Polyamine metabolism in brain tumours: diagnostic relevance of quantitative biochemistry

R-I Ernestus, G Röhn, R Schröder, T Els, Á Klekner, W Paschen, N Klug

Abstract

***Objective*—Activation of polyamine metabolism is closely associated with cellular proliferation. The purpose was to investigate whether the content of the polyamines putrescine, spermidine, and spermine, and the activity of the first metabolic key enzyme of polyamine metabolism, ornithine decarboxylase (ODC), represent biochemical markers of malignancy in brain tumours.**

***Methods*—The concentration of putrescine, spermidine, and spermine, and the activity of ODC were biochemically quantified in tissue samples obtained during open microsurgery of 670 patients with brain tumours. Biochemical analysis and histopathological classification were carried out in serial tumour samples.**

***Results*—The activity of ODC was very low in peritumorous non-neoplastic brain tissue (0.9 (SD 0.6) nmol/g/h). It was significantly higher in gliomas and it significantly increased with a higher grade of malignancy (grade I 2.7 (2.8) nmol/g/h, grade II 3.1 (4.0) nmol/g/h, grade III 5.7 (5.6) nmol/g/h, grade IV 10.6 (11.7) nmol/g/h). High enzyme activity was also found in medulloblastomas (25.5 (15.1) nmol/g/h), malignant lymphomas (52.1 (42.1) nmol/g/h), and metastases from carcinoma (14.9 (22.1) nmol/g/h). Lowest values were measured in epidermoid cysts (0.5 (0.2) nmol/g/h), craniopharyngiomas (1.2 (0.9) nmol/g/h), angioblastomas (1.6 (1.7) nmol/g/h), and neurinomas (2.0 (1.8) nmol/g/h). By contrast with ODC activity, polyamine concentrations did not correlate with the grade of malignancy. Correlation of regional biochemical and histomorphological data in rapidly growing neoplasms showed high enzyme activity in solid tumour parts and low activity in necrotic areas.**

***Conclusions*—Novel data relating ODC activation and polyamine concentrations to neuropathology is presented indicating that high ODC activity represents a biochemical marker of malignancy in brain tumours. This information is important for clinical and therapeutic investigations.**

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Department of Neurosurgery, University of Cologne, Joseph-Stelzmann-Straße 9, D-50924 Köln, Germany
R-I Ernestus
G Röhn
Á Klekner
N Klug

Institute for Pathology
R Schröder

Max-Planck-Institute for Neurological Research, Department of Experimental Neurology, Gleueler Strasse 50, D-50931 Köln, Germany
T Els
W Paschen

Correspondence to:
Dr R-I Ernestus
Ralf-Inzo.Ernestus@medizin.uni-koeln.de

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Biosynthesis of the polyamines spermidine and spermine and their metabolic precursor putrescine is mainly regulated by changes in the activity of the first key enzyme, ornithine decarboxylase (ODC, EC 4.1.1.17), which catalyzes the decarboxylation of the amino acid ornithine to the diamine putrescine (fig 1).[1 2] Polyamines are present in all eukaryotic cells, including cells of the CNS.[3–5] Their biosynthesis is closely associated with cellular growth processes, including physiological[3 4 6 7] and neoplastic cell proliferation.[8–11] It has been demonstrated that polyamines might be useful biochemical markers for diagnosis and follow up in patients with malignant neoplasms.[9 10 12] In human brain tumours increases in ODC activity[13–15] and putrescine concentrations[14–18] have been found. Goldman *et al*[19] showed that conversion of putrescine was greater in tumours than in normal brain and, furthermore, that putrescine metabolism could be related to the grade of malignancy. Moulinoux *et al*[17] found an increased spermidine/spermine ratio in tumour tissue from patients with glioblastomas.

In recent studies of experimental gliomas, we showed that ODC activity represents a reliable biochemical marker of neoplastic growth in the brain.[20 21] These data were obtained from highly malignant anaplastic gliomas.

