TBARS, Glutathione and Carnitine Levels in Human Brain Tumours

İNSAN BEYİN TÜMÖRLERİNDE TBARS, GLUTATYON, KARNİTİN DÜZEYLERİ

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Summary

Purpose: The aim of the present investigation was to determine the tissue lipid peroxidation (TBARS: thiobarbituric acid reactive substance), reduced glutathione (GSH) and carnitine levels in human brain tumours.

Results: The mean carnitine, GSH and TBARS levels for tumourous group were found to be 0.011±0.0047 μg/mg protein, 0.03±0.01 μg/mg protein and 2.3±1.3 μmol/mg protein respectively versus 0.019±0.002 μg/mg protein, 0.0387±0.01 μg/mg protein and 0.62±0.2 μmol/mg protein for normal brain tissues.

Carnitine and GSH levels were found to be significantly lower in human brain tumours ($p<0.001$, $p<0.05$) than in control group. Whereas the mean TBARS level of tumourous group was significantly higher than those of the control group ($p<0.001$).

Conclusion: In conclusion, elevated tissue TBARS level may be due to the decreased levels of carnitine and GSH in human brain tumours.

Key Words: TBARS, Glutathione, Carnitine, Brain tumours

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Özet

Amaç: Bu çalışmamızın amacı insan beyin tümörlerinde insan beyin tümörlerinde lipid peroksidasyonu (TBARS: thiobarbituric acid reactive substance) karnitin ve indirgenmiş glutatyon (GSH) düzeylerini tayin etmekti.

Bulgular: Ortalama karnitin, GSH ve TBARS-2 düzeyleri tümörlü grupta sırasıyla 0.011±0.0047 μg/mg protein, 0.03±0.01 μg/mg protein ve 2.3±1.3 μmol/mg protein, normal beyin dokularında ise sırasıyla 0.019±0.002 μg/mg protein, 0.0387±0.01 μg/mg protein ve 0.62±0.2 μmol/mg protein bulundu.

Kontrol grubuna göre insan beyin tümörlerinde karnitin ve GSH düzeyleri anlamlı olarak düşük ($p<0.001$, $p<0.05$) bulundu. TBARS düzeylerinin ise insan beyin tümörlerinde kontrol grubuna göre anlamlı olarak yüksek olduğu bulundu ($p<0.001$).

Sonuç: Kanaat olarak; insan beyin tümörlerinde TBARS düzeylerinin artması karnitin ve GSH düzeylerindeki azalmaya bağlı olabilir.

Anahtar Kelimeler: TBARS, Glutatyon, Karnitin, Beyin tümörleri

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Free radicals are potentially dangerous by products of cellular metabolism that have direct effects on cell growth and development, cell survival and have a significant role in the pathogenesis of atherosclerosis, cancer, aging and several other conditions, including inflammotory disease. Free radicals are generated by aerobic organisms during the production of ATP (adenosine triphosphate) in mitochondria. During the electron transport steps of ATP production, due to the leakage of electrons from mitochondria, reactive oxygen species (ROS) like superoxide anion and hydroxyl radicals are generated. These species lead to the production of hydrogen peroxide, from which further hydroxyl radicals are generated in a reaction that seems to depend on the presence of Fe(+2) ions. Free radicals have both benefical and harmful actions. They are needed for signal-transduction pathways that regulate cell growth, reduction-oxidation (redox) status, and as a first line of defense against infections by polymorphonuclear leukocytes. On the other hand, excessive amounts of free radicals can start lethal chain reactions, which can inactivate vital enzymes, proteins and other important subcellular elements needed for cell survival and lead to cell death (1). Moreover, ROS-induced lipid peroxidation has been implicated in malignant transformations (2-4). The oxidative destruction of polyunsaturated fatty acids known as lipid peroxidation, is particularly damaging because it proceeds as a self penetrating chain-reaction (5).

Organisms use various antioxidant defenses against free radical damage. Antioxidant can be defined as any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delay, or prevents oxidation of that substance (6,7). Some of these antioxidant agents are superoxide dismutase, catalase, glutathione peroxidase, α-tocopherol, β-carotene, glutathione, ascorbic acid, urate, bilirubin (8).

Reduced glutathione (S-glutamyl sisteinylglcyine: GSH) plays an important role in protection of cells against

damage from endogenous and exogenous free radicals and oxidants (9). Also, several recent studies in have suggested that carnitine and some of its esters protect against ischemia-reperfusion injury of the heart (10-12).

L-propionylcarnitine, a propionyl ester of L-carnitine, increases the intracellular pool of L-carnitine. It exhibits a high affinity for the enzyme carnitine acetyltransferase and, thus, is readily converted into propionyl-coenzyme A and, free carnitine. L-propionylcarnitine might reduce the hydroxyl radical production in the Fenton system, by chelating the iron required for the generation of hydroxyl radicals (13). On the other hand, *in vitro* evidence would support the concept that acetyl-L-carnitine might posses a direct antioxidant activity (6,12).

There are only few reports on oxidant-antioxidant profile in human brain tumours. We therefore, undertook the present study to assess the extent of tissue TBARS (indicator for tissue lipid peroxidation), carnitine and GSH in patients with brain tumours.

