

Original Article

Cytochrome Oxidase Activity and ATP Levels in High-Grade Gliomas and Meningiomas

ABSTRACT

OBJECTIVE: Malignant brain tumors have a diminished respiratory rate coupled with increased aerobic glycolysis when compared to low-grade brain tumors. However, there are a limited number of studies that compare the cytochrome oxidase activity and adenosine three-phosphate production in low and high-grade brain tumors.

METHODS: Here, we measured the activity of cytochrome oxidase and adenosine three-phosphate production in high-grade brain tumors (9 patients), meningiomas (grade I, 10 patients), and normal brain tissues (10).

RESULTS: Cytochrome oxidase activity was markedly higher in high-grade gliomas than both meningiomas and controls. The difference between high-grade gliomas and meningiomas was statically significant ($p = 0.0011$). A statistically significant difference was also found between meningiomas and the control groups ($p = 0.0002$); however, no statistically significant difference was demonstrated between high-grade gliomas and the control group ($p = 0.23$). The mean level of adenosine three-phosphate was also higher than meningiomas and controls. Statistical calculation demonstrated the "p" value as 0.014 between the high-grade gliomas and meningiomas groups. When comparing high-grade gliomas and meningiomas to the control group, a statistically significant difference was found.

CONCLUSION: It is clear from these data that high-grade tumors have higher respiratory rate than low-grade tumors as evident by the increased enzyme activity and adenosine three-phosphate levels, suggesting that a treatment regimen targeted towards the energy metabolism of brain tumors may be beneficial.

KEY WORDS: ATP, Brain tumors, Cytochrome oxidase, Gliomas, Meningiomas

INTRODUCTION

With regard to brain tumors, there seems to be general agreement that tumor cells with high grades of anaplasia exhibit rather low levels of respiration when compared to benign tumors and that this is associated with an excessive rate of aerobic glycolysis. Moreover, it has been found that several tumors show defects in the components of the electron transport chain of respiration and a decrease in the number of mitochondria per cell (5, 6, 8, 9).

A number of investigators have determined cytochrome oxidase (COX) in various brain tumors using in vitro tissue slices. COX is a terminal step in the electron transport chain and significant reductions could well limit the rate of respiration. Allen (1) showed that well-differentiated astrocytomas and ependymomas have extremely low levels of COX in comparison to either gray or white matter, whereas glioblastoma multiforme (GBM) had somewhat higher levels. A defect in respiration proximal to cytochrome b complex has also been shown in several pituitary adenomas and ependymomas (10). With regard to pituitary adenomas and meningiomas, Lichtor and Dohrmann (9) reported COX values considerably lower than those found in high-grade astrocytoma and metastatic squamous cell carcinoma. Recently, it was demonstrated that COX activity was decreased in the deep layers of peritumoral cortex, and almost absent in the tumoral cortex, the most

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superficial layer of tumor, in patients with glioblastoma (6). Moreover, Dmitrenko (5) demonstrated that levels of mitochondrial transcripts for adenosine three-phosphate (ATP) synthase were decreased in glioblastomas as compared to tumor-adjacent histologically normal brain. Overall, data summarized above suggest that there is conflicting data regarding respiratory enzyme activity in both low- and high-grade brain tumors and the more rapidly growing or poorly differentiated a tumor becomes, the more its energy metabolism approaches a fetal-like state; the mitochondrial content is reduced and the glycolytic enzymes become elevated.

We investigated whether there was a difference in COX and ATP production at two ends of Democles' sword: grade I meningiomas and high-grade gliomas (HGG). In addition, the results were compared to values from normal brain tissues.

MATERIALS AND METHODS

Patients and control group

Ethical approval for this study was obtained from the Human Investigations Committee at Istanbul University and all patients, or the next of kin if the patient was unconscious, provided informed consent. Specimens of brain tumors were obtained from patients undergoing for surgery for brain tumors. Immediately upon removal, the tumors were placed liquid nitrogen to be frozen and stored at -70 °C until assayed. After histopathological diagnosis was made, the tumor specimens were assayed. Ten patients with meningiomas (grade I) and 9 patients with high-grade gliomas were included in this study. (Table I) summarizes the clinical data of the patients.