In human brain tumours, individual tumour behaviour does not always correspond to the histologically defined neuropathology. Thus the objective of the present study was to investigate whether ODC activity and polyamine concentrations are biochemical markers of malignancy, which might be used for individual tumour characterisation in addition to conventional neuropathology. In this series, ODC activity and polyamine concentrations were measured for the first time in parallel in a large number of human brain tumours.

Clinical materials and methods

PATIENTS

The present study comprised 670 patients with primary (n=521) and recurrent (n=149) brain tumours surgically treated in the Department of Neurosurgery of the University of Cologne between 1990 and 1996. Informed consent of the patients was obtained according to the Helsinki declaration of ethical requirements. Only one patient with a primary glioblastoma multiforme and patients with recurrent gliomas had received chemotherapy, radiotherapy, or both before tumour removal.

TISSUE SAMPLING AND HISTOLOGICAL PROCESSING

During neurosurgery up to five samples from intracranial neoplasms were obtained and, immediately after excision, frozen in liquid nitrogen and stored at −80°C until analysis. For conventional histopathological evaluation and differentiation of solid and necrotic

tumour areas, 10 µm thick cryostat sections were taken from each sample and stained with haematoxylin and eosin. Tumours were classified according to the criteria of the World Health Organisation (WHO), revised in 1993.[22] Samples of peritumorous brain tissue, which were histologically free of tumour cells, served as reference tissue for gliomas.

BIOCHEMICAL ANALYSIS

Biochemical assays were performed in the same samples from which the cryostat sections for neuropathological evaluation were taken. Analysis of ODC was done by measurement of the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine (specific activity 52.6 mCi/mmol; ARC Biotrend, Cologne, Germany). Tumour samples were homogenised at 4°C with 25 vol (w/v) of Tris/HCl buffer (50 mmol/l, pH 7.2, supplemented with 5 mmol/l dithiothreitol (DTT) and 0.1 mmol/l EGTA). The assay was carried out in sealed tubes containing centre wells. The test mix was composed of tumour tissue homogenate (4 mg tissue), pyridoxal-5-phosphate (54 µmol/l), and ^{14}C-ornithine (74 µmol/l) in a total volume of 130 µl.[23]

For determination of polyamine concentrations, tumour samples were homogenised with 0.1 mol/l HCl in methanol at −20°C. Tissue homogenates were extracted twice with 0.6 mol/l $HClO_4$. After centrifugation the supernatants were neutralised with 3 mol/l KOH. After derivatisation with *o*-phthalaldehyde polyamines were separated on a reverse phase Partisil 10 ODS 3 high performance liquid chromatography column and quantified by fluorescence detection.[24]

Histopathological grading of malignancy generally refers to the tumour part exhibiting the highest malignancy. For statistical analysis we considered, therefore, in cases with several samples from the same tumour only the highest ODC and polyamine values measured in one specimen of solid tumour tissue. Data are given as means (SD). Mathematical differences were quantified using Student's *t* test for unpaired samples and the Bonferroni correction for multiple comparisons.

Results

HISTOLOGY

The 670 brain tumours included in the present series were histologically classified as follows (table 1):

