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RESEARCH ARTICLE

INFLUENCE OF TAURINE AND GLUTATHIONE ON THE CYTOMETRY MEASUREMENT OF LIVER CELLS AND ITS NUCLEUS OF THE MERCURY INTOXICATED RATS

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ABSTRACT

The aim of the present study is to investigate the effect of taurine and glutathione on mercury intoxicated liver tissue of rats, *Rattus norvegicus*. The rats were divided into six groups, keeping group I as a healthy control. Rats of groups II received mercuric chloride orally at the rate of 5 mg per kg body weight daily for 30 days. Group III and IV of animals received Taurine and Glutathione followed by mercuric chloride treatments. Group V and VI of animals received Taurine and Glutathione alone treatments. In the present study, cytometric measurement of liver cells and its nucleus size were observed. Due to the mercury intoxication the cell and nucleus size of hepatocytes were increased in the liver tissue. After the scheduled treatment, the mercury intoxicated rats again dosed with taurine and glutathione (5 mg per kg body weight) for 15 days respectively. The result shows that the increased size of the cell and nucleus were decreased to near normal size. Concomitant administration of taurine and glutathione were found to reduce the cell and nucleus size of liver cells is mainly due to their hepatoprotective action.

INTRODUCTION

Mercury is released naturally from the earth's crust by mining, fossil-fuel combustion and other industrial activities. In the non-occupationally exposed population, however, dental amalgam is typically the major source of mercury (WHO, 1989). Methyl mercury is recognized as a hazardous environmental pollutant (Jagadesan, 2004; Takeuchi *et al*., 1962). Mercury is found in the environment in three basic states elemental mercury or mercury vapor, inorganic mercury and organic mercury (ethyl-methyl alkyl or phenyl mercury). Each form has an individual toxicological profile and metabolic fate (NRC, 2000). Taurine (2-amino ethane sulfonic acid) is the major intracellular free β-amino acid, which is normally present in most mammalian tissues (Chesney, 1985). It is not utilized in protein synthesis, but rather is found free or in simple peptides. It plays various important physiological roles including osmoregulation, bile acid conjugation modulation of the proliferation; viability and prevention of oxidant induced injury

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in may tissues (Chesney, 1985; Huxtable, 1992; Redmond *et al*., 1996; Sankar Samipillai, 2004; 2005). The beneficial effects of taurine as an antioxidant in biological systems have been attributed to its ability to stabilize biomembranes (Wright *et al*., 1986; Jagadeesan and Sankar Samipillai, 2007; Sankar samipillai and Jagadeesan, 2005;Sankar Samipillai *et al.,* 2010). Scavenge reactive oxygen species (Wright *et al*., 1985) reduced the production of lipid per oxidation end products (Huxtable, 1992). Glutathione (GSH) is a low molecular weight sulfhydryl-containing compound in mammalian cells (Janaky *et al*., 1999). GSH is an essential tripeptide made up of the amino acid such as glutamate, cysteine, and glycine (Sankar Samipillai and Jagadeesan,2009; Huxtable, 1986). The glutathione (GSH) is a cellular thiol, which is present in all mammalian tissues (Cooper, 1997). It provides a reducing milieu for the maintenance of protein thiols and antioxidant, reduction of ribonucleotides and protection against oxidative and free radicals-mediated damage and other types of toxic injury (Deleve and Kaplowitz, 1990; Meister, 1991; Margarat and Jagadeesan, 2000; Jagadeesan, 2004; Sankar Samipillai *et al.,* 2010). Cell swelling is one of the most conspicuous features of cellular damage (Kinter and Pritchard, 1977). Ballatori *et al*. (1988) reported that the inorganic mercury produces cytotoxicity in animals by altering ion and non-electrolyte transport and regulation of cell and nucleus cell volume. Disturbance in the volume of cell by the toxicants reflects cellular communication and metabolic functions by altering the transmembrane ion, solute and electrical gradients. So, cell volume plays a vital role in the physiological signal for cellular proliferation, metabolic control and gene expression (McManus *et al.,* 1995).

MATERIALS AND METHODS

Chemicals: Mercuric chloride, Taurine, Glutathione and all other necessary reagents of analytical grade were bought from HiMedia laboratories Ltd. Mumbai, India

Animals: The Wister strain rats (45 days old) of the Wister strain weighing ranging from 200±5g were used in this experiment. They were divided at random into four groups (each of six rats). All the animals were fed on a standard rat feed and water ad Libitum. Experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of RMMCH, Annamalai University. Wistar albino rats were divided into four groups each consisting of six animals: Group-I saline (0.9% NaCl)-treated control group ;Group-II Mercuric chloride (2 mg/kg orally.,for 15 days single dose)-treated group (Hg); Group-III Mercuric chloride (2 mg/kg orally single dose) + Taurine (50 mg/kg daily orally. for 15 days) treated group (Hg +taurine), Group IV Mercuric chloride (2 mg/kg orally single dose) + Glutathione (50 mg/kg daily orally. for 10 days) treated group (Hg +Glutathione), Group-V taurine (50 mg/kg daily for 15 days)-treated control group Group VI Glutathione (50 mg/kg daily for 15 days)-treated control group. The animals were sacrificed under light ether anesthesia and tissues were dissected.

Cytometry measurement in liver tissue: After the staining the cell and nucleus, the size of cell and nucleus was measured by using the ocular micrometer. Statistical significance was evaluated by using ANOVA followed by Duncan Multiple Range Test (DMRT) Duncan (1957).

RESULTS

At sub-lethal dose of mercuric chloride, the liver tissue showed a significant decrease in the size of nucleus (0.96 ± 0.14μm) when compared with control liver. During the taurine treatment ($HgCl_2$ followed by taurine), the reduced size of nucleus increased to near normal size (1.52 ± 0.20 μm). During the glutathione treatment ($HgCl_2$followed by glutathione), the same increasing trend was noticed. The size of nucleus in the liver was 1.46 ± 0.20 μm.

