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Elevation of Monoacetylated Polyamines in Human Breast Cancers*

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Abstract—*Normal tissues contain only trace amounts of monoacetylated polyamines.* N^1*-Acetylspermidine is present in high concentrations in mouse liver cells damaged by hepatotoxins and is also found in specialised cells of the hamster epididymis. In the present study human breast cancers were analysed for the presence of monoacetylated polyamines because* N^1*-acetylspermidine is selectively elevated in human colorectal cancers. Free and monoacetylated polyamines* (N^1*-acetylspermidine,* N^8*-acetylspermidine and* N^1*-acetylspermine), measured by high-performance liquid chromatography, were expressed as* nmol/g *wet wt of breast cancers* (n = 54) *or normal breast tissue* (n = 15). *Putrescine and monoacetylated polyamines were absent from normal breast tissue. Mean total content of monoacetylated polyamines in breast cancers* 14.9 ± 5.3 (*S.E.*) *exceeded the mean total content of free polyamines* (8.3 ± 1.0) *in normal breast tissue. Detectable levels of at least two of the monoacetylated polyamines were found in all breast cancers:* N^1*-acetylspermidine was present in* 51 (13.1 ± 6.3), N^8*-acetylspermidine in* 32 (0.6 ± 0.1) *and* N^1*-acetylspermine in* 28 *tumours* (1.2 ± 0.3). *There was no correlation between monoacetylated polyamine content of breast cancers and factors known to affect survival, i.e. tumour size, histological grade, oestrogen receptor status and node status. Monoacetylated polyamines are present in human breast cancers but not in normal breast tissue, implying that polyamine catabolism in breast cancers differs from that in normal breast tissue.*

INTRODUCTION

PUTRESCINE, spermidine and spermine, biologically active products of polyamine metabolism, facilitate DNA synthesis and cell replication in normal and neoplastic cells [1, 2]. Acetylspermidine and acetylspermine, intermediary products of polyamine catabolism, are major excretory products in urine [3]. Increased quantities of putrescine, spermidine and spermine are present in human cancers compared with normal tissue [4-6], and some patients with cancers excrete increased quantities of acetylated polyamines in urine [7, 8]. Human breast cancers contain higher concentrations of spermidine and spermine than normal breast tissue [9, 10], and more rapidly proliferating breast tumours contain the highest concentrations [11].

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The polyamine content of cells is controlled by the activity of biosynthetic and catabolic enzymes [12-14]. In rodent tissues three enzymes regulate cellular polyamine content: biosynthesis is rate-limited by ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC); catabolism is rate-limited by spermidine/spermine N^1-acetyltransferase [15]. N^8-Acetyltransferase is a nuclear enzyme with low activity [14]. Because N^1-acetylspermidine and N^1-acetylspermine are very susceptible to catabolism by tissue polyamine oxidase (PAO) [15] these compounds are not usually detectable in tissues except under conditions of extreme stimulation of N^1-acetyltransferase activity, such as occurs in the livers of mice treated with hepatotoxins [16]. Nevertheless, the accumulation of N^1-acetylspermidine does occur in normal tissues: hamster epididymis contains high levels [17], probably because augmented polyamine production in the male genital tract is required to inhibit seminal protein coagulation in the urethra [18]. In

addition, under conditions of polyamine-depletion and cessation of cell division in rat hepatoma cells cellular growth and cellular spermidine content can be restored by the addition of N^1-acetylspermidine or N^1-acetylspermine [19]. The elevation of N^1-acetylspermidine in human colorectal cancers was therefore an unexpected finding [20] and one which merits further investigation in other human tumours.

MATERIALS AND METHODS

Patients

Breast cancers from 54 women were studied. Clinical follow-up was for a minimum of 2 yr to detect recurrence of disease. Tumour size was assessed according to the UICC classification. Biopsies for polyamine analysis were collected fresh from the operating theatre and stored at -70°C for later analysis. Tumours were graded histologically from the degree of glandular differentiation, nuclear pleomorphism and mitotic index. Nodes removed at operation were assessed histologically for the presence of metastatic tumour. Oestrogen receptor status was measured in fmol oestradiol bound/mg total protein.

Normal breast tissue was obtained from 15 women undergoing reduction mammoplasty.