One hundred and three low grade gliomas (WHO grade I/II), 157 high grade gliomas

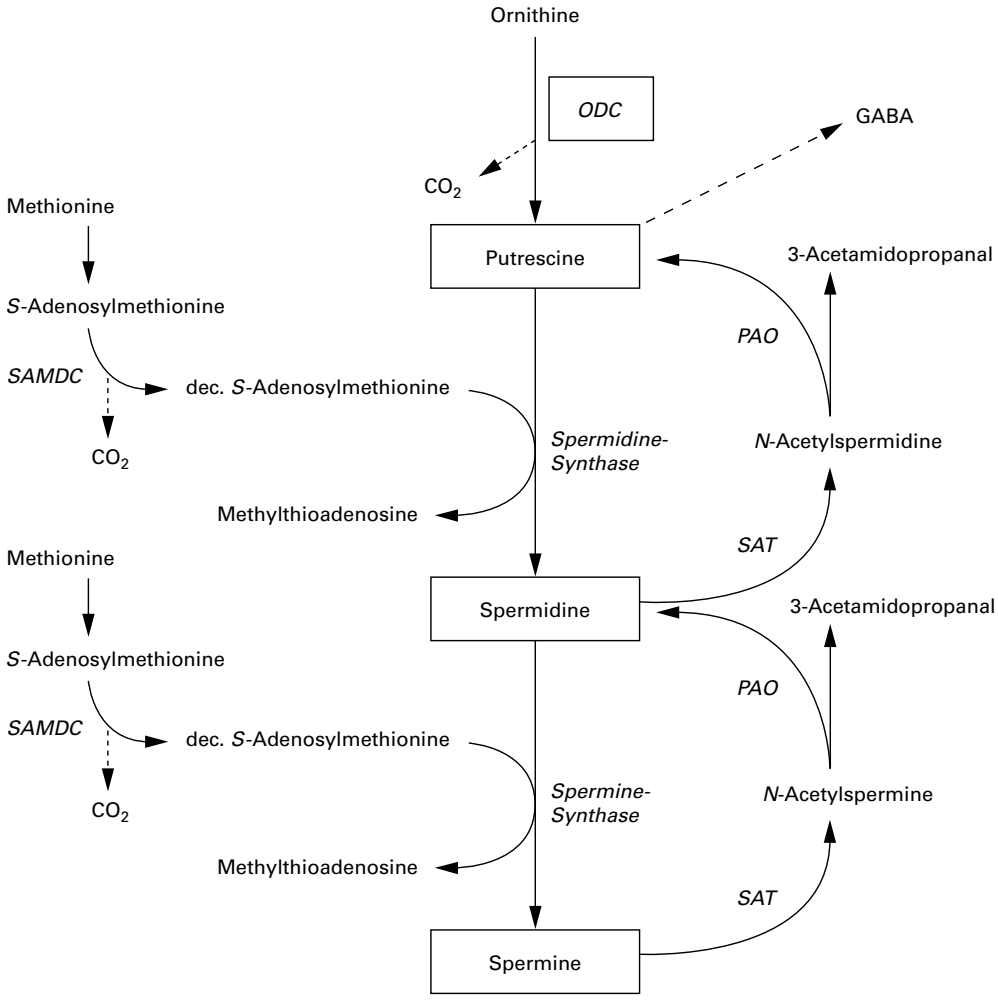

Figure 1 Pathways of polyamine metabolism and interconversion. ODC=Ornithine decarboxylase; SAMDC=S-adenosylmethionine decarboxylase; dec S-adenosylmethionine=decarboxylated S-adenosylmethionine; SAT=spermidine/spermine N¹-acetyltransferase; PAO=polyamine oxidase; GABA=γ-aminobutyric acid.

Table 1 ODC activity and polyamine concentrations in 670 brain tumours

Histology and grade of malignancy	*n*	*ODC (nmol/g/h)*	*Putrescine (nmol/g)*	*Spermidine (nmol/g)*	*Spermine (nmol/g)*
Non-neoplastic brain tissue	75	0.9 (0.6)	46 (27)	242 (124)	86 (45)
Glioma I	16	2.7 (2.8)*	61 (41)	328 (183)	156 (108)‡
Glioma II	87	3.1 (4.0)*	117 (103)*	328 (169)†	72 (78)
Glioma III	37	5.7 (5.6)*	123 (85)*	357 (254)‡	170 (130)†
Glioma IV	108	10.6 (11.7)*	161 (134)*	365 (271)†	248 (214)*
Glioma I and II	103	3.0 (3.8)	108 (100)	328 (170)	85 (87)
Glioma III and IV	145	9.4 (10.7)§	151 (124)¶	363 (265)	227 (197)§
Gliosarcoma IV	12	7.3 (5.8)*	141 (97)*	279 (83)	113 (35)
Ependymoma II	10	11.9 (14.3)	154 (130)	310 (285)	170 (157)
Ependymoma III	3	20.0 (6.1)	31	50	280
Medulloblastoma IV	14	25.5 (15.1)	167 (82)	251 (148)	240 (206)
Neurinoma I	25	2.0 (1.8)	174 (117)	355 (149)	148 (79)
Meningioma I	189	6.2 (9.3)	64 (49)	326 (161)	306 (211)
Atypical meningioma II	9	12.4 (10.2)	55 (37)	340 (203)	338 (166)
Anaplastic meningioma III	3	17.5 (9.7)	462 (225)	324 (152)	244 (39)
Angioblastoma I	5	1.6 (1.7)	37 (37)	288 (110)	311 (193)
Malignant lymphoma IV	6	52.1 (42.1)	138 (82)	350 (135)	280 (174)
Epidermoid cyst	7	0.5 (0.2)	5 (3)	19 (6)	20 (27)
Pituitary adenoma I	56	4.8 (4.8)	13 (8)	61 (43)	232 (263)
Craniopharyngioma I	3	1.2 (0.9)	59 (31)	123 (81)	149 (146)
Metastasis (carcinoma) IV	71	14.9 (22.1)	71 (71)	196 (113)	245 (269)
Metastasis (melanoma) IV	9	6.1 (5.2)	22 (14)	199 (119)	345 (381)

