CHAPTER-V
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EFFECT OF ACRYLAMIDE ON CHICK EMBRYONIC HIPPOCAMPUS REGION OF BRAIN – A HISTOLOGICAL STUDY

Introduction

The environment plays a predominant role in the perception and causation of cancers. It includes various drugs, xenobiotics, radiation, viruses and dietary constituents. Cell transformation by chemical and physical carcinogens involves multiple steps with two phases, initiation involves changes in the genome and promotion stimulates cell division and in turn leads to malignant transformation or normal metabolism. Induction of cell transformation results in the elevation of enzymatic activities coupled with histological studies provides a reliable study for monitoring the severity of chemical carcinogenesis.

Acrylamide is a potential carcinogen to cause nervous system damage (Arisseto et al., 2007). Ingestion of foodstuffs containing acrylamide appears to be one of the most common methods of exposure for the general public. Average estimated intake of acrylamide from food sources ranged from 0.8 to 6.0μg/kg bw/day for short-term exposure and 0.3 to 0.8μg/kg bodyweight/day for long-term exposure (WHO, 2002; WHO, 2003). Children may be susceptible to food-borne exposure 2–3 times that of adults on a body weight basis (WHO, 2002; WHO, 2003). Based on evidence of early structural and functional damage, it was suggested that nerve terminals were of the primary sites to acrylamide action and that synaptic dysfunction and subsequent degeneration by acrylamide neurotoxicity.

Histopathological evidence of acrylamide induced peripheral neuropathy has been observed in rats receiving oral doses as low as 1mg/kg/day for 3months; the observed degenerative effects in peripheral nerve fibers at such dose levels have been shown to be completely reversible within a few months following the cessation of exposure. Considering all the facts experiments were conducted on chick embryos to study the effect of acrylamide on developing brain.
Objective:

To study histological changes of control, acrylamide treated 11th day old chick embryonic brain in a dose and time dependent manner.

Materials and methodology of this chapter were mentioned in the chapter “Materials and Methods”.

Results

Histopathological studies

The results of control and treated chick embryonic hippocampus region of brain histology studies were presented in the Figure-5.1 to 5.7. In these results the control and 0.1mg AC treated (24, 48 and 72h) hippocampus of brain showed normal cytoarchitectural Figure-5.1 and 5.2. The 0.2, 0.3mg AC treated showed mild degenerative changes in hippocampus region of brain tissue Figure-5.3 and 5.4, 0.4mg AC treated showed mild and structural damages Figure-5.5. The chick embryonic brain treated with 0.5mg AC at 24, 48h shows structural damages and 72h shows mild hemorrhages (lesion), structural damage shown in Figure-5.6. The 0.6mg AC treated showed structural damages at 24, 48h and 72h showing necrotic damage, formation of vacuole shown in Figure-5.7.

Figure-5.1: Histological analysis of brain section (11th day old chick embryo) under the light Microscope (10X) showing Normal architecture (NA) of Hippocampus region (Control).
**Figure-5.2:** 0.1mg AC treated 11th day old chick embryonic brain, A) 24h, B) 48h, C)72h showing Normal architecture (NA) of hippocampus region (no damage).
**Figure-5.3:** 0.2mg AC treated 11th day old chick embryonic hippocampus region., A) 24h, B) 48h showing no damage C) 72h showing mild damage (MD).
**Figure-5.4**: 0.3mg AC treated 11th day old chick embryonic hippocampus region, A) 24h, B) 48h C)72h-showing mild damages (MD).
**Figure-5.5:** 0.4mg AC treated 11th day old chick embryonic hippocampus region., A) 24h, B) 48h showing mild damage (MD). C) 72h showing structural damage (SD).
**Figure-5.6**: 0.5mg AC treated 11th day old chick embryonic hippocampus region, A) 24h, B) 48h structural damages (SD), C) 72h showing mild hemorrhages (MHG) (blue line), structural damage (SD).
**Figure-5.7**: 0.6mg AC treated 11th day old chick embryonic hippocampus region, A) 24h, B) 48h structural damages (SD), C) 72h showing necrotic damage (ND), (blue line), formation of vacuole (VC).
Discussion