Tissue slices adjacent to biopsy specimens sent for biochemical assay were examined histologically to ensure a high degree of cellularity in breast tumours, and a high content of normal glandular breast tissue in mammoplasty specimens. Biopsies of breast tumours that were not highly cellular or that were heavily infiltrated with macrophages were discarded. Likewise, normal breast tissue with a heavy fatty infiltrate was discarded.

Polyamines and their acetyl derivatives were determined by a modification of the method of Seiler and Knodgen [21]. Samples were separated using gradient elution and ion-pair chromatography on a reversed-phase C_{18} Microbondapak column (Waters Associates, U.S.A.). Samples were quantified by relative fluorescence (excitation wavelength 347 nm and emission wavelength 465 nm) after reaction with *o*-phthalaldehyde (400 mg/l) using the method of external standard quantification. The flow rate was 1.5 ml/min and the separation took 55 min. Authentic samples of free and acetylated polyamines were purchased from Sigma Chemical Company Ltd, Poole, Dorset, U.K.

RESULTS

Polyamine content in breast cancers compared with normal breast tissue (Fig. 1)

Putrescine, N^1-acetylspermidine, N^8-acetylspermidine and N^1-acetylspermine were not detected in normal breast tissue. Mean spermidine content of breast cancers was 2.6 times and mean spermine content 2.8 times higher than that found in normal breast tissue. Total content of free polyamines (putrescine, spermidine and spermine) was 3 times higher in breast cancers than normal breast tissue. Total polyamine content (free, and acetylated derivatives) was 4.8 times higher in breast cancers than in normal breast tissue. The total content of acetylated polyamines in breast cancers (14.9 ± 5.3 nmol/g wet wt) was higher than the total content of free polyamines in normal breast tissue (8.3 ± 1.0).

N^1-Acetylspermidine, N^8-acetylspermidine and N^1-acetylspermine contents of breast cancers (Fig. 2)

In only three tumours were levels of N^1-acetylspermidine undetectable: in all of these tumours there were detectable levels of N^8-acetylspermidine and N^1-acetylspermine. Of the total mean content of acetylated polyamines, 88% was present as N^1-acetylspermidine (13.1 ± 6.3 nmol/100 mg wet wt), 4% as N^8-acetylspermidine (0.6 ± 0.1) and 8% as N^1-acetylspermine (1.2 ± 0.3). Large variations in content of acetylated polyamines were detected: levels of N^1-acetylspermidine ranged from 0.1 to 79.3, levels of N^8-acetylspermidine ranged from 0.1 to 2.1 and levels of N^1-acetylspermine ranged from 0.05 to 5.0 nmol/100 mg wet wt. Less than 1 nmol/100 mg wet tissue of acetylated polyamine was detected in 19 of 51 N^1-acetylspermidine-containing tumours, in 26 of 32 N^8-acetylspermidine-containing tumours and in 18 of 28 N^1-acetylspermine-containing tumours.

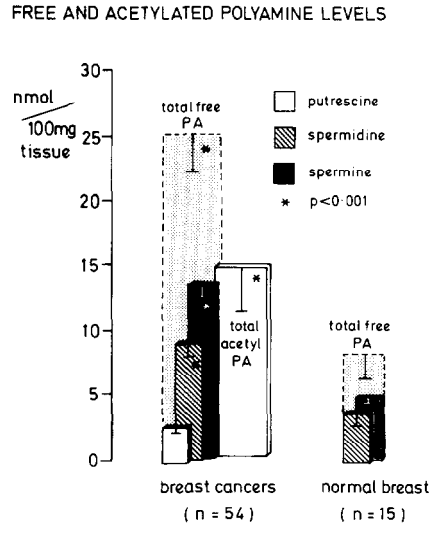

Fig. 1. Free and total monoacetylated polyamine content of breast cancers and normal breast tissue (mean ± S.E.).

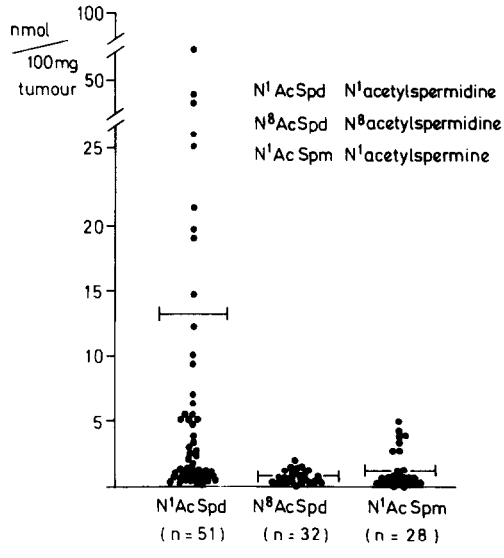

Fig. 2. Differential monoacetylated polyamine content of breast cancers (mean ± S.E.).