Methods

24 samples of brain tumour (6 mixed glial tumour; 8 meningioma; 2 craniopharingoma; 1 astrocytoma; 1 Acustic neurinoma; 4 hypophyseal adenoma; 1 chordoma; 1 sarcoma) and 10 normal brain samples were investigated.

Tumour tissue samples were obtained from the Neurosurgery Department immediately after surgical intervention. Macroscopically homogenous pieces of the tumour were selected and samples were sent to the Department of Pathology for identification. Normal human brain tissues were obtained at autopsy. All tissues were stored at –70°C until analysis.

Tumour and normal tissue samples were homogenized in phosphate buffer at 4°C forming a %10 (w/v) homogenate. Homogenization was performed with a tissue grinder which had been fitted with a Teflon pestle at a speed at 1000 rev/min for 10 min.

Carnitine was determined by the DTNB method (14). TBARS was measured by the thiobarbituric acid assay method (15). TBARS concentrations were calculated by using $1{,}56x10^5$ $M^{-1}cm^{-1}$ as molar absorption coefficient. GSH was determined by the method of Ellman based on the development of a yellow color when 5,5'-dithiobis (2-nitro-benzoic acid) (DTNB) is added to compounds containing sulfhydryl groups (16). Total proteins were assayed by the Lowry procedure (17).

Radiological data of the patients were obtained by the evaluation of computed tomography and magnetic resonance imaging scans before operation.

The mean tissue values of GSH, TBARS, carnitine between the groups were compared by commonly used statistical techniques for the comparision of sample means (student's t test) and p values equal to or less than 0.05 were considered significant.

Results

In Table 1 the mean carnitine, reduced glutathione and TBARS levels in tumours and control brain tissues are seen. The mean carnitine and reduced glutathione levels in the patients group were significantly lower than in control group ($p<0.001$, $p<0.05$) whereas tissue TBARS levels in the patients group were significantly higher than in the control group ($p<0.001$).

Discussion

Molecular oxygen and its reaction products, including the superoxide radical and hydrogen peroxide can cause injury to biological organisms through a variety of mechanisms. Carcinogenesis is thought to be a multistep process (18) and oxidative damage may be lined to tumorigenesis through several mechanisms (19).

Molecular oxygen reaction products induce point mutations, deletions and gene amplification and rearrangement in mammalian cells, which may result in protooncogene activation and-or tumour supressor gene inactivation (20,21).

Free radical alterations of unsaturated lipids in cell membranes may result in loss of membrane fludity and can lead to cell lysis (11,18,22). Also, the oxidative degradation of polyunsaturated fatty acids has been shown to create aldehydes; such as TBARS which can cause cross links in nucleic acids, proteins and lipids (11).

Our findings indicate that TBARS level in cancerous tissue is higher than those in normal tissue. These findings are in agreement with the findings of other researchers (23,24).

Antioxidative defense mechanism which protect

Table 1. Carnitine, GSH and TBARS levels in patients with brain tumours and control group (mean ± SD)

Group	n	Carnitine (µg/mg protein)	GSH (µg/mg protein)	TBARS (µmol/mg protein)
Brain tumours	24	0.011±0.0047*	0.03±0.01**	2.3±1.3*
Control	10	0.019±0.002	0.0387±0.01	0.62±0.2

* $p<0.001$, when compared to control group
**$p<0.05$, when compared to control group
n: Number of patients

against oxidation include enzymes such as glutathione peroxidase, superoxide dismutase, catalase, a selenium-dependent free radical scavenger and smaller molecules such as the carotenoids, ascorbic acid, glutathione, bilirubin, urate and the tocopherols (8,11,12,22,25). These antioxidants are proposed to protect cells and their moleculer components from oxidative stress by scavenging free radicals and quenching lipid peroxidation chain reactions (7,11,25).

Many new experimental and clinical data concerning the physiological and pathopysiological role of carnitine have accumulated in recent years (26-28).

It is now generally accepted that the major function of L-carnitine in the eukaryotic cells is to transfer acyl compounds from the cytosol to the mitocondrial matrix where these acids are further metabolized. Heart and skeletal muscles have the highest content of carnitine and rat brain tenth of that (29). However, the activity of palmitoylcarnitine transferase in brain is comparable with that in kidney and skeletal muscles (29,30).

In previous studies showed that L-propionylcarnitine was able to scavenge superoxide anion, to inhibit the lipoperoxidation of linoleic acid, and the protect Pbr 322 DNA from cleavage induced by hydrogen peroxide UV-photolysis (13).

On the other hand intracellular glutathione play an important role in the protection of cells against damage from free radicals and also influences cytotoxicity to some kind of chemotherapeutic agents (31). In this reaction, reduced glutathione (GSH) transforms oxidized glutathione (GSSG) by glutathione peroxidase, and GSSG returns to GSH by glutathione reductase (redox cycle) (32,33). Cells are protected against cellular injury by this redox cycle.

In our study, both carnitine and reduced glutahione levels (GSH) in human brain tumors were found to be significantly lower than in control group.

In conclusion; elevated levels of TBARS in cancerous tissues may be due to decreased levels of carnitine and glutathione as part of the antioxidant defense system which might alter various physiological properties of the nerve cell.

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