INTRODUCTION

1. INTRODUCTION

1.1 Glyoxalase System

Biology is a molecular science as the biological systems are built of molecules. It is therefore logical to believe that all biological reactions have to be molecular. While proposing the electronic theory of cancer, Szent–Gyorgyi (1967) introduced the glyoxalase as a vital molecular system playing an important role in transformed cell. Indeed his theory, was centered around the glyoxalase system. He described cancer cells as a car without brakes on the steep slope. He suggested the significance of searching and understanding the brakes, for the affective application. The glyoxals were identified for this possible role, since these compounds, carbonyl in nature, had an ability to bind reversibly with proteins to form the charge transfer complexes leading to arrest of cell division. Therefore, the levels of free glyoxals in cells were considered to be crucial and needed to be regulated. Szent–Gyorgyi assigned this function to the enzyme known as glyoxalase.

The glyoxalase system is considered to be vital for biological functions (Thornalley, 1990, 1993, 1993a). An active glyoxalase system is known to be present throughout embryogenesis, tissue maturation and persists until cell death (Mclellan and Thornalley 1989). Although, this system came into lime light due to the electronic theory of cancer, it was independently discovered by Dakin and Dudley (1913) and Neuberg (1913). It was considered as a major metabolic pathway for conversion of glucose to D-lactate. GSH (glutathione) was demonstrated to be essential co-factor for its activity (Lohman, 1932). Quastel and Jovett (1933) showed the spontaneous formation of intermediate complex between GSH and methlyglyoxal, which is converted to D-lactate by glyoxalase system. Further studies by Racker (1951, 1954) lead to conclusion that the glyoxalase system involves two discrete steps catalyzed by two enzymes. It is now well established that the glyoxalase system consists of glyoxalase I (EC 4.4.1.5, lactoylglutathione lyase), glyoxalase II (EC 3.1.2.6, hydroxyacyl glutathione hydrolase) and GSH as a co-factor. Glyoxalase I catalyzes the isomerization of hemithioacetal formed non-enzymatically from GSH and a-oxoaldehydes to S-2-hydroxyacyl

glutathione derivatives. The glyoxalase II catalyzes the hydrolysis of S-2-hydroxyacyl glutathione derivatives to aldonates and generates GSH consumed in glyoxalase I catalyzed reaction (Uotila, 1989, Thornalley, 1993). The glyoxalase has wide range of substrate specificity towards glyoxal compounds.

The electronic theory of cancer generated tremendous interest in the glyoxalase system as a regulator of cell division. Subsequently, a large body of evidences was accumulated from melage of experimental system including both plants and animals leading to accept its involvement in cell proliferation and differentiation. Enhanced activity of glyoxalase I in rapidly proliferating cell lines (Nathan *et al.*, 1993), growth arrest in tumor cells induced as a result of inhibition of glyoxalase system (Allen *et al.*, 1993a), inverse co-relation between glyoxal level and the activity of glyoxalase I in Douglas fir (Smith and Johnson, 1981) and direct co-relation between glyoxalase I and mitotic index in different system have been reported.

Moreover, it was suggested that the glyoxalase system also carry out detoxification of cytotoxic a- oxoaldehyde which are produced in glycolysis bypass, acetone metabolism, threonine catabolism and peroxidation process (Kalapos, 1994) to non toxic aldonates. The a- oxoaldehyde are known to be involved in mutagenesis, inactivation of proteins and formation of crosslinked adducts similar to advanced glycation end products which contributes towards various pathophysiological conditions (Thornalley, 1998).

Meanwhile, evidences started emerging in support of showing responsiveness of the glyoxalase system towards environmental stress conditions such as salt stress in plants (Espartero *et al.*, 1995; Veena and Sopory, 1999; Jain *et al.*, 2002), tumor implantation in animals (Strzinek *et al.*, 1970) and glycol load to bacteria (Freedberg *et al.*, 1998). The realization of its ubiquitous and extant nature through all living organism suggested the need to examine the response of glyoxalase system under various stress conditions including effect of ionizing radiation. The evolution was accompanied by ionizing radiation if not guided. Since the glyoxalase system was retained through evolution, it might have experienced the irradiation and perhaps responded to the same.

