Short report

Elevated levels of free putrescine and N¹-acetylspermidine in cyst fluids of malignant brain tumours

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SUMMARY The contents of free and conjugated polyamines in cyst fluids of brain tumours were analysed. The putrescine and N¹-acetylspermidine levels in cyst fluids of malignant brain tumours were significantly higher than those of benign tumours. The free spermidine levels in malignant and benign tumours were about the same, while the total spermidine levels were higher in malignant than those in benign tumours. These results suggest that polyamine levels in cyst fluids reflect the altered polyamine metabolism in brain tumours.

Polyamines such as putrescine, spermidine and spermine are present in high concentrations especially in rapidly growing tissues, including neoplasms.^{1,2} These polyamines have been shown to play important roles in the regulation of cell proliferation and differentiation.^{1,2} They are thought to be useful for the diagnosis of malignant tumours and in monitoring the effects of anticancer treatment.^{3,4} Recently conjugated polyamines such as acetylated forms are claimed to be better tumour markers than free polyamines.^{5,6}

Polyamines are present in various body fluids and exudates, though their levels are usually lower in body fluids than in most tissues.^{7,8} Polyamines in the cerebrospinal fluid are shown to be excellent markers for the detection of the recurrence of meningeal dissemination of cerebellar medulloblastoma.⁹ Not only free polyamines but also some peptide-bound conjugated polyamines have been found in the human amniotic fluid.¹⁰ These findings prompted us to analyse polyamines in cyst fluids from patients with brain tumours.

Materials and methods

Chemicals. N¹- and N⁸-acetylspermidine were purchased from Sigma (St. Louis, USA). Isoputrescine and hypusine were kindly supplied by Drs. A. M. Astoor of Westminster Hospital, London, and T. Shiba of Osaka University, respectively. Putrescine was purchased from Calbiochem-Behring Corp (La Jolla, USA).

Specimens. Cyst fluids were collected by aspiration at surgery from patients with various brain tumours and then centrifuged at 900 g for 20 min to remove cells and cell debris. The supernatants were stored at -80°C until assayed. To 450 µl of the cyst fluid 50 µl of 6 N perchloric acid (HClO₄) was added and thoroughly mixed. After centrifugation, the supernatants were taken for polyamine analysis. Some specimens were hydrolysed at 110°C in the presence of 6 N HCl for 16 h. Polyamines found in the hydrolysates were referred to as total polyamines. Free and acetylated polyamines were measured with the use of high-performance liquid chromatography (HPLC) as described previously.¹¹

Results

Patients with intracranial tumours examined included 25 cases of benign and 17 of malignant tumours. The former group included seven craniopharyngiomas, eight acoustic neurinomas, six haemangioblastomas, two meningiomas and pituitary adenomas. The malignant tumours were two malignant astrocytomas and

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Table *N*¹-Acetylspermidine and free polyamines in cyst fluids of brain tumours

	<i>N</i> ¹ -acetylspermidine	Putrescine	Spermidine	Spermine
	(nmol/ml cyst fluid)			
Benign tumour (25)*	0.14 ± 0.04†	0.73 ± 0.15	1.21 ± 0.18	0.12 ± 0.04
Malignant tumour (17)	2.43 ± 0.63‡	2.23 ± 0.66§	1.24 ± 0.26	0.20 ± 0.08

*Numbers in parentheses, number of determinations.

†Mean ± SE.

‡Differs significantly from benign tumour ($p < 0.001$).

§Differs significantly from benign tumour ($p < 0.01$).

15 cases of glioblastoma multiforme. In all the cyst fluids obtained from patients with these tumours, putrescine, spermidine and *N*¹-acetylspermidine were detected, while only trace amounts of spermine were found. The levels of these polyamines in cyst fluids were much higher than those of cerebrospinal fluids.⁷⁻⁹ Several unknown peaks were observed on the chromatograms, none of which corresponded to putrescine, isoputrescine, hypusine or *N*¹-acetylspermine. The table shows that the concentrations of both putrescine and *N*¹-acetylspermidine in cyst fluids were significantly higher in malignant than in benign tumours. The free spermidine levels were nearly the same in both malignant and benign tumours.

Upon acid hydrolysis of cyst fluids the *N*¹-acetylspermidine peak and some other peaks disappeared and putrescine and spermidine increased in height. The concentration of total putrescine and spermidine were higher in malignant than in benign tumours (data not shown).

Discussion

The present study has shown that the concentrations of *N*¹-acetylspermidine and putrescine in cyst fluids of brain tumours are higher in malignant than in benign tumours. Since the concentrations of putrescine, spermidine and spermine increased after acid hydrolysis, these polyamines may also be present in some conjugated forms. We could not identify these compounds except *N*¹-acetylspermidine. Possibly these free and conjugated polyamines are released from adjacent neoplastic tissues. In human amniotic fluid several peptide-bound polyamines were found.¹⁰

The elevated urinary levels of *N*¹-acetylspermidine and the higher ratio of *N*¹-acetylspermidine to *N*⁸-acetylspermidine have been reported in patients with various cancers.^{5,6} In colonic adenocarcinomas higher levels of *N*¹-acetylspermidine than in adenomas or control mucosae were observed.¹¹ These

findings suggest that the formation of *N*¹-acetylspermidine is accelerated in malignant tumours and that *N*¹-acetylspermidine is preferentially released from neoplastic tissues. The measurement of free and conjugated polyamines in cyst fluids of brain tumours can be of some use for the detection of malignancy.

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