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O^6-METHYLGAUNINE-DNA METHYLTRANSFERASE ACTIVITY IN CEREBRAL GLIOMAS

A guidance for nitrosourea treatment?

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The activity of O^6-methylgaunine-DNA methyltransferase (O^6-MT), which removes O^6-methyl residues from O^6-methylguanine-DNA leading to cell death, has been reported to correlate with sensitivity to nitrosoureas used for chemotherapy of gliomas. We determined O^6-MT activity in tumors and matched brain tissue from patients with gliomas. Histological diagnoses were: six malignant astrocytomas, two glioblastomas, two oligodendrogliomas, one ependymoma, and one medulloblastoma. In all cases but one, the activity ranged widely from 39 to 258 fmol/mg protein extract. The wide range of activity of the tumor tissue may indicate varying degree of sensitivity to nitrosoureas. The activity of brain tissue, available from the peritumoral region of five cases, varied between 38 to 415 fmol/mg. Four of the five regions showed a higher value than the respective tumor, and one showed a lower value.

Nitrosoureas including carmustin (BCNU), semustin (MeCCNU), and nimustin (ACNU) have been frequently used for treatment of gliomas because of their blood–brain barrier permeability and cell-cycle non-specificity. However, clinical trials have shown that the benefit of adjuvant BCNU or MeCCNU chemotherapy is too small to significantly prolong survival (1, 2). The poor results may be due to non-selective application of nitrosoureas. If nitrosourea treatment could be limited to nitrosourea-sensitive tumors, the therapeutic results might improve. Tests are therefore needed that can predict the drug sensitivity of the tumors.

Recently, nitrosoureas have been shown to induce damage or modifications at positions of guanine on DNA strands via alkylation (3, 4). Of critical importance for living cells are O^6-methylguanine adducts, which the enzyme O^6-methylguanine-DNA methyltransferase (O^6-MT) repairs by transfer of methyl groups from O^6 position to cysteine residues. Reports on O^6-MT are, however, too scarce to fully understand distribution and clinical implication of this enzyme in human brain tumors (5, 6).

We now report the O^6-MT activity of tumors and adjacent non-neoplastic brain tissue in a series of patients with gliomas.

Material and Methods

Twelve patients with gliomas were studied, including six malginant astrocytomas, two glioblastomas, two oligodendrogliomas, one ependymoma, and one medulloblastoma. Histological diagnosis was classified according to the WHO classification (7). There were six males and six females with a mean age of 34 years (range 3–67). Tumor tissue was obtained at surgery. In five patients, surrounding brain tissue was removed by necessity for the surgical approach or as part of extensive resection. For enzymic measurement, we chose tissue, which was histologically free of tumor cells.

The tissue, kept frozen at −80°C until assay, was cut into pieces on a block of dry ice. The methods of preparation for crude protein fraction have been reported elsewhere (8). Briefly, the tissue pieces were dissolved in Buffer C (50 mM Tris-HCl, pH 7.8, 1 mM EDTA, 5 mM 2-

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mercaptoethanol, 1 mM phenylmethyl sulfonyl fluoride, 10 mM potassium pyrosulfite, 1 μg/ml leupepton, 25 μg/ml aprotinin, and 1 mM dithiothreitol). Pepstatin A and leupepton were purchased from Peptide Institute Inc. (Minoh, Japan), and aprotinin from Sigma Chemical Co. (St. Louis, MO). The tissue was homogenized by a Polytron PT-10 (Kinematica, Switzerland) and a Potter-Elvehjem homogenzier. Then, the homogenate was sonicated at 4°C three times for 30-s bursts and 30-s intervals with a Bioruptor (UCD-1303, CosmoBio, Tokyo). The lysate was then spun at 15 000 rpm for 10 min with a centrifuge (Tomy MR-150, Tokyo). The supernatant was collected as a crude protein extract; the protein content was measured according to the modified Lowry's method (9).

The standard target DNA containing methyl-^{3}H-labeled O^6-methylguanine was prepared as described previously (8). High performance liquid chromatography of the substrate DNA after hydrolysis revealed that ^{3}H-labeled O^6-methylguanine constituted more than 70% of the radioactivity.

The protein extract from tissue was reacted with the target DNA in Buffer for 60 min. After addition of bovine serum albumin, the reaction mixture was precipitated with 0.8 M trichloroacetic acid. The precipitate was heated at 90°C for 30 min, and was then filtrated through a GF/C filter (Whatman Ltd., England). The filter dissolved into NCS tissue solubilizer (Amersham, IL) was incubated at 55°C got 2 h. The filter was counted in xylene-based scintillation liquid. The O^6-MT activity as a function of protein concentration in the extracts was calculated by least square regression analysis, and was expressed as fmol of ^{3}H-methyl group transferred. O^6-MT values were determined by at least three independent measurements.

Results

The Table summarizes the O^6-MT activity of tumors in 12 patients with gliomas. One patient with malignant astrocytoma showed an undetectable level of the enzyme activity, and the other 11 patients a value between 39 and 258 fmol/mg protein extract. Six cases (50%) had a value less than 100 fmol/mg and three cases (25%) a value less than 60 fmol/mg. A medulloblastoma patient had the highest value. No significant difference in the enzyme activity was noted between low- and high-grade gliomas.

Table

O^6-MT activity in patients with gliomas

Pathology	O^6-MT activity (fmol/mg)	(Mean ± SD)
High-grade glioma (n = 9)		118 ± 84
Malignant astrocytoma (n = 6)	ND, 91, 110 143, 152, 215	
Glioblastoma (n = 2)	39, 54	
Medulloblastoma (n = 1)	258	
Low-grade glioma (n = 3)		99 ± 44
Oligodendroglioma (n = 2)	61, 147	
Ependymoma (n = 1)	90	

ND: not detected.
The enzymic activity was expressed as fmol of ^{3}H-methyl transferred per mg protein.