Correlation between free and acetylated polyamine levels in breast cancers (Table 1)

A significant correlation was found between the N^8-acetylspermidine content and the spermidine content, and a significant correlation was found between the N^1-acetylspermine content and the spermine content of breast cancers.

Prognostic factors and recurrence compared with levels of acetylated polyamines in breast cancers (Figs 3–7)

Levels of N^1-acetylspermidine, N^8-acetylspermidine and N^1-acetylspermine were not affected by tumour size, histological grade, oestrogen receptor status, node status or tumour recurrence at 2 yr. There were 11 tumours containing more than 10 nmol N^1-acetylspermidine/100 mg wet tissue, and these tumours were analysed separately for correlation with prognostic factors and recurrence: there were no identifiable poor prognostic signs, nor evidence of early recurrence (2 yr) in these 11 tumours. There was a similar statistical failure to find signs of poor prognosis in six tumours containing more than 1 nmol N^8-acetylspermidine, and ten tumours containing more than 1 nmol N^1-acetylspermine.

Table 1. Correlation between free and monoacetylated polyamine levels in breast cancers

	Correlation coefficient (r)	Significance (P value)
Spermidine: N^1-acetylspermidine	0.22	N.S.
Spermidine: N^8-acetylspermidine	0.54	$P < 0.02$
Spermine: N^1-acetylspermine	0.65	$P < 0.01$
Total free polyamines: total acetylated polyamines	0.32	N.S.

DISCUSSION

Undisturbed polyamine biosynthesis is essential for the growth of experimental breast cancers. In rats inhibition of ornithine decarboxylase (ODC) activity with α-difluoromethylornithine not only dramatically reduces the yield of chemically induced mammary tumours [22], but also inhibits the growth of transplanted murine mammary EMT6 sarcomas [23]. Moreover, hormonally induced growth of *N*-nitrosomethylurea (NMU)-induced rat mammary tumours *in vitro* [24] and hormonally controlled growth of normal mouse mammary gland is mediated by polyamines [25]. In addition, the cytotoxic action of the antioestrogen tamoxifen on NMU-induced

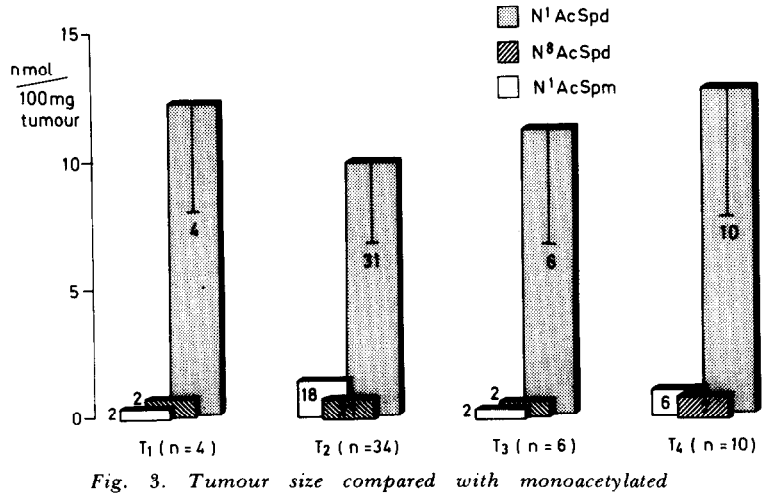

Fig. 3. Tumour size compared with monoacetylated polyamine content of breast cancers (mean ± S.E.).

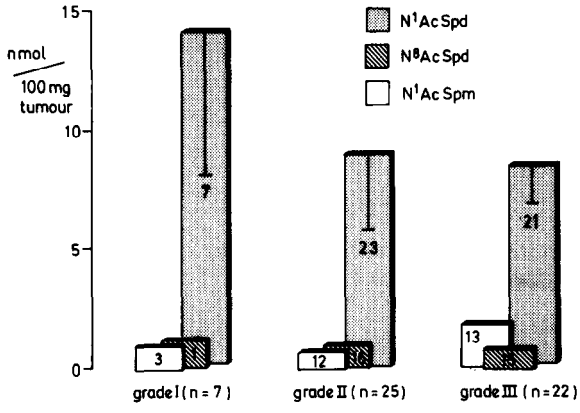

Fig. 4. Histological grade compared with monoacetylated polyamine content of breast cancers (mean ± S.E.).

