1[b]	12 ± 2[c]	17 ± 2	0.05
Haemoglobin (g/l)	119 ± 3[a,b]	113 ± 4[c]	139 ± 4	0.05
Creatinine (μmol/l)	92 ± 5[a]	125 ± 9[c]	86 ± 3	0.05
ASAT (μkat/l)	0.9 ± 0.2	0.7 ± 0.1	0.4 ± 0.1	n.s.
ALAT (μkat/l)	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	n.s.
ALP (μkat/l)	8.6 ± 1.8	2.7 ± 0.3	3.9 ± 0.7	n.s.
Bil (mmol/l)	58.1 ± 21.8	15.8 ± 2.5	10.8 ± 1.6	n.s.
Albumin (g/l)	30.0 ± 1.0[b]	31.2 ± 1.2	36.6 ± 1.1	0.05
Plasma proteins (g/l)	63 ± 3[a]	50 ± 6[c]	63 ± 5	0.05
ESR (mm/h)	46 ± 5[b]	29 ± 10	16 ± 3	0.05
CRP (μg/ml)	63 ± 11[b]	91 ± 12[c]	5 ± 2	0.05

Abbreviations: AMC = arm muscle circumference; TSF = triceps skin fold; ASAT = asparate-amino-transferase; ALAT = alanine-amino-transferase; ALP = alkaline phosphatase activity; Bil = bilirubin concentration; ESR = erythrocyte sedimentation rate; CRP = c-reactive protein.
[a] = significantly different compared with the aneurysm group.
[b,c] = significantly different compared with the control group.

Cancer patients

The study group of patients included 18 females and 20 males with a mean weight loss of 14 ± 2%, metabolically representing a rather homogeneous group of cancer patients with overt cachexia and systemic tumour-host alterations (14). All patients displayed advanced tumours with local and/or distant metastases. No attempt was made to quantify tumour burden objectively. Instead, survival was used as an integrated measure of tumour progression and functional host reserves. None of the patients received any specific tumour treatment. Provision of analgesics was the only treatment during the 6 months preceding the study. The patients suffered from carcinoma of the oesophagus (2), stomach (2), bile ducts (2), liver (2), pancreas (8), colon and rectum (14), breast (2), kidney (1), malignant melanoma (3) and retroperitoneal sarcoma (2). Diagnoses were confirmed by ultrasonography, computerized tomography, biopsy, biochemical tests and clinical examination. Clinical and nutritional values are presented in Table 1.

Reference patients

The 15 reference patients without metabolic stress were investigated as a part of routine clinical assessments before surgery. They were hospitalized for minor surgical procedures. The diagnoses were; gallstone disease (6), inguinal hernia (4), benign mammary tumour (1), varicose veins (1), intermittent claudication (2) and Crohn's disease without any signs of active inflammation (1). Ten male patients with infrarenal aortic aneurysms who were investigated represented a defined population with a postoperative metabolic stress. These patients had undergone surgery with general anaesthesia in combination with a peri-operative epidural blockade. They were investigated on the fourth postoperative day to achieve a rather defined degree of metabolic stress.

Energy expenditure, nutritional state and biochemical tests

Resting-energy expenditure (REE) was measured using indirect calorimetry (Deltatrac® Datex, Helsinki) in the morning after an overnight fast during the days of urine collection (13, 15, 16). Triceps skin fold (TSF) and arm muscle circumference (AMC) were measured as described elsewhere (14). Routine blood tests including haemoglobin concentration, erythrocyte sedimentation rate (ESR), C-reactive protein concentration (CRP), liver function tests (ASAT, ALAT, ALP, S-Bil) and plasma albumin were routine hospital tests.

Determination of urinary polyamines

Urine samples were collected from all patients during 24 h in the presence of 60 ml 3 mol/l HCl during three consecutive days, because significant diurnal variations of various urine polyamines have been reported (17). The mean values of three days' sampling were used in each patient. Urine polyamines were measured using a high-performance liquid chromatography (HPLC) technique, as described by Minchin et al. (18). Briefly, 1,6 diaminohexane

Table 2

Energy expenditure, body temperature and heart rate. Mean ± SEM

	Cancer	Reference patients		ANOVA
		with stress	without stress	
REE (kcal/d)	1 435 ± 44[a]	1 797 ± 144	1 497 ± 74	0.05
REE (kcal/kg/d)	22.8 ± 0.6	23.0 ± 1.4	22.0 ± 1.0	n.s.
RQ	0.80 ± 0.01[a]	0.91 ± 0.02[c]	0.80 ± 0.02	0.05
Heart rate (beats/min)	77 ± 3	82 ± 4	69 ± 5	n.s.
Body temp (°C)	37.1 ± 0.1[a,b]	38.2 ± 0.2[c]	36.7 ± 0.1	0.05

RQ = respiratory quotient
[a] = significantly different compared with control patients with stress.
[b,c] = significantly different compared with control patients without stress.

