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URINARY EXCRETION OF 6-HYDROXYMETHYLPTERIN IN BRAIN TUMOURS

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The urinary 6-hydroxymethylpterin(Pt-6-CH_2OH) excretion was determined in 87 patients with brain tumours and in 50 control patients. The Pt-6-CH_2OH levels were significantly elevated in all patients with tumours. No difference was observed when malignant tumours were compared with benign neoplasms. Following therapy, the Pt-6-CH_2OH levels were partially reduced when compared with control patients and their pre-operative values.

Various pterin derivatives are known to be excreted in the urine (1). Halpern et al. (2) described a blue fluorescent compound which was later identified as 6-hydroxymethylpterin[3, Pt-6-CH_2OH] and this compound is known as a characteristic folate degradation product of various cancer cells in tissue culture. The compound has also been found to be uniquely excreted in the urine of cancer patients (2). During the last few years, interesting results have been reported on alterations of folate and pterin metabolism in tissues and body fluids of patients with malignant tumours (3). Neither oral folate consumption nor folate deficiency has been shown to affect the urinary pterin excretion or tissue pterin cofactor activity in healthy non-malignant subjects (1, 4). Rao et al. (5) have reported elevated Pt-6-CH_2OH excretion during malignancy and liver regeneration. It has also been stated that urinary Pt-6-CH_2OH levels accurately monitor response to chemotherapy in acute myeloblastic leukemia (6). However, there are no reports so far on pterin excretion in patients with brain tumours.

The objective of the present study was to measure the levels of this unconjugated pterin in the urine of brain tumour patients with various intracranial space-occupying lesions.

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Material and Methods

Eighty-seven patients with intracranial tumours or tumour-like lesions were included in the study (53 males and 34 females). The age ranged from 10–70 years with a median of 35 years. Fifty age and sex matched healthy controls were also studied during this period.

All patients underwent a detailed clinical examination and the nature of the brain lesion was in all cases confirmed by histology. The resection was regarded as macroscopically complete in all the patients. The study material included 41 gliomas (31 males, 10 females); 19 meningiomas (9 males, 10 females); 12 acoustic neurinomas (4 males, 8 females) and 15 of other types (9 males, 6 females), which included 4 cases of medulloblastoma, 4 cases of secondary tumour, 2 cases of craniopharyngioma and one case each of tuberculoma, pineoloma, neuroblastoma, subdural hydroma and lipoma. The gliomas, medulloblastomas and secondary tumours were classified as malignant.

Most gliomas had high grade of malignancy. Two of them were reported as glioblastoma multiforme and 33 were classified as grade III and IV astrocytomas. Three patients with malignant ependymomas were also included. In the remaining three patients in the glioma group, specific grade could not be given due to the presence of different cell types in the biopsies.

After resection of the tumour mass, the patients received dexamethasone 16 mg/day (for 72 h in benign and for 4

weeks in malignant cases respectively). Furthermore, antiepileptic drugs (dilantin, 3 mg/day or phenobarbitone, 90–120 mg/day) were also administered to these patients.

Urine was collected from the controls and study patients at any time of the day, since it has been previously established that excretion of pterins remain relatively constant throughout the day (7). Aliquots were kept frozen at −70°C until use. Individuals ingesting multivitamin tablets were not included in this study. No dietary restrictions were imposed on the controls or the study patients prior to collection of the samples. Postoperative urine samples were obtained from the glioma patients when they were re-examined at a follow-up following radiotherapy (6 cases after 5 weeks and 3 cases after more than 12 weeks). These patients received 45 Gy whole brain irradiation with an additional 10 Gy to the tumour over four weeks in 20–25 fractions, starting from the 7th to 10th day after operation. After radiotherapy, these patients received chemotherapy consisting of PCV regimen (procarbazine 100 mg/day for 7 days, and lomustine 40 mg/m^2 and vincristine 1.5 mg/m^2 once every 2 weeks for 12 weeks). During chemotherapy, no steroids were given. In 3 cases of meningioma and one case of acoustic neurinoma postsurgical samples were obtained after 12 weeks. (Out of the 87 patients, only 13 were investigated in this way).

Spectrophotofluorometric estimation of urinary Pt-6-CH_2OH was carried out in activated charcoal eluates by the method of Rao et al. (5) with slight modifications. Quinine sulfate served as the reference standard. creatinine levels were determined colorimetrically (8).

Statistical analysis was carried out according to Student's paired and unpaired t-tests.

