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Vitamin C against Concomitant Exposure to Heavy Metal and Radiation : A Study on Variations in Hepatic Cellular Counts

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Abstract : The objective of this study was to screen for prophylactic use of ascorbic acid against radiation and heavy metal intoxication. For this purpose, male Swiss albino mice were intoxicated with lead acetate (20 mg/kg b. wt.) intraperitoneally 1 hr. before exposure to 4.80 Gy gamma radiation in the presence (experimental) or absence (control) of vitamin C (400 mg/kg b. wt.). Mice were sacrificed at various autopsy intervals (6 hrs. to 20 days) to examine quantitative changes in liver. It was observed that vitamin C administration, prior to combined treatment of lead acetate and radiation reduced the depletion in normal hepatocytes and the elevation in binucleate as well as abnormal hepatocytes in comparison to their respective controls, and moreover, it initiated a faster recovery to reinstate the normal cellular number by increasing glutathione level.

Key words : Metal toxicity, Gamma radiation, Chemical protection, Vitamin C, Liver, Swiss albino mice

Introduction :

With an improvement in the lifestyle of people by the application of advances in science and technology, man has to pay the penalty of accepting a certain degree of deterioration of the environment with its concomitant adverse effects on the health and well-being of the living beings. Metal toxicity and radiation effects on organisms are manifested in the form of various pathological, histological and biochemical alterations. Extensive research has been carried out on toxicity of heavy metal or radiation alone (Gajawat and Goyal, 2002), but a little has been emphasized on their combined interaction in living systems. Chemical protectors like 2-mercaptopropionyl glycine (2-MPG) (Ayene *et al.*, 1988), cysteamine (Bacq *et al.*, 1951), deoxyspergualin (Nemato *et al.*, 1995), WR-2721 (Grdina *et al.*, 1992), Liv. 52 (Ganapathi and Jagetia, 1995) have shown very promising results when tested in laboratories, but could not have practical utility owing to their high toxicity.

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Heavy metal and radiation have the potential to induce free radical formation and cause lipid peroxidation, leading to devastating effect on the structure and function of the cell and cell membrane by causing cellular death. Vitamin C (ascorbic acid), the major water-soluble antioxidant, is believed to decrease lipid peroxidation either directly or indirectly by regenerating vitamin E, the major lipid-soluble antioxidant (Frei *et al.*, 1989). Based on the above role and significance, the present study has been undertaken to assess the effectiveness of vitamin C (ascorbic acid) against quantitative hepatic cellular alterations by concomitant treatment of lead and radiation.

Materials and Methods :

Animal care and handling were performed according to guidelines issued by the World Health Organization (Geneva, Switzerland) and the Indian National Science Academy (New Delhi, India). Swiss albino male mice (*Mus musculus*), 6-8 weeks old and 25±2 gm b.wt. from an inbred colony, were selected for the study. They were maintained under controlled conditions of temperature and light (light: dark, 10 h: 14 h). They were provided standard mice feed (procured from Hindustan Lever Ltd., India) and water *ad libitum*. Tetracycline water was given for prevention against infection once a fortnight. The Departmental animal ethical committee approved the study.

Animals were divided into 3 groups. Group-I was administered with double distilled water (volume equal to vitamin C) and was considered as normal. Animals of Group-II (control) were intoxicated intraperitoneally with lead acetate, 1 hr. before irradiation. Animals belonging to Group-III (experimental) were pre-treated with vitamin C (once in a day for 7 consecutive days) and lead acetate was injected intraperitoneally, 1 hr. before exposure to gamma radiation. Animals were sacrificed and livers were collected from each group at various autopsy intervals between 6 hrs. to 20 days. Microscopic slides were prepared by routine procedure and cellular quantitative studies were made by counting normal, binucleate and abnormal cell population with respect to total hepatocytes.

