CHAPTER IV

GENERAL DISCUSSION

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As stated in section I of mortality and Introduction the results of section I were used to decide the H_2O_2 (0.5 mM) and vitamin C (3 mg) doses so that H_2O_2 mediated alterations in early hrs of the embryo during differentiation and development of the brain can be studied. In the histological section, the alterations revealed that H_2O_2 treatment stopped the cell migration and associated growth and development of brain vesicular three regions viz. Ependymal, Mantle and marginal layers. The stunted axial and dendritic extensions could be observed which opened up the intracellular spaces.

The cavity and tissue Kinetics studied in chick embryo during brain growth (24-120 hrs) showed brain enlargement 8.5 times, cavity expansion of 9.8 times and tissue growth 7.6 times (Pacheco *et al.,* 1985). This kinetics was retarded by 60% in H_2O_2 treated embryos but simultaneous treatment of vitamin C had altered this kinetics and remained similar to normal indicating there is abnormal growth of brain in presence of H_2O_2 + vitamin C (3 mg).

These results indicated that vitamin had mediated the removal of stability of brain growth and associated alterations but had not altered the GAGs distribution within the sites or in quantity indicating this effect free of any adverse alterations to brain.

It has also been shown that during 48-96 hrs of development brain cells were incapable to differentiate in culture (Tonzet *et al.,* 1975) and other growth factors *in vivo*. In present condition H_2O_2 mediated stability of differentiation was protected in presence of vitamin C (3 mg) indicating the potency of the cells to respond vitamin C (3 mg).

During early stages of development fibroblast growth factor -2 and other isoform of these factors from cerebrospinal fluid regulates the neuroepithelial cell behavior i.e. cell protects and neurogenesis proliferation. *In vivo* and *in vitro* indicating complementary regulation by neuronal and extra-neuronal factors (Martin *et al.,* 1990). In present observations, also vitamin C (3 mg) treatment given simultaneously protected the normal development of brain and death of embryo. Since H_2O_2 induced abnormalities and survivals and death were observed (Section I). Thus, vitamin C directly or through its mediated metabolism stimulated and

regulated the growth of brain under stressed condition in vivo indicating neuronal cells stimulatory potency of vitamin C.

In our other simultaneous work it has been shown, that TCA-TBA reactive substances were increased associated with the alterations in glutathione content, which under the influence of vitamin C showed alterations that managed the H_2O_2 generated free radicals (Toraskar, 2008). These results are supported by the earlier observations where it has been shown that glutathione peroxidase activity is involved in the neuronal defense against H_2O_2 toxicity on 7^{th} and 21^{st} day of development (i.e. 144 hrs of development) of chick embryo (Wakai and Hirokawa, 1981).

The alterations in GAGs studied in early development of chick embryo included neutral GAGs (NGAG), acidic GAGs (AGAG), sulphated GAGs (SGAGs) and hyaluronan glycan (HA) and sialic acid (SA).

NGAG altered in trace to intense quantity in different experimental protocol. H_2O_2 increased NGAGs as a response to stress such type of response was also observed in neuronal cells of chick embryo in culture (Arisaka *et al.,* 1995). This seems to be the most emidiate response to the stress, which was noted on short duration exposure to H_2O_2.

This response was managed by vitamin C when given simultaneously by the dose used.

The quantitative and location wise alterations in nonsulphated AGAGs were hyalurons the glycans which were restricted to the extending tips of axons and dendrites, which were directly involved in the migration of the cells. H_2O_2 mediated stunted growth the axons and dendrites accumulate Hyaluronan at the tips which were not removed or digested indicating the stabilization of migration and associated growth. This effect was protected by vitamin C (3 mg) but it had not altered any of the localization or quantity in protective effect and acted as external regulatory factor.

Presence of SGAG in normal embryo itself had two levels of distribution at the early development phase (40-72 hrs) and late phase i.e. 72-96 hrs. In early phase, it was similar to Hyaluronan but was extended on the remaining cell surfaces of extensions, which may be due to the extracellular domain of syndecan-3 (heparin sulphate) which functions as receptor for fibrocyte growth factor, which is regulator of early chick brain development (Gould *et al.,* 1995).

While in late phase (72-144 hrs), SGAGs were concentrated in cytoplasm. Their localization was not altered due to H_2O_2 treatment but with stunted cytoplamsic extensions, they were aggregated as rubber caps, which were stained intensely (due to accumulation). Vitamin C (3 mg) managed the H_2O_2 induced alteration and kept distribution and amount as in normal. Thsese results indicate the transitory phase SGAG which are found in normal brain after 144 hrs when growth phase and vesicular dialation go hand in hand which are the consequences of the hydrophilic pressure created within the vesicles by SGAG viz. dermatin sulphates, chondroitin sulphates, heparin sulfate, which were contributed further by neuronal and matrix cells.

The hrs at which the chick embryo had been studied in present project appeared to be phase where cells are preparing for the growth phase by production of SGAGs, which will help to grow the vesicles of brain, which occurs after 144 hrs of development. This phase had been stabilized by H_2O_2 without other alterations and vitamin C protected it by not changing its status as observed.

Sialic acid studied is the important part of membrane gangliosides. Similarly it has been revealed that (Introduction) other transmembrane proteins involved in cell-cell adhesion. N-CAM are known to contain polysialation. Therefore, the alterations observed are sialic acid belonging to both of these molecules.

Its cell surface distribution in early chick embryo was trace to weak in amount but, on exposure to H_2O_2 it was increased significantly at cell surfaces indicating the stabilization of migration have affected the membranes by free radicals to reveal sialic acid present both in gangliocide and as N-CAM part.

PAS +ve sialic acid had been observed both at cell surfaces and cytoplasm in normal embryos, which was increased by H_2O_2 induced free radical stimuli. Vitamin C protected its distribution various sites and also its distribution at various sites and also in amount.

All these results indicated that the H_2O_2 induced free radicals seem to alter the configuration of sialic acid (in lipid glycoproteins) to expose them at cell surfaces. This seems to interfere with the cell migration and axoneuronal network building (Chia-Ron *et. at.*, 2000). But vitamin C seem to manage the H_2O_2 generated free radicals to maintain the normal developmental condition.

From all these results it can be stated that early development hrs of chick embryo involve glycans, glycosaminoglyacans in restricted sites and in

comparatively low amount. Sialic acid containing glycolipids and glycoproteins do not seem to expose sialic acid residues at exterior domains so that it is available for histological staining especially on the cell bodies. But they were moderately stained in migratory layer where cellular axonal network was present. But H_2O_2 exposure had influenced all those configurations to expose them at cell surfaces. The sialic acid content was proportionally increased intensity with increasing hrs of development.

SGAG seem to play role in late hrs and are not influenced their staining intensity whatever observed might be aggregation effect.

Vitamin C plays role without altering the normal status of brain including status of proteoglycans, glycans and sialic acid. Thus, it seems vitamin C play its role through influencing the metabolism, which play its role in free radical scavenging and are not influencing all the metabolisms that are related with the synthesis and metabolism of proteoglycans, glycans and sialic acid.