Tumour classification and grading was performed according to the World Health Organisation (WHO) proposal of 1993.[21] Data are expressed as mean values (SD). Mathematical differences were quantified by the *t* test for unpaired samples and the Bonferroni correction for multiple comparisons. n=Number of tumours.
*p<0.001 glioma *v* non-neoplastic brain.
†p<0.01 glioma *v* non-neoplastic brain.
‡p<0.05 glioma *v* non-neoplastic brain.
§p<0.001 glioma III and IV *v* glioma I and II.
¶p<0.05 glioma III and IV *v* glioma I and II.

(grades III and IV, including 12 gliosarcomas), 13 ependymomas grades II and III, 14 medulloblastomas, 25 neurinomas, 201 meningiomas including nine atypical tumours (grade II) and three anaplastic meningiomas (grade III), five angioblastomas, six malignant lymphomas, seven epidermoid cysts, 56 pituitary adenomas, three craniopharyngiomas, and 80 metastases from primarily extracerebral neoplasms including nine malignant melanomas. Seventy five specimens from the tumour surroundings without neoplastic cells served as reference tissue.

ACTIVITY OF ODC

Activity of ODC was different between the various histological groups. In general, mean ODC activity was higher in anaplastic than in slowly growing tumours. Thus, highest ODC activity was found in malignant lymphomas (52.1 (42.1) nmol/g/h), followed by medulloblastomas (25.5 (15.1) nmol/g/h), anaplastic meningiomas grade III (17.5 (9.7) nmol/g/h), and metastatic carcinomas (14.9 (22.1) nmol/g/h). Lowest enzyme activity was measured in epidermoid cysts (0.5 (0.2) nmol/g/h), in craniopharyngiomas (1.2 (0.9) nmol/g/h), angioblastomas (1.6 (1.7) nmol/g/h), and neurinomas (2.0 (1.8) nmol/g/h).

In the glioma group, mean ODC activity was significantly increased in all tumour grades compared with non-neoplastic peritumorous brain tissue. Furthermore, it rose with a higher grade of malignancy. Thus, it was significantly higher in grade III and IV (9.4 (10.7) nmol/g/h) than in grade I and II gliomas (3.0 (3.8) nmol/g/h; p<0.001). There were no differences between astrocytomas, oligodendrogliomas, and mixed gliomas; nor between primary and recurrent gliomas (data not shown).

Mean ODC activity of low grade ependymomas (11.9 (14.3) nmol/g/h) was also increased in comparison with non-neoplastic brain (0.9 (0.6) nmol/g/h; p<0.001), but, furthermore, it was also higher than in other grade II gliomas (3.1 (4.0) nmol/g/h; p<0.001). Anaplastic ependymomas grade III exhibited a very high mean enzyme activity of 20.0 (6.1) nmol/g/h, which was also higher than in grade III gliomas (5.7 (5.6) nmol/g/h; p<0.001). However, two patients in the group of ependymomas grade II also showed individual values of 39.6 and 36.6 nmol/g/h.