(20 μg/ml) was added to urine samples as an internal standard, before hydrolysis of urine in the presence of 0.5 mol/l HCl final conc. at 100°C for 16 h. Protein precipitation was carried out using perchloric acid followed by dansylation with dansyl chloride at 60°C in darkness for 60 min. After extraction in methanol on a C_{18} sep-pak cartridge, the samples were analysed by HPLC on a reverse-phase column (Waters, Mass., USA). Polyamine concentration in the original urine sample was calculated, taking the internal standard into account. The overall variation coefficient for these procedures did not exceed 5%. Urine creatinine excretion was quantified according to routine hospital measurements. Urinary polyamine excretion was normalized to urinary creatinine in order to minimize variability in diuresis and urine sampling (17).

Statistics

Results are presented as mean ± SEM. Comparisons among the groups were made by one-factor analysis of variance. A post hoc test, Fischer PLSD, was used to test significance among subgroups. Statistical analyses were also done based on the hypothesis that polyamine excretions in cancer patients are related to malnutrition, activation of the immune system and growth of malignant cells. Factorial analyses obtained with ANOVA in a randomized block design were performed accordingly (19). Multivariate analyses were done using multiple regressions and ANOVA.

RESULTS

Patient characteristics

All the cancer patients displayed metabolic alterations associated with cachexia and progressive growth of solid malignant tumours such as anorexia, malnutrition and increased acute-phase reactions. They were malnourished, with significant weight loss, decreased body weight, triceps skin fold (TSF) and arm muscle circumference (AMC) compared with the reference control patients (Table 1). Increased erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and decreased haemoglobin, albumin and total protein concentration indicated pronounced systemic inflammation in cancer patients. Cancer and control patients were well matched according to sex. Liver function tests did not differ significantly among the study and the reference groups, although cancer patients tended to have higher bilirubin concentration and alkaline phosphatase activity with mean values above the normal range (Table 1). Patients operated on for aortic aneurysm had increased plasma creatinine concentration postoperatively. Plasma concentrations of electrolytes were normal and did not differ among the groups (data not shown).

Energy metabolism and body temperature

Whole-body, resting-energy expenditure was significantly higher in reference patients with stress compared with both cancer patients and reference patients without stress. This elevation was associated with increased body temperature and respiratory quotient, while resting heart rates did not differ among the groups. However, the difference in whole-body energy expenditure among patient groups was not apparent when body weight was taken into account, which, on the whole, suggests that stressed non-cancer patients had elevated whole-body metabolism, primarily resulting from heat dissipation (Table 2). Elevated respiratory quotient in postoperative patients, indicating a shift in net substrate utilization towards carbohydrate oxidation, was probably due to postoperative provision of dextrose.

Urine polyamine excretion

Polyamine excretion was numerically higher in cancer and postoperative patients compared with reference patients without metabolic stress but this difference did not reach statistical significance when the three patient groups were compared by analysis of variance according to the study design, either on an individual basis or when polyamine excretion was normalized to creatinine excretion (Table 3). However, polyamine excretion was significantly increased in both cancer and stressed patients when compared directly to reference patients by one-tailed Student's t-test. There were no significant differences in excretion of

Table 3

Urinary excretion of polyamines based on 24 h sampling. Mean ± SEM

	Cancer	Reference patients		ANOVA
		with stress	without stress	
Putrescine (nmol/mg creat.)	29.6 ± 5.3	28.4 ± 3.1	15.6 ± 2.7	n.s.
Spermidine (nmol/mg creat.)	9.3 ± 1.7	11.8 ± 6.0	7.5 ± 0.8	n.s.
Spermine (nmol/mg creat.)	3.4 ± 2.2	1.7 ± 0.6	1.7 ± 0.4	n.s.
Sum of polyamines (nmol/mg creat.)	42.1 ± 5.2[a]	39.6 ± 6.8[a]	23.9 ± 3.41	n.s.

[a] $p < 0.05$ when compared using a one-tailed *t*-test versus reference patients without stress.

polyamines among cancer patients with low and intermediate tumour extension (regional metastases) versus patients with extensive metastases (liver and lung secondaries).

Related host factors

Multivariate analysis in cancer patients showed that sex, plasma protein and plasma albumin concentrations, liver function tests as aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase as well as liver metastases contributed to the variation in excretion of all polyamines ($p < 0.004$). Since body composition was different among the groups, correlations between anthropometric measures and polyamine excretion were also analysed, without any observed significant relationship. Factors reflecting inflammatory response as C-reactive protein, haemoglobin concentration and erythrocyte sedimentation rate were all insignificantly related to polyamine excretion in cancer patients, while these factors predicted polyamine excretion in reference patients without metabolic stress. Plasma protein concentration and liver function tests (ASAT, ALAT) were also significantly related to polyamine excretion in patients without stress ($p < 0.05$) No single factor predicted excretion of polyamines in reference patients with postoperative metabolic stress.

Survival

Patient records were reviewed over a 5-year observation time. There was no correlation between survival and urinary excretion of polyamines in cancer patients. The mean survival in all cancer patients was 8 ± 2 months (mean ± SE.), while the median survival was 4 months. Patients with a survival between 0.5 and 6 months had the same mean polyamine excretion [45 ± 9 nmol/mg creatinine (mean ± SE), 12–168 (range)] as patients with a more than one-year survival [49 ± 21 nmol/mg creatinine (mean ± SE), 19–134 (range)].