Normal brain tissues for control group were collected from the Institute of Forensic Medicine 2-8 hours after death without brain trauma and stored at -70 °C until prior to assay.

Isolation of mitochondria

Following the histopathological diagnosis, tumor and normal brain specimens were placed on ice, and all the following procedures were carried out at 4 °C (2). The specimens were minced and subsequently suspended in STEH buffer (0.4 M sucrose; 0.01 M Tris-HCL; 0.001 M ethylenediaminetetra-acetic acid (EDTA); and 0.02 % heparin, pH 7.4). Specimens were then homogenized with 15 strokes of a Teflon pestle in a smooth glass-surfaced Thomas tissue grinder. The homogenate was centrifuged at 1100 G

Table I. Summary of the clinical parameters of the patients included in this study

High-grade gliomas	Age (yrs)	Gender	Main complaint	Location	Histopathological diagnosis (grade)
1	48	Male	Aphasia	Left FT	GBM
2	17	Female	Headache	Right F	A A (III)
3	46	Male	Seizure	Right FP	AO (III)
4	33	Male	Hemiparesia	Right FP	GBM
5	67	Male	Headache	Left P	GBM
6	25	Male	Diplopia	Left C	GBM
7	25	Female	Seizure	Right T	GBM
8	49	Male	Dysphasia	Left TP	GBM
9	55	Male	Hemiparesia	Right FP	AO (III)
Meningiomas					
1	63	Female	Headache	Left FP	Grade I
2	27	Female	Seizure	Right T	Grade I
3	65	Female	Hearing loss	Right CPA	Grade I
4	61	Female	Headache	Right F	Grade I
5	33	Male	Diplopia	Right SW	Grade I
6	52	Female	Headache	Right F	Grade I
7	47	Male	Headache	Left SW	Grade I
8	59	Male	Headache	Left T	Grade I
9	43	Male	Headache	Left F	Grade I
10	32	Female	Seizure	Right P	Grade I

AA: Anaplastic astrocytoma; AO: Anaplastic oligodendroglioma; C: Cerebellum; CPA: Cerebello-pontine angle; F: Frontal; GBM: Glioblastome multiforme; P: Parietal; SW: Sphenoid wing; T: Temporal.

for 10 minutes to separate nuclei, unbroken cells, and large cytoplasmic debris. The pellet was resuspended in STEH buffer, homogenized again, and centrifuged as before. Both supernatants were combined and sedimented at 17,000 G for 20 minutes. The pellet of mitochondria thus obtained was washed with STEH buffer plus 8 % Ficoll and recentrifuged at 17,000 G for 20 minutes. The pellet was then suspended in STEH buffer.

Protein determinations

All protein concentrations were determined according to the method of Lowry, et al (1).

Cytochrome c oxidase assay

The method of Sakaii (13) was employed for the measurement of COX. The assay mixture contained 0.0017 mM cytochrome c in 80 mM phosphate buffer

Table II. Values of each specimen belonging to the patients

High-grade gliomas	Histopathological diagnosis (grade)	COX activity nmol/min/mg protein)	ATP levels $\mu\text{mol/g}$ protein
1	GBM	0.30	3.7
2	AA (III)	0.46	3.76
3	AO (III)	0.33	4.44
4	GBM	0.73	4.3
5	GBM	0.68	4.54
6	GBM	0.81	6.12
7	GBM	0.78	5.18
8	GBM	0.79	5.32
9	AO (III)	0.78	4.51
	Mean\pmSD	0.62\pm0.20	4.65\pm0.77
Meningiomas			
1	Grade I	0.38	4.41
2	Grade I	0.41	3.36
3	Grade I	0.32	4.18
4	Grade I	0.46	3.2
5	Grade I	0.52	3.5
6	Grade I	0.35	3.52
7	Grade I	0.16	3.78
8	Grade I	0.22	3.1
9	Grade I	0.16	2.88
10	Grade I	0.29	4.54
	Mean\pmSD	0.32\pm0.12	3.64\pm0.56
Control	Mean\pmSD	0.54\pm0.07	1.82\pm0.35

AA: Anaplastic astrocytoma; AO: Anaplastic oligodendroglioma; ATP: Adenosine three-phosphate; COX: Cytochrome oxidase; GBM: Glioblastome multiforme.

at pH 7.0. Assays were performed on mitochondria that had been stored frozen at -20 °C until just prior to use. Activity was expressed as nM cytochrome c, oxidized/min/mg of protein.

