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Lipid Peroxidation in Tin Intoxicated Partially Hepatectomized Rats

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In recent years the toxic effects of tin have been the subjects of numerous studies. The salient features of tin intoxications have been reviewed by BARNS AND STONER (1959). Studies from this laboratory have demonstrated that tin alters mitochondrial function, reduces the content of sulfhydryl groups and subsequently inhibits the activity of sulfhydryl containing enzymes (DWIVEDI et al 1983). Tin has also been known to alter the δ-amino-levulinic acid synthetase, δ-aminolevulinic acid dehydratase, heme oxygenase, drug biotransformation mechanisms related to decrease in cyto-P_{450} content in endoplasmic reticulum and glucuronidation and deglucuronidation capacity of the hepatic cells (DWIVEDI et al 1983). Various studies have been done to account for the cellular toxicity and its altered metabolism produced by metals in vivo and in vitro system (WEBB, 1977).

It was suggested that a possible mechanism for the altered physiology of the cells may involve lipid-peroxidation process (KINTER AND PRITCHARD, 1977). A relationship between lipid peroxidation and cellular toxicity has also been investigated in isolated hepatocytes, where there is some indication of a positive correlation between the two (STACEY AND KLASSEN, 1981). It has been reported that glutathione a cofactor for the selenium dependent GSH-peroxidase which reduces the content of lipid peroxides has been implicated in the defence mechanism against lipid peroxidation (MC CAY et al, 1976).

The present work was designed, therefore, to investigate the effects of tin-tartrate on normal and regenerating hepatic tissue and to determine the extent of any association with lipid peroxidation and hepatic

glutathione content. Regenerating rat liver-a growing model system, was taken in to account to assess the health risk due to heavy metals pollution in growing population.

METHODS AND MATERIALS

Male albino rats 200-250 g, the Sprague-Dawley strain inbred in Industrial Toxicology Research Centre Colony were housed in an air conditioned room at least one week before the experimentation. Partial hepatectomy was performed according to the procedure of HIGGINS AND ANDERSON (1939) and rats were treated as follows :

Group I : Sham-operated, receiving only 0.5 ml 0.15 M NaCl/100 g body wt.
GroupII : Sham-operated, receiving 0.5 ml 0.15 M NaCl and 2 mg Tin(II) tartrate/100 g body wt.
GroupIII: Partially-hepatectomized rats receiving only 0.5 ml 0.15 M NaCl/100 g body wt. immediately after the operation.
GroupIV : Partially-hepatectomized rats receiving 0.5 ml 0.15 M NaCl and 2 mg Tin(II) tartrate/ 100 g body wt. immediately after the operation.
Group V : Unoperated controls receiving 0.5 ml 0.15 M NaCl/100 g body wt.
Group VI: Unoperated controls receiving 0.5 ml 0.15 M NaCl/and 2 mg tin(II) tartrate/100 g body wt.

Overnight fasted animals were sacrificed in the following morning at 24 hrs of the treatment by decapitation and livers were immediately excised out, washed in cold 150 mm potassium chloride, blotted and weighed. Homogenates (10% w/v) were prepared according to the procedure described by Sharma and Krishna Murti (1976) for the measurment of lipid peroxides formed.

Lipid peroxides formation was determined by assaying the presence of thiobarbituric acid (TBA) reacting substances according to the method of WILBER et al (1949) as described by SHARMA AND KRISHNA MURTI(1976). One ml liver homogenate was aerobically incubated at $37^{\circ} \pm 1^{\circ}$ in a metabolic shaker (Scientronic Model SSI-239) 120 strikes/min (amplitude 1 cm) for 3 hrs. One millilitre of 10% (w/v) trichloroacetic acid was added at the end and after thorough mixing; the

reaction mixture was centrifuged at 800 x g for 10 min. Samples oneml of the clear supernatent were mixed with one ml of 0.67% 2-thiobarbituric acid and held in a boiling waterbath for 10 min. After cooling samples were diluted with one ml distilled water. The absorbance of the solution was read at 535 nm and the results oxpressed as malonyldialdehyde (MDH) using 1.56×10^5 as the extinction coefficient (UTLEY et al, 1967). TBA values= 10 x n mole of MDH formed per mg protein per 3 hrs.

Assay for hepatic glutathione was performed by using Ellman's Reagent as described by JOLLOW et al.(1974). A 0.50 ml aliquot of homogenate was mixed with 0.50 ml of 4% (w/v) sulfosalicylic acid at $4^{\circ}C$. The samples were stored overnight at $4^{\circ}C$. The samples were stored overnight at $4^{\circ}C$ and centrifuged at 3000 g for 10 min at room temp. A 0.4 ml aliquot of the clear supernatant was mixed to 1.6 ml of Ellman's Reagent and the absorbance at 412 mμ was determined after 10 min. Results were expressed as n moles of GSH/gm fresh wet weight of tissue.