Results and Discussion :

Table 1: Variation in hepatocytes (%) in mice after concomitant treatment of lead acetate and radiation in the presence (experimental) and absence (control) of vitamin C

Post-irradiation intervals	Group	Types of Hepatocytes (%)		
		Normal	Binucleate	Abnormal
6 hrs.	Cont.	85.74±0.55^{c}	9.99±0.22^{c}	4.27±0.22^{c}
	Expt.	87.21±0.51^{a}	8.79±0.25^{c}	4.00±0.25
12 hrs.	Cont.	83.70±0.56^{c}	10.51±0.26^{c}	5.79±0.23^{c}
	Expt.	85.11±0.54	9.27±0.38^{b}	5.62±0.24
1 day	Cont.	81.54±0.28^{c}	10.75±0.33^{c}	7.71±0.27^{c}
	Expt.	83.54±0.31^{c}	9.96±0.29	6.50±0.31^{b}
2 days	Cont.	78.75±0.38^{c}	11.30±0.27^{c}	9.95±0.11^{c}
	Expt.	80.09±0.39^{b}	10.71±0.34	9.20±0.22^{b}
5 days	Cont.	78.99±0.59^{c}	9.90±0.18^{c}	11.11±0.18^{c}
	Expt.	80.29±0.57	9.70±0.29	10.01±0.32^{b}
10 days	Cont.	80.69±0.10^{c}	10.31±0.17^{c}	9.00±0.27^{c}
	Expt.	81.91±0.25^{c}	9.34±0.19^{c}	8.75±0.30
20 days	Cont.	86.96±0.32^{c}	8.59±0.19^{c}	4.45±0.18^{c}
	Expt.	88.33±0.49	7.77±0.29^{b}	3.90±0.43
	Normal	90.18±0.193	7.36±0.162	2.38±0.194

Each value represents mean ± SE
Cont = Lead acetate + 4.8 Gy
Expt = Vit. C+ Lead acetate+ 4.8 Gy
Normal = No treatment

Statistical Comparison	Significance Level
Control v/s Normal	$^{a}p<0.05$
Experimental v/s Control	$^{b}p<0.01$
	$^{c}p<0.001$

The present study shows insignificant variations in the number of different hepatocytes in the animals treated with vitamin C alone, as compared to normal group (DDW treated). When animals were exposed to 4.80 Gy gamma rays after lead acetate treatment, normal cells of liver declined upto day 5 in both control as well as in experimental groups, but decreased thereafter till day 20 (Table 1). On the contrary, abnormal cells increased upto day 5 after such treatment. Binucleate hepatic cells showed a biphasic mode of elevation, first peak was observed on day 2 and second on day 10 in both the groups.

The histopathological observation revealed that the reason for an early increase of the binucleate cells before degeneration is due to the fusion of liver cells. Observations on the second day post-treatment exhibit that radiation or lead caused the death and removal of binucleate cells which resulted in the depletion of such cells and some of these even form mononucleate giant cells. The second elevation in binucleate cells in control (lead+ radiation) group caused during the recovery period may be due to the failure of complete telophase separation of the post-mitotic cell or inhibition of cell division by radiation or lead induced G_2 block.

The histopathological alterations exhibited a correlation with the number of abnormal cells in the present study. The elevation in their number is associated with an increase in radiation or lead induced lesions and these cells declined during the recovery phase. Similar observations were made by others (Matsuda, 1956; Gajawat and Goyal, 2003), who suggested that the percentage of dead and abnormal cells serve as good indicators of teratogenic sensitivity of liver cells.

Table 2 : Glutathione (GSH) level in mice after concomitant treatment of lead acetate and radiation (4.8 Gy) in the presence or absence of vitamin C

Treatment	GSH level (mean± SE)	
	Blood (µg/ml)	Liver (µg/ml)
Normal	3.94±0.196	6.89±0.187
Lead + Radiation	2.05±0.156[b]	4.91±0.188[b]
Vitamin C + Lead + Radiation	2.51±0.091[a]	5.40±0.096[a]

Each value represents mean ± SE

Significance levels [a]$p< 0.05$; [b]$p< 0.001$

Radiations interact with biological molecules and produce free radicals leading to DNA and membrane damage. Lead binds with the thiol groups (-SH) of the cellular components, responsible for protecting repair system against damage caused by radiation induced free radicals (Muller *et al*., 1985), thus making free -SH groups not available for protection which in turn contributes to an increased risk during combined exposure to lead and radiation. Glutathione is a versatile protector and executes its function through free radical scavenging, reduction of peroxides and maintenance of protein thiols in the reduced state. The depletion of GSH in both blood and liver as observed in the present study (Table 2) is one of the main causes of lead as well as radiation induced hepatic toxicity. Vitamin C protects the biological systems by increasing sulphydryl groups as well as endogenous glutathione level of blood and liver (Dreyfus, 1985). Thus, it is concluded that the prophylactic application of vitamin C is quite effective during lead intoxication and irradiation.

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