Investigation of Serum and Tissue Lipid Peroxidation and Serum Ascorbic Acid, and Iron Levels in Human Brain Tumour

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Abstract : The present study aimed the biochemical relationships between human brain tumours and serum lipid peroxide, ascorbic acid and total iron levels. Tissue lipid peroxide levels in tumour tissue itself and in the surrounding tissue were also measured. Serum lipid peroxide levels were found to be significantly high ($p<0.001$) in patients with brain tumour while the serum ascorbic acid levels were significantly low ($p<0.001$) compared with the control group. Serum iron concentrations in patients with brain tumours were also found to be high but the results were nor statistically significant. On the other hand lipid peroxide levels in the tumour tissues were significantly lower than in the surrounding tissue ($p<0.001$).

We believe that high serum lipid peroşide levels in patients with brain tumours are the result of decreased serum ascorbic acid concentrations and the pressure effect of tumours on surrounding tissues which cause lipid peroxide levels in peripheral tissues and in the serum to increase.

Key words : Ascorbic acid, Brain tumour, Iron, Lipid peroxidation.

INTRODUCTION

There is increasing evidence to suggest that the increase of lipid peroxidation in membranous structures causes many pathological states in humans by damaging the membranes and interfering with the function of organelles and tissues (20,21,25,28). Biomembranes and subcellular organalles are particularly sensitive to oxidative attacks as they carry specific amounts of polyunsaturated fatty acids (PUFA) in their membrane phospholipids (15,20,21,30).

In recent years, lipid peroxidation in tumour cells has been investigated by several research groups (2,3). Malignant tumours and regenerating tissue exhibit a low degree of "peroxidizability" that has been shown to be ralated to the growth rate of the tumour (4,7,14). Altered lipid composition of the cellular membranes which have decreased PUFA causes important changes in the static and dynamic properties of the membranes and is associated with low susceptibility to peroxidation. Cellular oxy-radical scavenging enzymes are markedly reduced (11). Lipid peroxidation can be induced by iron ions as reported by Kappus (10). In previous studies the interrelation of ascorbic acid and iron with lipid peroxidation has been investigated experimentally within the tissues (13,15). Lack of information regarding the levels of lipid peroxide in serum and brain tumour tissues and also the serum iron and ascorbic acid level interrelation with brain tumours directed us to investigate this area. In this study we examined the serum ascorbic acid and iron levels of patients with brain tumours and measured serum and tissue lipid peroxide levels in these patients.

MATERIALS AND METHODS

This study was carried out on 30 patients with brain tumours between the ages 20-65. The control group included 30 healthy people with similar age variations. Brain tumours and surrounding tissues which had been removed surgically were washed in saline solution and homogenized with cold 1.15% KCl to make 10% homogenates.

Tissue lipid peroxide was determined using the method of Uchiyama and Mihara (24). Briefly, 3 ml 1% phosphoric acid and 1 ml 0.5% thiobarbituric acid (TBA) were added to a 0.5 ml homogenate and the mixture was kept in a water bath a 95 C for 45 minutes. The coloured reaction product was extracted with 4 ml n-butanol and the difference of absorbances at 535 and 520 nm was recorded. The breakdown product of 1,1,3,3 tetraethoxypropane was used as standard and the values were expressed as nmol malondialdehyde / gram (MDA/g) tissue.

Serum lipid peroxide levels were measured using the method described by Yagi (27). 20 ul serum was mixed with 4 ml N/12 sulphuric acid and then 0.5 ml 10% phosphotungstic acid was added. After standing at room temperature for 5 minutes, the mixture was centrifuged at 3000 rev/min for 10 minutes. The supernatant was discarged and the sediment was mixed with 2 ml N/12 H_2SO_4 and 0.3 ml 10% phosphotungstic acid before centrifugation was repeated. The sediment was suspended in 4 ml distilled water and then 1 ml thiobarturic acid (TBA) reagent (a mixture of equal volumes of 0.67% TBA aqueous and glacial acetic acid) was added and heated the 95 C for 60 minutes in an oil bath. After cooling with tap water, 5.0 ml n-butanol was added and the mixture was shaken vigorously. After centrifugation an 3000 rev/min for 15 minutes, the n-butanol layer was taken for gluorometric measurement at 553 nm with excitation at 515 nm. By taking the fluorescence intensity as f and that of the standard solution, obtained by reacting 0.5 nm tetramethoxypropane with TBA, as F, the lipid peroxide (Lp) level was expressed in terms of malondialdehyde: Lp:0.5.f/F.1.0/0.02=f/F.25 (nmol/ml serum)

Serum ascorbic acid (AA) concentrations were determined using a modified 2,4-dinitrophenylhydrazine method (17). The results were expressed as milligrams AA/dl.