RESULTS AND DISCUSSION

Lipid peroxides formation at various stages of liver regeneration is shown in the figure 1. The data depicted in the figure reveal that formation of lipid peroxides in the fresh liver homogenate is affected by the different stages of the multiplication or mitotic cycle. At 24 hrs of the regeneration peroxidation of lipids was significantly reduced while a small rise in the peroxides level was observed at 36 hrs. It is noted that the process of peroxidation is further inhibited at 48 hrs of the operation. In later period of regeneration a normalising trend in the formation of lipid peroxides is found. Results indicate that the peroxide formation is inhibited at 24 and 48 hrs of the regeneration.

The production of lipid peroxides is believed to be mediated by a nonenzymic reaction since more lipid peroxides are formed from the preheated homogenate than by the unheated (SHARMA AND KRISHNA MURTI,1963). In view of this observation, in a parallel set, aliquots of the liver homogenates at different time intervals were held at the temperature of boiling water bath for 10 min. This was employed for following any changes in the rate of peroxide formation,

consequent on exposing the tissues to high temperature at various phases of regeneration. The results are shown in Table 1. It was found that non enzymic peroxide formation is considerably enhanced in the heated homogenate during the course of liver regeneration.

From the results as shown in figure 2, it appears that there is a significant increase in the glutathione content at 24 hrs after the operation. It may be noted that at the time maximum cell proliferation when the mitotic cycle is at its peak i.e. 24 hrs the content of glutathione increases while that of lipid peroxides decreases. After 24 hrs of the operation, glutathione content starts decreasing to the process of regeneration and reaches to approximately normal level at 168 hrs of the operation.

Administration of tin to partially hepatectomised rats immediately after the operation affects theformation of lipid peroxides and glutathione content at 24 hrs of the regeneration. The data are shown in figure 3. Almost 13% increase in the formation of lipid peroxides in sham operated fresh and heated homogenate and 24% increase in the partially hepatectomised rats resulted. Glutathione content was simultaneously depleted to 24% and 21% in tin treated sham and partially hepatectomised rats respectively. It is evident that tin produces more pronounced changes in the formation of peroxides and glutathione content at the time when cellular proliferation is maximum. These changes are more or less related to each other.

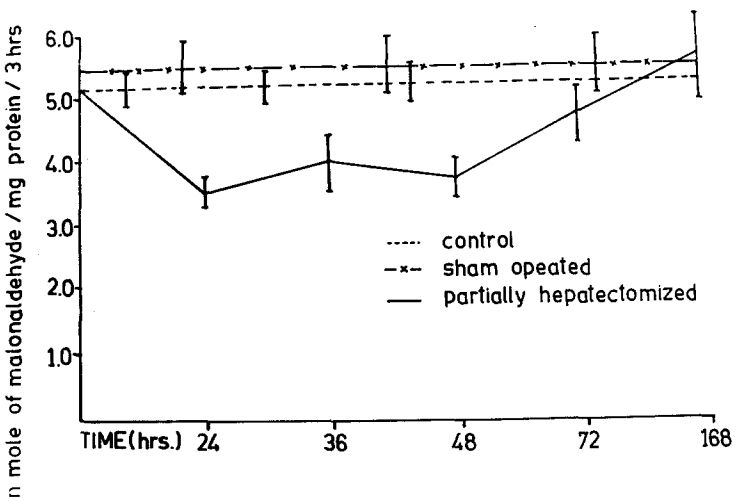

Fig.1. Changes in lipid peroxidation during liver regeneation at various time intervals.

Table 1. Lipid Peroxidation in Partially Hepatectomized Rat Liver at Various Time Intervals of Regeneration Process.

Parameters	Control	Sham	hrs after partial hepatectomy (pH) 24	36	48	72	168
Lipid peroxidation TBA reactants (n moles of malonaldehyde/mg protein/3hr) X 10	Fresh						
	5.22 ± 0.25	5.49 ± 0.40	3.50[a] ± 0.20	3.95[b] ± 0.36	3.65[a] ± 0.24	4.65 ± 0.39	5.60 ± 0.75
	Heated						
	6.45 ± 0.45	7.60 ± 0.66	4.10[a] ± 0.40	4.75 ± 0.60b	4.50[a] ± 0.80	5.80 ± 0.65	6.30 ± 0.38

Each value represents ± SEM of 6 Rats.
Significance levels is [a]$p < .001$, [b]$p < .05$ versus controls.

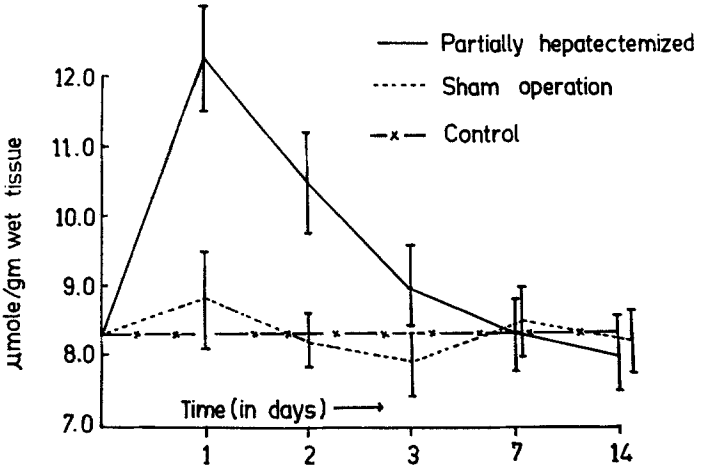

Fig.2. Glutathione content in Regenerating rat liver at various time intervals.