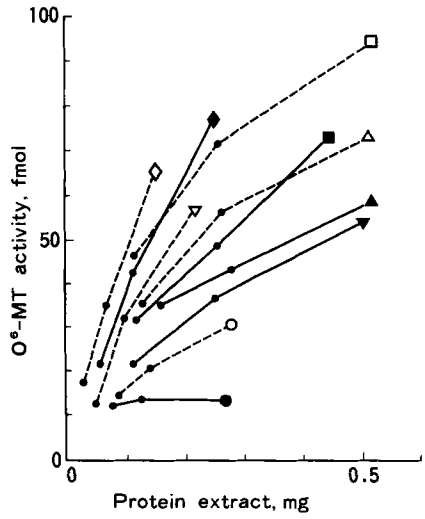

Figure. O^6-MT activity in gliomas and surrounding brain tissue in relation to protein concentration. Solid symbols and solid lines represent tumor tissue whereas open symbols and dotted lines represent surrounding brain tissue from the respective patients. In four cases the values were higher in the brain tissue than in the tumor whereas in one case the values were higher in the tumor than in the brain.

As demonstrated in Figure, the enzyme activity of the surrounding brain tissue available in five cases ranged from 38 to 415 fmol/mg with a mean of 175 fmol/mg. Four of these cases had a higher value of O^6-MT in the surrounding brain tissue than in the tumor tissue. In the remaining case, the value of the brain tissue was lower than that of the tumor.

Discussion

Antitumor nitrosoureas have been adminstrated to glioma patients as a single adjuvant or drug combination. Since clinical introduction of nitrosourea treatment of gliomas in the early 1970's, survival rates have not improved singificantly (1, 2). MeCCNU used alone produced no survival benefit. A group reveiving BCNU plus radiation or MeCCNU plus radiation had only slightly superior survival rate compared to a group receiving radiation alone (1). Polychemotherapy including BCNU did not lead to significant improvement in survival (2). A recent overview of 17 randomized clinical trials in malignant gliomas suggested that the survival rate in patients treated with chemotheraphy following radiation increased on average by 10% and 6% at 12 and 24 months respectively, as compared with patients treated with radiation alone (10).

Several alkylating agents might have been responsible for this modest survival benefit. Selective application and combination of existing drugs might enhance the therapeutic effectiveness.

In the present study, O^6-MT activity showed a wide range from an undetectable level to 258 fmol/mg. No significant difference was observed between low-grade and high-grade gliomas. O^6-MT activity seemed unrelated to the histological malignancy. A varying O^6-MT activity in gliomas may be due to histological and biological heterogeneity. Malignant gliomas comprise histological components such as necrosis, which could modify O^6-MT activity. Multiple measurements in several portions of a tumor are therefore important.

Chemosensitivity tests must be rapid and accurate to be clinically useful. In vitro sensitivity tests on cultured cells takes 6–8 weeks from surgery. It is also difficult to establish cell strains from human solid tumours for long-term culture (11). O^6-MT enzyme assay takes only one day which should be a great advantage in the clinical situation.

In vitro and in vivo studies have revealed a correlation between the enzymic activity of O^6-MT and nitrosourea sensitivity. Resistance of ACNU increases linearly as function of O^6-MT activity in human tumor cell lines (12). We previously described a linkage between O^6-MT activity and cellular sensitivity to ACNU or ranimustin (MCNU) (8). Cultured brain tumor cell lines sensitive to the nitrosoureas showed a lower level of the enzyme. No correlation was found between the enzyme and the sensitivity to other DNA-damaging agents such as bleomycin (BLM), noecarzinostatin (NCS), cis-diamminedichloroplatinum (II) (CDDP), and etoposide (VP-16). O^6-MT activity seems to be a useful indicator of sensitivity to nitrosoureas. Tumor xenografts with Mer^- phenotype lacking O^6-MT activity were much more sensitive to ACNU than tumors with Mer^+ phenotype possessing high activity (13).

The level of O^6-MT activity in gliomas might be important to define whether a tumor is sensitive or resistant to nitrosoureas. A 9L glioma cell strain showing 52 fmol/mg was markedly sensitive to ACNU and MCNU. Subcutaneously transplanted xenografts of this strain regressed rapidly or disappeared at a high frequency after ACNU treatment. In contrast, C6 gliomas showing 160 fmol/mg grew progressively after transient response to ACNU (14). Thus, a level around 60 fmol/mg may represent the dividing line between sensitivity or resistance to ACNU.

In a recent paper a relationship was shown between O^6-alkylguanine-DNA alkyltransferase (a synonym of the O^6-MT enzyme) and sensitivity to procarbazine (PCZ), which also generates O^6-methylguanine on DNA in human brain tumor xenografts (6). Tumors with O^6-MT activity above 100 fmol/mg showed less growth delay after PCZ treatment, compared to tumors with undetectable activity.

Wiestler et al. (5) tested the O^6-MT activity of human brain tumors and brain samples. Activity was detectable in a variety of brain tumors and in normal brain samples, with the highest values found in benign tumors, such as meningiomas and neurinomas. Most tumors with the exception of some gliomas had higher activity than normal brain. In the present study, one case with medulloblastoma had the highest value among the tumors. The activity of peritumoral brain tissue available in five cases varied between 38 and 415 fmol/mg. One case showed a lower value in the brain tissue than in the tumor which could imply vulnerability to nitrosoureas. However, further in vitro studies and prospective clinical trials are needed for evaluation of the possible predictive value of O^6-MT activity in connection with nitrosourea treatment of gliomas.

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