Results

In the 50 healthy individuals (controls) no significant differences were found between males and females or between young adults and older individuals.

The mean urinary excretion of Pt-6-CH_2OH was significantly elevated in patients with gliomas ($p < 0.05$), meningiomas ($p < 0.001$), acoustic neurinomas ($p < 0.001$) and the other types of lesions ($p < 0.001$), when compared to the controls (Table 1). Both the malignant group and the benign group had significantly higher Pt-6-CH_2OH excretion than the controls ($p < 0.01$ and $p < 0.001$ respectively (Table 1)). There was a tendency towards higher excretion in the patients with benign lesions compared to those with malignant tumours but the difference was not statistically significant (Table 1).

No significant differences were found when Pt-6-CH_2OH post-treatment excretion levels were compared with controls and with the preoperative levels (Table 2). However, in most individual malignant cases there was a tendency towards lower levels after treatment than before the resection (Table 2).

Table 1

Urinary excretion of 6-hydroxymethylpterin (mean ± SD) in brain tumours

Clinical condition	Pt-6-CH_2OH μg/mg creatinine (range)
Controls (n = 50)	1.08 ± 1.11 (0.05 – 4.83)
Glioma (n = 41)	2.06 ± 2.92[b] (0.08 – 14.53)
Meningioma (n = 19)	4.30 ± 6.0[a] (0.16 – 22.71)
Acoustic neurinoma (n = 12)	3.58 ± 4.18[a] (0.12 – 13.54)
Other types (n = 15)	3.75 ± 3.39[a] (0.42 – 13.67)
All benign cases (n = 38)	3.61 ± 4.87[c] (0.12 – 22.71)
All malignant cases (n = 49)	2.60 ± 3.31[d] (0.08 – 14.53)

[a)] $p < 0.001$; b) $p < 0.05$; c) $p < 0.001$; d) $p < 0.01$.
Benign vs malignant: NS
(Student's unpaired t-test).

Table 2

Pt-6-CH_2OH excretion before and after treatment

Patient No.	Period after surgery (days)	Pt-6-CH_2OH μg/mg creatinine	
		Preoperative	Post-treatment
Glioma			
1	25	0.12	0.73
2	25	1.44	0.69
3	25	1.10	0.39
4	25	2.12	0.04
5	30	0.18	0.05
6	30	1.22	0.73
7	90	0.38	0.16
8	120	1.14	0.41
9	270	0.75	0.48
(Mean ± SD			0.41 ± 0.28*)
Meningioma			
10	90	0.16	0.41
11	150	0.07	0.49
12	270	1.12	0.13
(Mean ± SD			0.34 ± 0.18*)
Acoustic neurinoma			
13	240	0.01	0.63

* Not significant with respect to controls and preoperative values (Student's paired and unpaired t-test)

Discussion

In the present study an elevation of urinary Pt-6-CH_2OH excretion was observed in benign as well as malignant brain tumours when compared to a control group. There was a tendency for non-malignant tumour

patients to have slightly higher excretion of Pt-6-CH_2OH than those with malignant tumours. This observation is difficult to interpret since so far little is known about pterin excretion and brain tumours. In the patients with malignant tumours the levels seemed to be decreased after treatment but this was not statistically significant. Possibly the decrease can be related to the prognosis but this question remains to be studied.

An increase in the catabolism of folic acid may be responsible for the folate deficiency observed in many types of cancer and in other pathological conditions (9, 10). It has been reported that pterin-6-aldehyde can be reduced to Pt-6-CH_2OH by the action of growing cancer cells (11). According to Halpern et al. (2) active folate degradation appears to be a unique characteristic of cancer cells in tissue culture. It has also been proposed that Pt-6-CH_2OH is an active inhibitor of xanthine oxidase (2). Furthermore, there are reports suggesting that the presence of malignant disease is accompanied by striking disturbances in the biosynthesis of some pterins and, consequently, in their catabolism (12–14). This phenomenon may play a role in the 'biochemical imbalance' observed by other investigators (15) in cancer cells whereby the enzymatic machinery of the cell is reprogrammed to accelerate and to maximize the growth rate of the cancer.

Although urinary 6-hydroxymethylpterin levels seem to be associated with some neoplastic conditions, more metabolic studies will have to be carried out before it is understood why the compound is excreted in increased amounts in patients with tumours. The value of urine 6-hydroxymethylpterin as a marker for monitoring response and for prognostication must also be further explored.

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