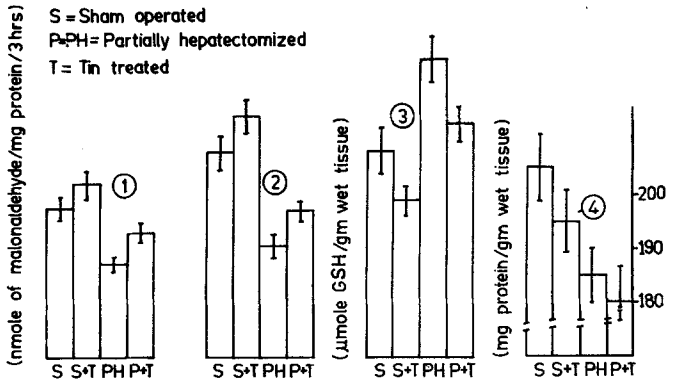

Fig.3. Effects of Tin(II) tartrate on lipid peroxides accumulation (in fresh-1, and heated-2, homogenate) reduced glutathione(GSH) content-3, and total protein content-4, in Regenerating Rat Liver.

The results of the present study demonstrate that the peroxide formation is reduced during the rapid phase of regeneration. This is evident from the data that at 24 hrs of the regeneration when cells are in their maximum capacity for multiplication, peroxide formation and its accumulation is lowered. It is also noted at the same time that the level of hepatic glutathione (GSH) is induced which might be of helpful in protecting the newly born hepatocytes from the damage following lipid peroxidation (ANUNDI et al, 1979). Also production of lipid peroxide is reduced at 48 hrs of the operation which is the time of 2nd mitotic peak. A lowering at 36 hrs of the operation and normalising trend after 48 hrs of the operation in the content of lipid peroxides suggest that lipoperoxidation rate is reduced when cell proliferation takes place at the maximum value. These findings are in the agreement of an earlier report of ZICHA et al.(1966) who reported the reduced formation of lipid peroxides during the process of cellular proliferation. Besides regenerating normal liver, some experimentally induced tumors are the examples of growing tissue in which the equilibrium of the so called "Peroxidative balance" is shifted towards the antioxidant site. This biological environment has been described in mouse hepatoma 22-A (BURLAKOVA et al, 1965), in Novikoff hepatoma (THIELE AND HUFF, 1960), in ascites tumor of Ehrlich (LASH, 1966) and of Yoshida (UGAZIO et al, 1968).

In the past years KINTER AND PRITCHARD (1977) has reported that the formation of lipid peroxides is associated with metal toxicity. Induction of lipid peroxidation process by mercuric chloride has already been established in rats. Further more lipid peroxidation has been associated with kidney and testicular injury in response to cadmium treatment <u>in vivo</u> (GABOR et al, 1978).

Administration of tin-tartrate in our experimental study resulted the depletion in the level of GSH and induction of the lipid peroxide formation in normal (Sham) and regenerating rat liver. This might be due to the fact that tin combines with sulfhydryl groups resulting ultimately in a deficiency of glutathione (GSH). This decrease in GSH content reduced the degradation of lipid peroxides to hydroxy acids leading to the accumulation of the former (TAPPEL,1970). It has been documented earlier that glutathione protects against lipid peroxidation (HOGEBERG,1975) and there exist a close correlationship between low level of GSH and accumulation of the malonaldehydes

(ANUNDI et al, 1979). It can be speculated, therefore, that the content of lipid peroxides will be increased after the exposure of tin salt to the liver. An increase in the formation of lipid peroxides in regenerating rat liver due to the exposure of tin could be explained on the basis that regenerating rat liver apparently has the necessary unsaturated fatty acids and the catalyst (FEX,1975) for the process of peroxidation in the architecture of the cell itself which are readily available for the reaction with molecular oxygen favouring the production of toxic lipid peroxides (THIELE AND HUFF,1960). These lipid peroxides in turn damage the biomembranes and subcellular organelles of the cellular components which are thought to be the principal target site for their interaction. Cytotoxicity to the metabolically active cells is due to an effects on cellular membranes as proposed by KINTER AND PRITCHARD (1977).

It might, therefore, be suggested that tin causes a decrease in GSH which then allows an increase in the process of lipid peroxidation, damaging the hepatocytic membranes. This is in agreement with previous reports for other toxic chemicals too (ANUNDI et al, 1979). Thus on the basis of direct involvement of GSH depletion and increased lipid peroxidation cytotoxic response of tin could be expected like other chemicals including cadmium (STACEY et al,1980). Further work is in progress to find out whether glutathione depletion due to tin exposure, is at its synthetic level.

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