Acrylamide may pose more than a neurotoxic health hazard to exposed birds which shall have signs of impaired neurological performance in central and peripheral nervous systems that include impaired motor function and muscle weakness. Acrylamide and its principal metabolite, glycidamide (epoxide), react with various biologically significant targets. The AC enhances the production of reactive oxygen species and potentially affects brain. Two compounds, glutathione and vitamin-c plays a critical role in protecting cells from oxidative stress and xenobiotics, in the central nervous system (Dringen, 2000) with a particular emphasis on the mechanism by which neuronal GSH synthesis is regulated. In brain GSH depletion has leads to an increased production of superoxide, hydroxyl and \( \text{H}_2\text{O}_2 \) radicals (Gupta et al., 2000). The intracellular GSH pool is important for limiting oxidative stress–induced neuronal injury. GSH as neuromodulator is required for cell proliferation and neuronal differentiation (Sagara and Makino, 2008). In fact, brain GSH is reduced in some age-related neurodegenerative diseases. For example Alzheimer’s disease (AD) is a leading age-related neurodegenerative disease, which pathogenically involves oxidative stress (Christen, 2000). The depletion of total glutathione is one of the earliest biochemical change seen in the Parkinson’s disease (PD). The primary neuropathological feature of PD is the loss of dopaminergic neurons of the substantia nigra pars compacta (SN) that projects to striatum (Dauer and Przedborski, 2003). The ascorbate content of developing rat brain doubles in late pregnancy, which fits well with results of studies showing that ascorbate enhances the differentiation of embryonic stem cells into neurons in a culture medium (Lee et al., 2000). This is associated with increases in expression of genes involved in neurogenesis, maturation, and neurotransmission (Shin et al., 2004). Ascorbate at 200\( \mu \text{M} \) concentration induces synaptic maturation of the neurons, based on findings increased number of miniature excitatory post-synaptic currents in the cultured neurons.

Chick embryos have been used in the past for several years to investigate the effect of environmental chemicals and radiations on developmental effects, morphogenesis, etc. Avian embryonic tissues are characterized by the accumulation of polyunsaturated lipids during the final week of in ovo development. During development the embryo brain differs from other tissues in being a highly aerobic and
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totally oxygen-dependent tissue and may be especially at risk from free radical attack, because this tissue is characterized by a low content of natural antioxidants and generates a greater amount of free radicals per gram of tissue than any other organ (Reiter, 1995).

Acrylamide induced free radical production in neurons has been suggested to be responsible for the oxidative brain damage. This fact proves the intensification of lipid peroxidation in patients with central nervous system (Levchenko and Demchuk, 1999) as obtained similar results in patients operated on for a brain tumor glioblastoma multiforme and meningioma. In the opinion of the authors the growth of a tumour may be a cause of the disturbance of the equilibrium of redox reactions intensification. Louw et al., (1997) showed an increase in the lipid peroxidation process in patients with astrocytoma. Some reports (Arora et al., 1996; Landolt et al., 1994) indicate that a higher level of oxygen-derived free radicals may be responsible for the intensification of the proliferation of neoplastic cells, which is followed by the development of a tumour. Arora et al., (1996) showed that the addition of a well-known free radical scavenger as lazaroids to brain tumour cell cultures leads to inhibition of neoplasm growth. Also our (Figure-5.1 to 5.7) results showed increase degenerative changes, mild damage, hemorrhages, structural damages, necrotic damage, and formation of vacuole shows damage in the hippocampus region of chick embryonic brain tissue.

On the basis of results obtained I conclude that acrylamide cause disturbances in the oxidative status and the effect was pronounced with the high doses, indicated enhanced brain damage risk during exposure to acrylamide. In chick embryo brain GSH depletion leads to increased production of superoxide, hydroxyl radicals, and also H₂O₂. The intracellular GSH pool is important for limiting oxidative stress–induced neuronal injury and suppression of cell proliferation and neuronal differentiation. Because of reduction of glutathione and vitamin-c the free radical scavenger’s role was suppressed and histological analysis indicates acrylamide may probably involved in propagation of toxicity to hippocampus region of chick embryonic brain, hippocampus damage impairs memory for the order of a series and inhibits the nerve impulse transmission.