AN INHIBITOR OF LIPID PEROXIDATION IN DEVELOPING HUMAN FOETAL BRAIN

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Summary

Supraependymal cells are phagocytic cells which prevent the membrane lipid damage by removing the superoxide radicals through superoxide dismutase (SOD) and catalase enzyme systems. Aim of this study was to find out the correlation between the increase in number of supraependymal cells with increasing age and the change in lipid peroxidation in human foetal brain.

Scanning electron microscopy (SEM) of vagal triangle of fourth ventricle and lipid peroxidation studies were done on fresh aborted human fetal brain. Brain homogenates were shaken and mixed with equal volume of 10% trichloro acetic acid (TCA) solution .The resultant thio-barbituric acid reactive products were estimated in the protein free supernatant by the method of Wilbur, calculated as nanomoles of malonaldehyde. Proteins were estimated by the Folin-Lowry method (1951).[1]

Considerably high amounts of lipid peroxides were produced by fourth ventricle of human fetal brain upto 23rd week. During 26th and 27th week, the formation of lipid peroxides suddenly dropped with simultaneous appearance of a few supraependymal cells. The lipid peroxidation was further reduced to approximately half during 34th and 35th week as compared to 22nd and 23rd week indicating the presence of an inhibitor of lipid peroxidation, accompanied by the development of numerous supraependymal cells.

The present study indicates that there is a correlation between the lipid peroxidation activity and the number of supraependymal cells of the brain as their appearance proportionately decreased the formation of lipid peroxides.

Key Words: Lipid Peroxidation- Developing Brain-superoxide –electron microscopy-antioxidant

Running Title: Inhibitor of Lipid Peroxidation in Developing Human Foetal Brain

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Introduction

Due to the high energy demand of brain cells, the brain is equipped with an excessive vascular supply carrying oxygenated blood. The lipid contents of brain being very high shows a very high rate of lipid peroxidation which is a causative factor for membrane damage.[2-4] Among these, peroxy radicals derived from PUFA has special significance because it is the most common indicator of processes producing free radical in living system and causative factor for membrane damage .The process of lipid peroxidation is divided into three stages, initiation, propagation and termination.[5]To prevent this process, some naturally occurring antioxidants and inhibitors are present which are produced by the cells in some tissues. Antioxidants fall into two classes:

1. Preventive antioxidant which reduces rate of chain initiation like catalases, peroxidases that reacts with ROOH and chelators of metal ions such as EDTA and DTPA. *In vivo*, the principal chain breaking antioxidant is SOD , it acts in aqueous phase to trap superoxide free radical (O_2^{---}). Urate and vitamine E acts in lipid phase to trap ROO° radical. Peroxidation is catalysed *in vivo* by heme compounds and by lipoxygenase found in platelets and leukocytes.

2. Chain-breaking antioxidants which interfere with chain propagation..[6,7]

Macrophages and phagocytic cells are efficient in peroxidative metabolism.[8,9] They act as scavengers and prevent the membrane lipid damage by removing the superoxide radicals through superoxide dismutase (SOD) and catalase enzyme systems.[10-12]

Supraependymal cells are considered to be the phagocytic cells in human brain. [13] However, their role during the development of human foetal brain is not fully understood and the studies on the fourth ventricle are mostly unexplored. The present studies were, therefore, conducted to find out the developmental changes in the fourth ventricle of brain during the early stages of the foetal development.

Materials and Methods

Human fetuses were collected from the patients admitted in the local hospitals at Lucknow, India, within 2 to 4 hours after being aborted. Only fresh fetuses having a shining translucent appearance and falling in the middle two weeks of every month were selected. The foetal age was calculated by measuring crown rump (CR) length .[14]Brain was removed from the cranium and the fourth ventricle was excised out and processed separately for scanning electron microscopy (SEM) and lipid peroxidation studies.

Scanning Electron Microscopy (SEM)

The vagal triangle region of fourth ventricle of foetal brain was fixed in the Karnovsky's fixative, washed in phosphate buffer (0.2M, pH –7.4),treated with osmium tetroxide for two hours, dehydrated through graded ethanol and amyl acetate, critical point dried in a Balzer's critical point drier, sputter coated with Polaron E-5000 Sputter Coater and finally scanned through scanning electron microscope, model 180(Phillips).[15]

Lipid Peroxidation

The tissue was washed in chilled 0.15M potassium chloride (KCl) solution. The extraneous adhering material was removed and the tissue was dried on a filter paper and weighed. A 10% (w/v) homogenate of brain tissue was prepared in chilled 0.15M KCl solution using a Potter Elvehjem type homogenizer giving ten gentle strokes.