ATP assay

ATP assay was employed according to spectrophotometric analysis (3). Levels were expressed as $\mu\text{mol/g}$ of protein.

Statistical analysis

Data were analyzed with the aid of the SPSS statistical program (SPSS, Chicago, IL). All data were provided as mean \pm standard deviation (mean \pm SD) and statistical analysis was performed using the "student t-test, two tails." Probability values less than 0.05 were considered statistically significant.

RESULTS

Nineteen tumors (9 HGG and 10 meningiomas) from the patients and 10 normal brain samples from the control group were obtained for this prospective clinical study. The samples were tested for the presence of COX and ATP.

In this study, the HGG group was composed of seven male and two female patients with a mean age of 40.55 years (40.55 \pm 16.43), and the meningioma group included six females and four males with a mean age of 48.20 years (48.20 \pm 14.01). There was no statically significant difference between the two patient groups with regard to age (p=0.28).

COX activity

COX activity differed markedly between patients in the HGG and meningioma groups. In the HGG group, the mean COX activity was 0.62 \pm 0.20 nmol/mg protein, while it was 0.32 \pm 0.12 in the meningioma group and the difference was statically significant (p=0.0011). The mean COX activity in the control group was 0.54 \pm 0.07 nmol/mg proteins. A statistically significant difference was also found between the meningioma and control groups (p=0.0002); however, no statistically significant difference was demonstrated between the HGG and control groups (p=0.23). (Figure 1) summarizes the statistical comparisons.

Levels of ATP

Regarding ATP levels, all comparisons demonstrated a marked difference between groups. The mean level of ATP was 4.65 \pm 0.77 $\mu\text{mol/g}$ protein in HGG, 3.64 \pm 0.56 $\mu\text{mol/g}$ protein in meningiomas, and 1.82 \pm 0.35 $\mu\text{mol/g}$ protein in control group. Two tailed student t-test demonstrated a "p" value of 0.014 between the HGG and meningioma groups. When HGG and meningioma groups were compared to the control group, a statistically marked difference was found (p=0.00001 for HGG/control; p=0.00001 for meningiomas/control) (Figure 2).

DISCUSSION

Our hypothesis in this study was that malignant cells have an increased respiratory rate coupled with an increased and excessive rate of aerobic glycolysis due to their high metabolic rate and more energy demand. The results in the present study supported our hypothesis that malignant brain tumors showed higher oxidative respiration than benign brain tumors. COX activity in HGGs was higher than either meningiomas or control, suggesting that the more rapidly growing or poorly differentiated a

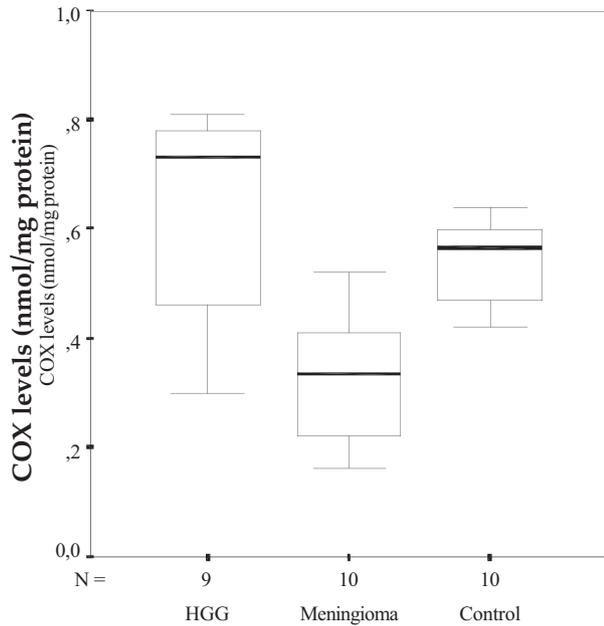


Figure 1. Box plot comparing the COX activity between HGGs, meningiomas, and controls. There is a considerable difference between HGG and meningiomas ($p=0.001$) and between meningiomas and controls ($p=0.0002$). This difference is not present when comparing HGGs and controls ($p=0.23$). Horizontal lines within the boxes represent median values and the boxes denote the interquartile range.