DISCUSSION

Cells are structural units that make up the animals and are small sacks mostly composed of water. The sacks are made up of phospholipid bilayer. The cell is a fluid like membrane that surrounds the contents of cell (Anderson *et al*., 1998; Child, 1995). The cells are basic units of life, which are

Table .1. Cytometric changes in the size of cell and nucleus in the hepatocyte's of liver tissue of rats treated with mercuric chloride followed by taurine and glutathione respectively.

Tissues	Group I Control	$HgCl_2$	$HgCl_2$ + Taurine	$HgCl_2$ + Glutathione	Taurine	Glutathione
Cell(μm)	3.68±0.34	3.36±0.19*	3.64±0.28*	3.60±0.32*	3.68±0.36	3.62±0.37
Nucleus μm)	1.40±0.15	0.96±0.14*	1.52±0.20*	1.46±0.29*	1.57±0.11	1.60±0.16

Mean± S.D of six individual observations; Group I compared with group II,III,IV,V and VI; Group II compared with group III and IV; * Significance at 0.05 level;

the smallest structures capable of basic life process. They require energy for a variety of functions including moving, building up and breaking down of molecule and transporting substances across the plasma membrane (Child, 1995). All cells must synthesize molecules and expel waste and synthesize the proteins, which is needed for cellular gates embedded in the membrane as ion channels and receptors (Cooper, 2000).

The nucleus also serves as the cell's command center and information library. The nucleus is bounded by a double membrane, which separates the contents of the nucleus from the cytoplasm (Anderson *et al.*, 1998). This helps to maintain the shape of the nucleus and assist in regulating the flow of molecules into and out of the nucleus through nucleus pore. The nucleus also controls the synthesis of proteins in the cytoplasm through the use of messenger RNA. This mRNA is produced in the nucleus of cells and travels to the cytoplasm through the pore of nuclear envelop (Cooper, 2000).A major mechanism by which inorganic mercury and its compounds produce cytotoxicity by the way of altering cell volume (Ballatori *et al*., 1988; Ballatori and Boyer,1996). The inability to regulate cell volume disturbs transmembrane ion, solute and electrical gradients, which in turn disrupt cellular communication, homeostatis and metabolic function. In addition, it is considered as a physiological signal for cellular proliferation, metabolic control and gene expression (McManus *et al.,* 1995). These normal signal transduction pathways could be compromised after mercury induced disturbed cell volume.

The cells demonstrate the ability to change their concentration of amino acid in response to volume. The cellular volume changes in response to a range of disturbances including disease and tissue damage. Mercury has the ability to cause changes at the cellular level. Its effect on microtubules has also been found in the tissue and results in disruption of the cell cycle (Falconer *et al*., 1994). Thus disturption can cause apoptosis in the tissues (Cavalleri and Gobba, 1998). The present study shows the reduced size of cells and nucleus in brain, liver and kidney tissues of rats when treated with sub-lethal dose of mercuric chloride for 30 days. The reduced size of cells and nucleus suggest that hereditary material is restricted to the nucleus by which the cells are not synthesized might be due to reduction of protein synthesis. Due to the reduction of protein, the cells may fail to hold the normal size and fail to perform their unique metabolic function. The reduced size of cells suggests that when cells are hypo-osmotic during hyponatremia, they might be swelling and might lyse if the hyponatremic state continued. In hypernatremic condition, the cells are usually shrunken or crenated and have a reduced fluid volume. There are many ways in which the cells of animals regulate their volume. The cells either release or accumulate (Na, K and Cl) to produce changes through ion specific channel and transport system across the cell membranes, and cells rely on organic osmolyte-molecules that create intracellular osmolarity without adversely affecting cell function for long-term maintenance of intra-cellular volume. Similarly, Garty *et al*., (1986) observed the reduced size of the cell. They suggested that the heavy metal process is sulfhydryl dependent.

During the recovery period (mercuric chloride followed by taurine and mercuric chlorite followed by glutathione), the reduced size of cells and nucleus was restored to near normal size in the brain, liver and kidney tissues of rats. These results may be due to prevention of swelling and

regulating fluid volume of the cells. In the tissue, metabolism of hypotaurine and taurine is coupled to ensure delivery of taurine which is needed to maintain osmolarity and it may be speculated that proteins in the blood pull fluid out of the body tissue in the blood stream. This supplementation of taurine and glutathione pull in or out of the cells and justify the conditions at the cellular level. This is also due to both taurine and glutathione which are important amino acid osmolyte and help to regulate osmolarity without causing additional perturbations of cellular volume.

In the present study, the accumulated amount of mercury ion in the respective tissues was completely eliminated (Table 1) by taurine and glutathione respectively, because taurine and glutathione directly bind mercury which is then not absorbed by tissue or is more rapidly excreted. Hence, dietary taurine and glutathione may play a vital role to reduce the toxic effect of mercury in the respective tissues of mercury- intoxicated rats. Once the toxic effect is withdrawn cells start to synthesize the protein. It helps the cells recover from the hyponotic condition. Similarly, Maar *et al*. (1999) reported that taurine functions to modulate the chemical and metabolic process. It regulates the most basic of cell function and it acts on osmoregulator (to balance cell volume) and neuromodualtor (protecting against cell death). This taurine has important role in maintaining the delicate balance of cellular fluid volume in the cell in the body. Trachtman *et al*., (1990) suggested that taurine reduces the bile salts by which cells increases in size at the cellular level. Therefore the present study concluded that taurine and glutathione protect the mercury induced cell swelling

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