Suitable aliquots of brain homogenates were incubated at 37ºC for one hour in a metallic shaker and then equal volumes of 10% trichloro acetic acid (TCA) solution added. The thio-barbituric acid reactive products were estimated in the protein free supernatant by the method of Wilbur, calculated as nanomoles of malonaldehyde .[16] Proteins were estimated by the method of Lowry, Rosebrough, Farr & Randell.[1]

Results

During 14 to 15 weeks of age, the floor of fourth ventricle in the vagal triangle region showed solitary and groups of cilia without any supraependymal cell (Fig.1).

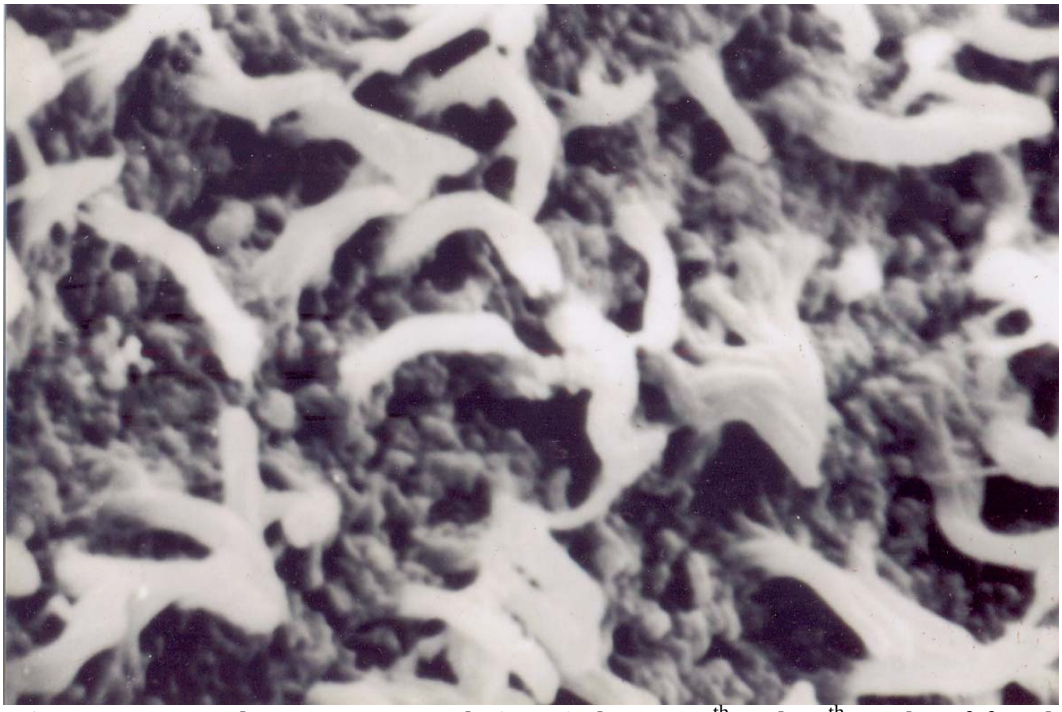

Fig.1: Scanning electron micrograph (SEM) during 14th and 15th weeks of foetal brain development showing floor of fourth ventricle in the vagal triangle region showed solitary and groups of cilia without any supraependymal cell (2300 x).