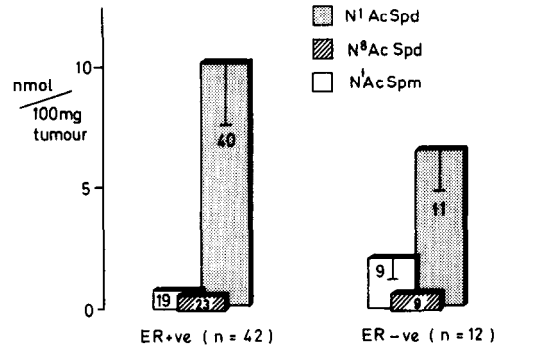

Fig. 5. Oestrogen receptor status compared with monoacetylated polyamine content of breast cancers (mean ± S.E.).

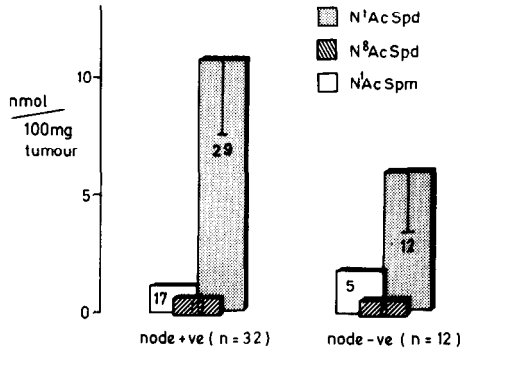

Fig. 6. Nodal status compared with monoacetylated polyamine content of breast cancers (mean ± S.E.).

tumours grown in soft agar can be rescued by administration of polyamines [26].

Accumulation of monoacetylated polyamines in breast cancers could take place by increased synthesis, decreased catabolism, increased absorption from extracellular fluids or a combination of

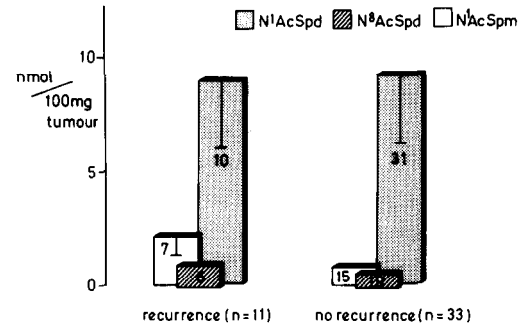

Fig. 7. Recurrence of breast cancer at 2 yr compared with monoacetylated polyamine content (mean ± S.E.).

these mechanisms. In mice treated with hepatotoxins early enhancement of hepatic putrescine levels is brought about initially by induction of N^1-acetyltransferase activity, followed by an increase in ODC activity to maintain putrescine levels [16]. It is unlikely that the moderately increased mitotic rate of human breast cancers requires augmentation of N^1-acetyltransferase activity to maintain polyamine levels and growth. Nevertheless, studies in experimental breast cancers with inhibitors of N^1-acetyltransferase [27] could provide circumstantial evidence for the role of polyamine catabolic enzymes in breast cancer growth. Low activity of the tissue catabolic enzyme polyamine oxidase (PAO) leads to accumulation of monoacetylated polyamines in mouse liver [15]. Inhibition of PAO activity leads to an increase in cellular proliferation in several cell lines grown in tissue culture [28]. However, in human tumours studied thus far, i.e. medullary thyroid carcinoma [29], small cell carcinoma of the lung [30], endometrial carcinoma [31], and ovarian carcinoma [32], diamine oxidase activity is increased. Takenoshita *et al.* [20] speculated that elevation of N^1-acetylspermidine in colorectal cancers could occur through absorption of luminal intestinal polyamines synthesised by bacteria, such as occurs in putrescine- and spermidine-depleted Ehrlich ascites carcinoma cells in mice [33]. Although absorption of monoacetylated polyamines in breast cancer could occur from surrounding extracellular fluids, this would require selective induction of polyamine transport mechanisms.

Before monoacetylated polyamines can be evaluated as tumour markers in breast cancer, further studies are necessary in benign breast disease.

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