Meningiomas grade I had a higher ODC activity (6.2 (9.3) nmol/g/h) than other grade I tumours—for example, neurinomas (2.0 (1.8) nmol/g/h) and angioblastomas (1.6 (1.7) nmol/g/h). In this group of 189 typical meningiomas, no biochemical differences could be detected between the different histological types of meningothelial, fibrous, transitional, psammomatous, angiomatous, and secretory tumours (data not shown). Atypical meningiomas grade II (12.4 (10.2) nmol/g/h) exhibited enzyme activity comparable with grade I meningiomas, whereas the highest ODC activity was found in three anaplastic meningiomas grade III (17.5 (9.7) nmol/g/h). No significant differences could be found between primary and recurrent meningiomas (data not shown).

In anaplastic tumours variability of ODC activity was not only found in different patients, but it was also evident in different samples from the same tumour. This corresponded well with the polymorphism of rapidly growing tumours: Low ODC values were measured in areas of necrosis or poor cellularity, whereas high enzyme activity was found in solid tumour parts with rich cellularity and numerous mitoses (fig 2).

POLYAMINE CONCENTRATIONS

Compared with peritumorous non-neoplastic brain tissue, polyamine concentrations were

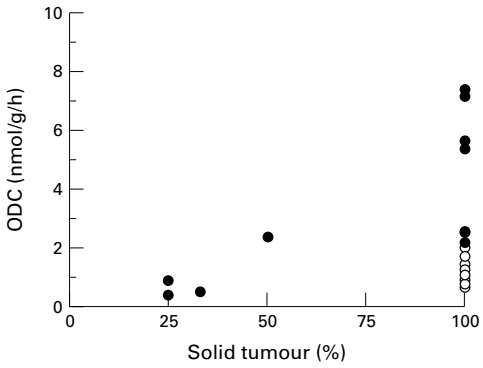

Figure 2 Correlation between histomorphology and ODC activity in 10 samples from a glioma grade II (open circles: 55 year old man, left temporal oligoastrocytoma grade II, ODC activity 0.7–2.1 nmol/g/h) and a glioma grade IV (filled circles: 66 year old woman, left frontal glioblastoma multiforme, ODC activity 0.4–7.3 nmol/g/h).

significantly higher in all gliomas, with the exception of putrescine and spermidine in grade I and spermine in grade II gliomas. Mean polyamine concentrations partially correlated with the histological grade of malignancy in gliomas (table 1). Between low grade and high grade gliomas a significant difference for putrescine (p=0.05) and spermine (p=0.001) was detected.

In non-glial tumours, high putrescine concentrations were measured predominantly in malignant tumours as anaplastic meningiomas (462 (225) nmol/g), medulloblastomas (167 (82) nmol/g), and malignant lymphomas (138 (82) nmol/g), but also in neurinomas (174 (117) nmol/g) and low grade ependymomas (154 (130) nmol/g). Spermidine and spermine concentrations as well as the spermidine/spermine ratio did not correlate with the grade of malignancy. Lowest polyamine content was found in seven epidermoid cysts.

Variability of polyamine content within the various histological groups was comparable with that demonstrated for ODC activity. Mean polyamine concentrations were not markedly different either between primary and recurrent tumours or between the various histological types of gliomas and meningiomas (data not shown).

Discussion

Since putrescine was identified as a growth factor for haemophilus parainfluenzae by Herbst and Snell in 1949, many reports have indicated a close connection of polyamine metabolism activation and cellular proliferation.[9 25 26] It has been suggested that activation of ODC is an early event that occurs during the expression of malignancy.[8] Induction of ODC has been shown to be a universal marker of the G1 phase of the cell cycle and a mandatory event for cells to traverse the G1 phase and enter the S phase.[7 27–29] Furthermore, recent investigations have shown that the ODC gene, which is localised on chromosome 7 and, near other oncogenes, on chromosome 2,[30] represents a (proto)oncogene for the regulation of cellular growth and malignant cell transformation.[31–33]