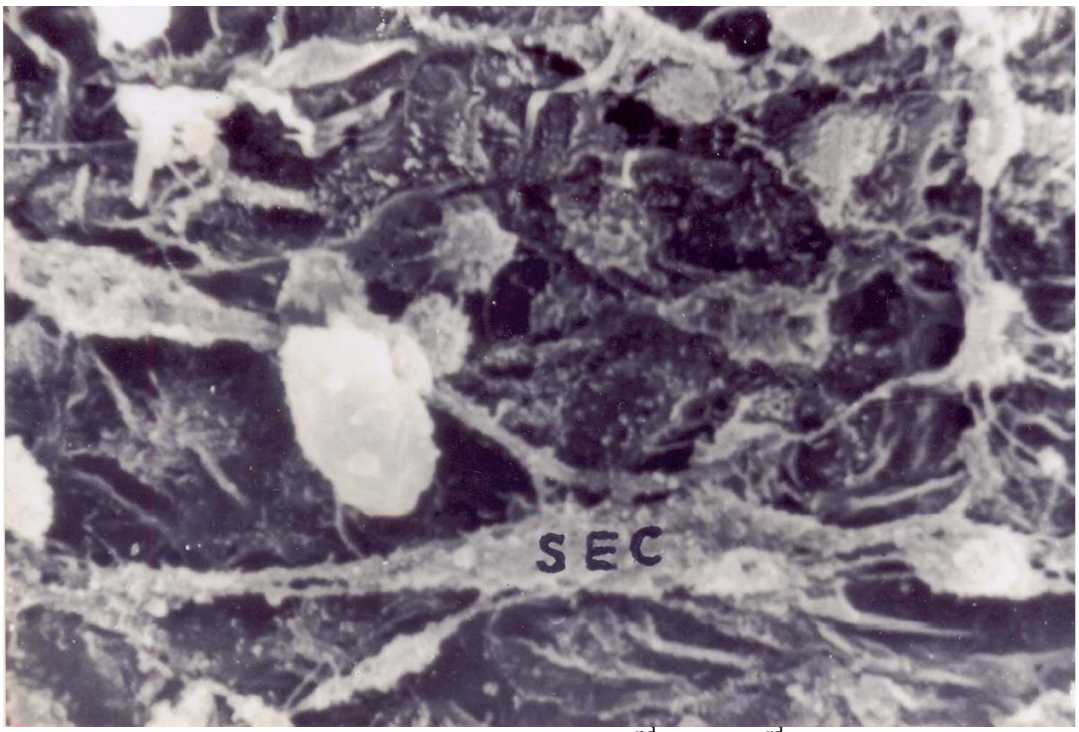

Fig.2: Scanning electron micrograph during 22nd and 23rd week of foetal brain development showing presence of ciliary groups and solitary cilia in different portions from surface of the ependymal cells (vagal triangle region, 3400 x)

Between the age of 22 to 23 weeks the presence of ciliary groups and solitary cilia in different portions from surface of the ependymal cells were apparent (Fig.2). Till this stage there was no indication of the presence of supraependymal cells.

However, at the age of 26 to 27 weeks, supraependymal cells (cells lying on the surface of ependymal cells) started appearing with pseudopodia in different directions (Fig.3). The supraependymal cells were of different size and shape. Each cell showed single or multiple pseudopodia intermingled with the pseudopodia of adjoining cells. The surface of these cells further revealed the presence of microvilli.

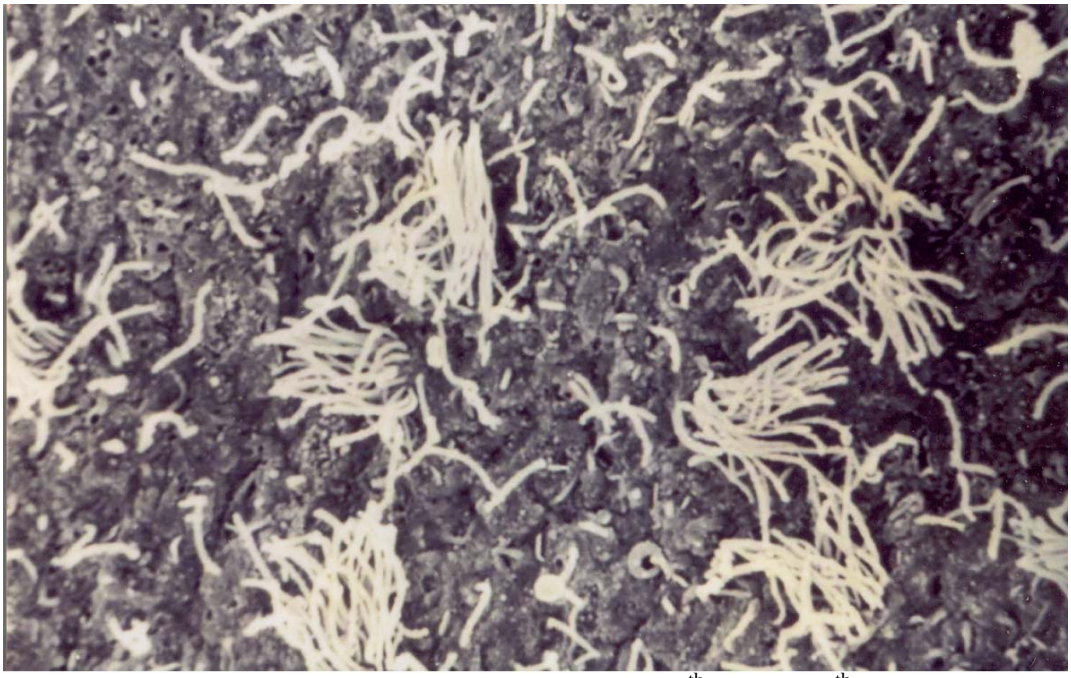

Fig.3: Scanning electron micrograph during 26^{th} and 27^{th} week showing supraependymal cells started appearing with pseudopodia in different directions (vagal triangle region, SEC=supraependymal cell,1250 x)

The vagal triangle at the age of 34 to 35 weeks showed non-cilliated polygonal ependymal cells. The number of supraependymal cells was considerably increased but the processes reduced with only few microvilli of small size (Fig.4).