1.2 Radiation-induced Stress

Ionizing radiation is detrimental to life. The biological effects of radiation are the end product of a long series of phenomena which are set in circular motion by the passage of radiation through the cell. Radiation damage involves either direct or indirect depend on the whether the energy is absorbed by the tissue molecules or the surrounding molecules. Since cells consist of about 60-80% of water, to a great extent the biological effects are mainly mediated through the action of radiation on water. As a cell contains about 10^{13} water molecules, a dose of 1 Gy (= 0.6×10^{18} eV Kg^{-1}) may be expected to cause 2×10^5 ionizations in a cell. These ionic product (ion pairs) are very active and get converted into free radicals. Radiolytically formed free radicals and molecular products (reaction 1) through decomposition of water, reacts with biomolecules and brings about change in structure and function. Therefore free radicals formation processes are considered to play very important role in detrimental effects of ionizing radiation.

$$H_2O \longrightarrow {}^{\cdot}OH, H^{\cdot}, e^{-}_{aq}, O^{\cdot -}_2, H_2O_2, H_2 \text{ etc.} \quad \ldots\ldots \quad (1)$$

These free radicals generated in the tissues as a result of exposure of animals to radiation, are likely to cause oxidative stress.

Recently, on the basis of findings of the effect of ionizing radiation on the xanthine oxido-reductase system, peroxidative damage and lactate dehydrogenase, it has been hypothesized that free radical generating systems could be activated in the radiolyitcally damaged cells. Therefore, free radicals formation is expected to continue in post irradiation period (Kale, 2003) and animals would experience oxidative stress for longer time even after exposure to radiation.

1.3 Glyoxalase System and Radiation

Radiation sensitivity of cells is known to be directly proportional to their reproductive activity and inversely proportional to the degree of differentiation. The glyoxalase system is known to be associated with the proliferation and differentiation processes. A direct relationship between the glyoxalase system and cell cycle has been shown (Hooper *et al.*, 1988a, 1988b).

The redox status is also considered one of the determinants of radiation sensitivity of biological system (Sun, 1998; Agrawal *et al.*, 2001a, 2001b; Bravard *et al.*, 2002). Glutathione (GSH) plays an important role in the redox state of cells. It is the most ubiquitous and abundant of non protein thiols. It has redox potential of around (-) 230 mV which makes GSH the primary nucleophile in the cell, capable of transferring electrons in variety of molecules including oxidants. It effectively scavenge most carbon, nitrogen and oxygen centred free radicals. GSH which is co-factor/co-enzyme in the glyoxalase system (Revez *et al.*, 1984). Many investigators (Astor *et al.*, 1984; Louie *et al.*, 1985; Vander Schans *et al.*, 1986; Vos *et al.*, 1986; Sauder *et al.*, 1991) have found that depletion of intracellular GSH increased radio-sensitivity of cells. GSH depletion leads to less detoxification of radiation–produced peroxyl radical or hydrogen peroxides (Biaglow *et al.*, 1984, 1986). In view of above facts the glyoxalase system seems to be intimately linked with the radio-sensitivity of the biological system.

Since a direct relationship among the glyoxalase system, cell division/cell cycle and differentiation is well established, the radio-modulation of this system is likely to have serious biological implications. Moreover, the glyoxalase system is closely linked with cancer, radiation effect on this system may have significance in radiation therapy. Therefore it was essential to understand radiation response of the glyoxalase system.

Considering the possible significance of glyoxalase system from radiation therapy point of view, an attempt has been done both at molecular and biochemical level which highlights importance of this system in radiation induced oxidative damage which may have significance in cancer therapy.

1.4 Aims and Objectives

From our earlier work, the modulatory effect of ionizing radiation on the glyoxalase system is now well established (Sharma and Kale, 1993, 1994, Choudhary *et al.,* 1999, Agrawal *et al.,* 2001a, 2001b; Tiku and Kale 2001). These findings are suggestive of the involvement of glyoxalase system in repair/regeneration of radiolytically damaged tissues. Further, the results are also suggestive of its antioxidant role against the oxidative stress induced by irradiation. Importantly, our findings are also suggestive of the close link between the glyoxalase system and radio-sensitivity of cells.

ıt work, we are interested to probe further and find out whether ionizing radiation ɛs transcription of the glyoxalase system.

'or this purpose, we have used glyoxalase I as its radio-modulation was found to stent. Therefore, the effect of different doses and dose-rates on modulation of se I mRNA in the liver of mice has been examined. The adaptive response and e effect on the glyoxalase I transcription was also studied. Furthermore, we have ıted whether transcription correlates with the specific activity of glyoxalase I and se II. Besides this, we have also evaluated the peroxidative damage and response , GST and catalase as representative of antioxidant system under similar ɛntal conditions.