In the present study, ODC activity and polyamine concentrations were biochemically quantified for the first time in parallel in a large series of human brain tumours. Mean ODC activity was significantly higher in all glioma grades compared with peritumorous non-neoplastic brain tissue. Thus, as known from experimental gliomas,[20 21] ODC activity represents a biochemical marker of neoplastic growth also in the human brain. Furthermore, enzyme activity was significantly higher in high grade than in low grade gliomas. High enzyme activity was also found in other anaplastic tumours such as malignant lymphomas, medulloblastomas, anaplastic meningiomas, and metastases. However, mathematical comparison between tumour groups of different histogenetic origin can be misleading as shown by similar values of meningiomas grade I and gliomas grade III (table 1). Thus, the findings of the present study indicate that ODC activity can be considered as a biochemical marker of proliferation and malignancy within neuropathologically defined entities.

Changes of ODC activity were more pronounced and correlated more closely with the grade of malignancy than those of polyamine concentrations. This corresponds well with the study of Scalabrino and Ferioli,[13] which showed that ODC activity reflects neoplastic growth rates better than activity of the second key enzyme of polyamine metabolism, S-adenosylmethionine decarboxylase (SAMDC). The spermidine/spermidine ratio, which has been found to be increased in glioblastoma multiforme by Moulinoux *et al*,[17] did not correlate with the grade of anaplasia, neither in gliomas nor in non-gliomatous tumours. Thus by contrast with previous studies of human brain tumours,[16 18] our results show that polyamine concentrations measured in tumour samples cannot be used as biochemical markers of malignancy.

A surprising finding of the present study was that ependymomas grade II exhibited very high ODC activity and polyamine concentrations corresponding to those found in anaplastic tumours. This was in accordance with the results of Kurihara *et al*,[18] who also found a high polyamine content in ependymomas. These tumours derive from ependymal cells, which arise directly from the neural tube.[34] On the other hand, development of nervous tissue is controlled, in part, by the ODC/polyamine system.[35 36] Thus, it may be suggested that high ODC activity and polyamine concentrations in ependymomas reflect the developmental characteristics and the proliferative potential of the ependymal and subependymal cell matrix.

Whereas the interpretation of mean values seems to be clear, the assessment of individual data becomes more difficult. Especially in anaplastic tumours, a large variability of ODC activity and polyamine concentrations became apparent, which correlated with histomorphological polymorphism in most cases. Such an intratumorous heterogeneity is also known from other proliferation markers.[37] Thus, individually high values might be an indicator of

malignancy, whereas low values do not prove that the tumour is benign.

Nevertheless, preliminary follow up investigations suggest that ODC activity[15] and the content of N^1-acetylspermidine[18] are related to the progression free survival in low grade gliomas. However, complete follow up documentation is required to estimate the prognostic significance of biochemical data quantified in brain tumour samples. These data are also important for clinical therapeutic studies using agents that inhibit ODC, as without knowledge of the relations between biochemistry and tumour type comprehensive evaluating of such therapies is not possible.[30]

The present study demonstrated that polyamine metabolism is activated in human brain tumours. Quantitative biochemistry disclosed that only activity of the first key enzyme ODC, but not polyamine concentrations, represent a biochemical parameter for the assessment of brain tumour malignancy. The level of ODC activation may contribute to the diagnosis of malignancy, not in general but in tumour groups defined by neuropathology. These results may lead to a better understanding of individual brain tumour pathology. However, the clinical value of these measurements is restricted due to extensive and expensive measurements on the one hand and to the already mentioned sampling errors caused by tumour heterogeneity on the other. The same problem has been shown for quantitative measurements of polyamine concentrations in the blood and CSF of patients with brain tumours.[17] Apart from the level of quantitative biochemistry, it was not possible, until now, to visualise the degree of the activation of polyamine metabolism in vivo, either by autoradiography or by positron emission tomography (PET).[38 39] For the future, immunohistochemical and molecular investigations are required to get more information about ODC regulation and to define the causal connection of activation of polyamine metabolism and cellular proliferation.[5 40] Especially, studies on the level of molecular biology might help to elucidate the possible role of the ODC gene as an oncogene in brain tumour growth,[11] and thus, to develop new therapeutic strategies.

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