The lipid peroxidation was substantial and increased gradually till reached maximum during 22^{nd} and 23^{rd} week. It significantly decreased between 26^{th} and 27^{th} week and attained a value nearby half the maximum during 34^{th} and 35^{th} week (Fig.5).

Fig.4: Scanning electron micrograph during 34th and 35th week showed non-cilliated polygonal ependymal cells. The number of supraependymal cells was considerably increased but the processes reduced with only few microvilli of small size (vagal triangle region, 4000x)

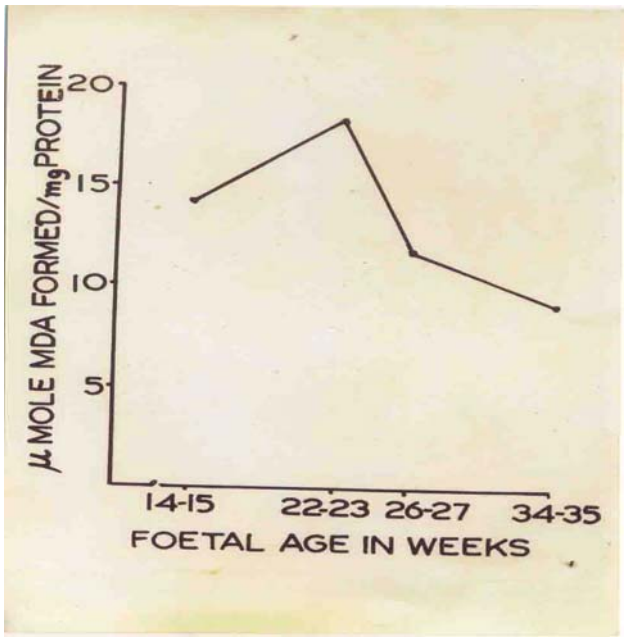

Fig.5: Graph showing lipid peroxidation in foetal brain at different age intervals.The lipid peroxidation increased gradually till reached maximum during 22nd and 23rd week. It significantly decreased between 26th and 27th week and attained a value nearby half the maximum during 34th and 35th week.

Discussion

Lipid peroxidation is considered to be a destructive process leading to multiple injuries to cell membrane and tissues *in vivo* causing cancer, inflammatory disease, atherosclerosis and aging .[6] The peroxidation in brain increases with age.[17] The tissues, therefore, may have a protective mechanism which might regulate lipid peroxidation through antioxidants or inhibitors. The major cellular antioxidants are vitamine C, E, carotenoids, selenium, catalases, peroxidases, glutathione peroxidase and the mechanisms includes:

A. Direct interaction with oxidants or oxidizing agents by ascorbic acid, glutathione and other reducing agents.
B. Scavenging of free radicals and singlet oxygen by vitamin-E, ascorbic acid, carotenoids, superoxide dismutase and other scavengers.[18,19]
C. Reduction of hydroperoxides by glutathione peroxidase and catalases.
D. Binding or removal of transition metals by ferritin, transferrin, ceruloplasmin, albumin and other chelators.
E. Separation or prevention of reactive oxygen species and other factors from reaching the specific site of action or reacting with essential cellular components by membrane barriers.
F. Repair of resulting damage by dietary nutrient and metabolic activities.[18-20]

During the present investigation, it was observed that lipid peroxidation gradually increased and reached maximum during 22nd and 23rd week of developing human foetal brain. Upto this stage there appeared no supraependymal cell in any part of the fourth ventricle including vagal triangle. However, the lipid peroxidation suddenly dropped to a considerable extent during 26th and 27th week with simultaneous emergence of a few supraependymal cells. Supraependymal cells are essentially the phagocytic cells in brain.[13,21] The phagocytic cells and macrophages are active in removing the superoxide radicals through their highly developed peroxidative system consisting of SOD and catalase enzymes.[22,10-12] As a challenge to oxygen, the superoxide dismutase of mitochondria and catalase in phagocytes increase significantly converting the partially reduced products of oxygen metabolism to less reactive molecules, thus acting as a scavenger of superoxide radicals and affecting the lipid peroxidation.[23,24]

The lipid peroxidation was further reduced and during 34th and 35th week, it reduced to approximately half of the maximum found during 22nd and 23rd week. This was accompanied with a considerably high increase in the number of supraependymal cells. The surface morphology of these cells was, however, altered which may be a functional adoption. The present study suggests that there is a correlation between the lipid peroxidation activity and the number of supraependymal cells of the brain as their appearance proportionately decreased the formation of lipid peroxides.

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ABBREVIATIONS:

PUFA-Polyunsaturated fatty acid

EDTA –Ethylene diamine tetraacetate

DTPA- Diethylenetriaminepentaacetate

SOD-Superoxide dismutase

CR length-crown rump length

SEM-scanning electron microscopy