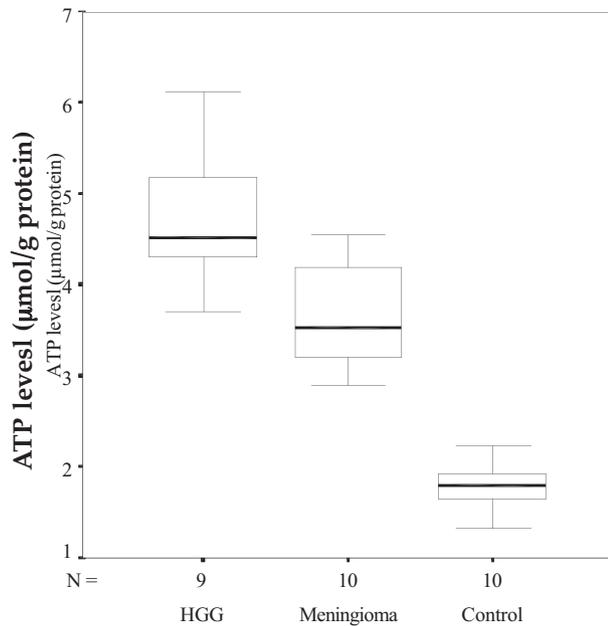


Figure 2. Box plot comparing the levels of ATP between HGGs, meningiomas, and controls. All comparisons showed marked difference ($p < 0.001$). Horizontal lines within the boxes represent median values and boxes denote the interquartile range.

tumor becomes, the more oxidative respiration and ATP production increase. ATP production in both the HGG and meningioma groups was higher than the control group in this study. Furthermore, there was more ATP production in HGG than in meningiomas. Although meningiomas showed lower COX activity than the control group, they showed high ATP levels. This discrepancy may partly be explained by increased glycolysis. It has previously been reported that low-grade astrocytomas have extremely low COX levels in comparison to normal cortex (1). Depressed respiratory capacity in both benign and malignant brain tumors has been attributed to either a decrease in mitochondria per cell or a defective respiratory mechanism (5, 6, 8, 9). A similar pattern has also been demonstrated in hepatoma cell lines (4, 11, 14,15). Therefore, such tumors that require high energy must rely on glucose utilization (1, 12) and this was reflected by positron emission tomographic scanning using FDG-PET (10). However, it has been demonstrated in certain human brain tumors that decrease in oxidative respiration and increase in glycolysis is not correlated with malignancy (8). There is no established correlation between the histological classification of brain tumors and respiratory function (9); however, some evidence does exist between increased glycolysis and malignancy in human astrocytomas both in vivo and in vitro (7). Garbossa et al. (6) demonstrated COX activity in the tumoral and peritumoral cortex in patients with GBM and metastasis of adenocarcinoma. They found that COX activity is very low both in tumoral and peritumoral cortex, independent of tumor type. They suggested similar to other reports that the neoplasm had depressed electron transport capacity coupled with compensatory elevated glucose utilization. In a recent paper, Dmitrenko (5) demonstrated that Northern blot hybridization revealed decreased expression of all mitochondrial genes coding respiratory chain subunits in GBMs as compared to the normal human adult brain. Our results demonstrated that there is no depression in respiratory capacity although we only measured COX activity. Therefore, based on the increased COX activity and ATP production in our study, we support the notion that diminished respiratory rate in malignant cells is neither unique to malignant tumors nor an essential characteristic of all varieties of cancer.

CONCLUSION

In conclusion, data suggest that there is induction of respiratory chain in HGGs together with an increase in enzyme activity, similar to ATP production, when compared to normal brain tissue. Induction of respiratory chain activity in brain tumors might be an answer to the increased energy demand. These findings may be relevant to designing new therapeutic approaches to malignant brain tumors. COX antagonists that selectively inhibit tumor respiration rate may induce apoptosis of tumor cells and hence tumor volume. However; further investigations are needed in order to improve our understanding of the oxidative metabolism of human brain tumors.

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