Serum iron was determined using the protein precipitation method; values were expessed in ugr/d (9).

The student's t test was employed for statistical comparison of the values.

RESULTS

The serum lipid peroxide, ascorbic acid and iron levels for patients with and without brain tumour are shown in Table I.

Results of peroxide values of brain tumour and surrounding tissue are shown Table II.

Tablo I : Comparison of Serum Lipid Peroxide, Ascorbic Acid and Iron Levels of Patients With and Without Brain Tumours.

Patients	Lipid Peroxide (nmol/ml)	Ascorbic Acid (mg/dl)	İron (ug/dl)
With tumours (n=30)	7.28+1.99a	0.32+0.095	95.27+ 11.58
Without tumours (n=30)	3.66§0.58	0.69+011	91.50+ 10.46
Significance	p<0.001	p<0.001	p<0.05

a Means + SEM

Table II : Comparison Between Levels of The Lipid Peroxide of Tumour Tissue and Surrounding Tissue, in Patients With Brain Tumours

Patients	Lipid Peroxide (nmol/g.tissue)
Brain tumour tissue (n=10)	17.80+89.4a
Surrounding tissue (n=10)	59.00+24.43
Significance	p<0.001)

a Means + SEM

DISCUSSION

Free radicals are formed during the normal metabolism of aerobic cells and free radical damage may cause some pathological states by either enhancing the production of the radicals or decreasing the antioxidative deferce mechanisms of the tissues (12,20,21,22).

Free oxygen radicals and lipid peroxides are short-lived products and difficult to measure directly. Malondyaldehyde on the other hand, is a more stable and long-lived degradative product of lipid peroxide and is often assayed as reflecting lipid peroxidation level (13,22).

When the fored lipid peroxides accumulate to a certain degree they leak from the tissue into the bloodstream and cause an increase in lipid peroxide levels in the serum. According to Yagi, the blood lipid peroxide level often correlates with the severity of the disease (27).

In this study, we compared serum and tissue lipid peroxide levels with brain tumours and found that the serum lipid peroxide levels of patients with brain tumours were significantly higher than those of the control group (p<0,001). The serum iron levels were

also found to be high in these patients though the results were not statistically significant.

Previous studies have concluded that lipid peroxidation is significantl decreased in tumour cells and surrounding tissues compared with the corresponding normal tissues (3). Peroxidative activity appears to be inversely related to the growth rate of the tumours. It is suggested thad the low susceptibility of tumour membranes to peroxidative agents may be a factor responsible for the high mitotic activity of these tissues (1,4).

Iron plays an important role in the biochemistry of oxygen species. It catalyses the Fenton reaction, can react with oxygen or superoxide anion and can participate in chain propagation lipid reactions Both Fe(II) and FE(III) are important in these processes. Extracellular iron is complex (transferrin, lactoferrin, haemoglobin/haeme) although free iron can be released at low pH. The brain contains large amounts of ferritin which is distributed heterogeneously (12,19). Rehncrona et al investigated the peroxidation of cortical phospholipids in vitro using Fe^{+2} and ascorbic acid stimulation (16). According to their reporte Fe^{+2} is an important pro-oxidant within the system.

Watson et al. reported the association of free radical processes with incomplete brain ischaemia in cat brain using decreased ascorbate levels as an indicator (26). In their report Flamm et al. found decreased tissue concentration ofascorbic acid during prolonged incomplete brain ischamia and claimed that this consumption of naturally occurring scaverger was due to pathological free radical reactions (6).

It is now well established that oxygen free radicals are generated in the ischaemic reperfused tissues (5,8,18,29).

Consequently, we believe that in patients with brain tumours the pressure caused by tumour tissue results in ischaemia of the surrounding tissue which causes an increase in lipid peroxidation and serum lipid peroxide level.

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