DISCUSSION

Polyamine synthesis is regulated by ornithine decarboxylase (ODC), a rate-limiting enzyme which is a prerequisite for induction of cellular growth in both benign and malignant conditions (1–3). It is therefore possible that excretion of polyamines could reflect tumour cell proliferation. Thus, polyamine synthesis has been proposed as an in vivo index of tumour growth, and there are several reports in the literature demonstrating elevated urinary concentrations of polyamines in cancer patients compared with healthy control subjects (5, 6, 21, 22). Conceptually, one should thus expect increased polyamine excretion in subjects with advanced progressive tumour growth, irrespective of tumour tissue origin. Therefore, our study was aimed at re-evaluating whether unselected patients with solid malignant tumours and progressive disease displayed increased urinary excretion of polyamines; and if so, to evaluate whether this phenomenon is related to tumour growth activity (survival) or is secondary to factors such as stress, systemic inflammation and host-tissue involvement such as cholestasis.

In the present study, we were unable formally to confirm published results of elevated urinary polyamine excretion in cancer patients when compared with groups of patients with and without stress, while polyamines may be regarded as significantly increased when compared only with rather healthy individuals without stress, systemic inflammation or host-tissue involvement.

Our measurements were based on 24-h urine samples collected for 3 days in all subjects, since diurnal variations of urine polyamines have been reported (22). Diurnal variation has generally not been accounted for in previous reports of polyamine metabolism in cancer patients in whom excretion measurements were based on isolated urine samples instead of consecutive 24-h sampling (20, 21). According to the results in the present study, it is important that patients groups are randomly well stratified in a study with the present hypothesis. Even stratification was achieved with exception for age, because reference patients without stress were significantly younger (Table 1). The difficulty in estimating tumour burden by indirect methods was in part circumvented by relating polyamine excretion to survival. Differences in anorexia and nutritional state among study and reference patients were accounted for by relating polyamine excretion to integrated dynamic variables such as whole-body metabolism, while differences in body weight and lean body mass were ac-

counted for by relating polyamine excretion to urinary creatinine excretion, which reflects lean body mass. Cancer and reference patients were comparable in REE, which previously has been identified as an important factor in explaining increased polyamine excretion in surgical patients (23). In normal individuals, REE is negatively correlated with age, a finding which is mainly due to alterations in the body composition of elderly subjects and REE is significantly elevated in cancer patients as well as in postoperative patients (16). However, REE was not correlated to urinary excretion of polyamines in our patients, indicating that elevated polyamine synthesis in cancer and stressed, non-cancer patients did not primarily reflect alterations in whole-body energy metabolism.

Multivariate analysis identified sex, plasma proteins and liver function tests as variables predicting urinary polyamine excretion in cancer patients. The observation that sex was a predictive factor in polyamine excretion is supported by a previous study with elevated urinary putrescine excretion in female subjects, irrespective of age (22), but this was not observed in our female reference patients. No correlation was found between age and polyamine excretion in the present study and all female subjects in the cancer group were post menopausal (24, 25). Therefore, we regard 'female' as a factor being significant by chance in our cancer patients. No correlation was found between anthropometric measures, systemic inflammation and polyamine excretion in cancer patients, which was also true for survival. However, liver function tests predicted polyamine excretion in both cancer and non-cancer patients without stress. Therefore, it is possible that urinary excretion of polyamines is significantly related to liver metabolism, with a more advanced functional influence of this phenomenon in cancer patients depending on either elevated hepatic acute-phase reactions with increased liver protein synthesis (26), or a more advanced influence of cholestasis in many cancer patients with progressive disease. This interpretation has also support in a negative correlation between plasma albumin, a negative acute-phase reactant, and polyamine excretion in both the present and previous studies (27).

Blood haemoglobin concentration correlated to urinary excretion of polyamines in reference patients without metabolic stress. Previous studies have demonstrated high polyamine content in erythrocytes, indicating that changes in plasma concentration of red blood cells may relate to whole-body polyamine content (27). Anaemia is a strong promoter of cell proliferation in bone marrow and of the synthesis of growth factors such as erythropoietin. In patients with normal responses to anaemia, a reduced blood haemoglobin concentration would thus result in increased polyamine production and excretion. However, in cancer patients erythropoietin production is often attenuated, partly due to cytokine activation (28). This fact may account for the disappearance of correlation between haemoglobin concentration and polyamine excretion in cancer patients.

In conclusion, this study confirms previous findings of increased polyamine excretion in unselected groups of cancer patients with advanced solid tumours, when compared with rather healthy individuals without stress. Secondary host factors, such as gender, plasma protein and albumin concentrations and abnormal liver function tests predicted urinary excretion of polyamines in cancer patients, whereas survival had no such influence. Therefore, previous reports of increased polyamine excretion in groups of cancer patients to some extent reflect host factors, primarily related to hepatic involvement; factors that do not directly determine survival.

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