Role of Ascorbic Acid on Chromium Induced Changes in Rat Liver - A Morphometric Analysis.

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This study was conducted with an aim of forming a morphometric base line for normal and treated liver tissue, establishing the comparative damage done by Chromium (Cr) alone and in combination with Ascorbic Acid in a time related sequence. A study involving 120 rats, randomly divided into 4 groups – A, B, C & D of 30 animals each. Every group subdivided in 3 subgroups each, according to treatment time i.e. 2,4 and 8 weeks. Group B was administered Cr. solution, intraperitoneally, group C was given Cr. solution ip(intraperitoneally) and Ascorbic Acid orally, group D received Ascorbic Acid alone. Control group A was administered distilled water intraperitoneally. Chromium toxicity exhibited as thickened liver capsule, dilated central vein. and increased hepatocytic dimensions, which got statistically significant with advance in treatment. Comparable group receiving both Cr. and Ascorbic Acid showed slightly milder form of the same changes. Chromium hepatoxicity in low doses is thus established thus confirming its potential health hazards. Role of Ascorbic Acid in Cr. poisoning needs to be exposed further in terms of administration route and dosage.

Key Words : Morphometric Analysis, Chromium, Hepatotoxicity, Rat Liver, Ascorbic Acid, Vitamin C..

Chromium (Cr), an essential trace element has numerous industrial applications[1,2]. Large amounts of metal are introduced into the environment through wastes of these industries .In addition to the workers employed in these, humans and animals living in nearby areas are constantly exposed to the metal through food and water intake. The higher toxicity of its more commonly used hexavalent form (Cr VI), is attributed to its strong oxidizing power and high transport rate through the cell membrane[3]. The subsequent intracellular reduction of Cr VI is important for its biochemical interaction and toxic manifestations[4]. If sufficient Cr VI reaches the target organ without being reduced to its trivalent form, acute damage may occur. Extracellular Cr reduction is thought to represent a detoxification mechanism[5]. Hepatotoxic and nephrotoxic actions of Cr VI[6] as well as its effects on lungs, blood circulation may contribute to its fatal outcome[7,8]. Hepatotoxicity leads to necrosis of the organ[9]. Dilatation and congestion of central vein, cloudy swelling of hepatocytes and marked necrosis of liver are time related histological changes[10,9]

Ascorbic acid, a water-soluble vitamin, is a potent antioxidant. A dose of 150 mg or more is required to be effective as a reducing agent[11]. It plays an important role in Cr VI reduction in tissues[12].

In this study a detailed morphometric analysis of various histological parameters was done to confirm and highlight the extent of damage done by the metal and also to assess the avoidance of these changes by giving antioxidants in supplemental doses.

Materials and Methods

One hundred and twenty male Sprague Dawley rats, mean weight 161±13gms were kept in animal house PGMI, Lahore. Fed on commercial diet and water ad libitum, care was taken regarding optimal light and temperature. The animals were given 2 weeks for acclimatization, and were then divided into 4 groups, namely A B C and D each comprising 30 rats. Group B animals received 1 mg/kg Cr i.p., ($Na_2 Cr_2 O_7$ --E.Merck) dissolved in 2 ml of 0.9 % Saline on alternate days. Group A animals received an equal amount of dist. water i.p., and served as control. Animals of Group D were given 1 mg / kg Cr i.p. , on alternate days and Ascorbic Acid 10 mg / kg body weight orally daily. The first dose of AsA was given 24 hours before the first does of Cr. Whereas Group C animals received Ascorbic Acid alone 10 mg/kg orally daily. The doses were adjusted on weekly basis in accordance with animals body weights. 10 animals each from all the four groups were sacrificed after 2, 4 and 8 weeks of treatment 24 hours following the last injection. This time period was given to enable excretion of unbound Cr from the body.

Liver of all animals was fixed in 10% formalin. Paraffin blocks were made and sections were cut at 4–5μm and stained with H and E by standard procedure[13]. Various morphometric parameters including capsular thickness, size of central vein, hepatocyte size, number of nuclie and nuclear dimensions were noted. Results were analyzed using analysis of variance. Significant differences between means were tested using Student's t–test.

Results *(see table)*

Livers of animals administered with Cr at 2 weeks showed capsule thickness at 5 – 7.5μm similar to control group. The central veins were considerably dilated (52.85μm) compared to the control group (33.98μm). Hepatocyte size was within normal range (15.9μm). Vesicular nuclie with a diameter of 6.2μm containing 1-2 nucleoli each were seen. At 4 weeks, capsule was still thin. Central vein dilatation progressed to 60.4μm, there was a significant increase in hepatocyte size (17μm). At 8 weeks capsular thickness was 12.5 to 16.5μm. The increase in hepatocytic size was statistically significant (19.4μm). Central vein diameter progressed further.

Table Effect of Sodium Dichromate (Chromium) and Ascorbic Acid alone and in combinations on different parameters of morphometric analysis of rat liver.

Groups	A Control			B Chromium Administration For:			C Ascorbic Acid Administration For:			D Chromium And Ascorbic Acid Administration For:		
	2 Weeks	4 Weeks	8 Weeks	2 Weeks	4 Weeks	8 Weeks	2 Weeks	4 Weeks	8 Weeks	2 Weeks	4 Weeks	8 Weeks
Diameter of[a] Central Vein µm (n=60)	33.9 ± 1.78	34.3 ± 1.38	34.3 ± 1.68	*** 52.85 ± 2.5	*** 60.4 ± 3.2	*** 64.1 ± 2.9	6.2 ± 1.5	39.7 ± 1.63	* 44.3 ± 2.08	*** 51.3 ±2.32	*** 53.8 ± 2.68	*** 63 ±3.05
Size of[a] Hepatocytes µm (n=90)	15.5 ± 0.22	15.3 ± 0.22	15.2 ± 0.26	15.9 ± 0.20	*** 17 ± 0.27	*** 19.4 ± 0.29	15.2 ± 0.20	15.1 ± 0.21	15.3 ± 0.25	15.4 ± 0.21	** 16.6± 0.27	*** 18.1 ± 0.27
Size of Nucleus µm (n=90)	6.25 ± 0.13	6.13 ± 0.13	6.16 ± 0.13	6.2 ± 0.14	6.15 ± 0.13	6.18 ± 0.14	6.18 ± 0.13	6.13 ± 0.13	6.25 ± 0.13	6.18 ± 0.14	6.16 ± 0.15	6.18 ±0.14
Number of Nucleoli/Nucleus (n=90)	1.3 ± 0.04	1.3 ± 0.02	1.5 ± 0.01	1.4 ± 0.03	1.6 ± 0.02	1.3 ± 0.04	1.5 ± 0.02	1.2 ± 0.03	1.4 ± 0.02	1.2 ± 0.02	1.1 ± 0.03	1.6 ± 0.04

a Mean ± SEM, students t-test ; p<0.05=*, p<0.01=**, p<0.001=***, For statistical significance in this table control groups have been compared to their respective experimental groups.

Ascorbic Acid alone at 2 weeks gave morphometric results similar to the control group. 4 weeks treatment showed slight increase in central vein diameter, which progressed to a significant value after 8 weeks of treatment (44.3μm). The rest of the parameters remained with in normal range even after 8 weeks treatment.

The combination treatment of Ascorbic Acid and Cr both at 2 weeks showed no increase in capsular thickness, central veins were dilated (51.3μm), hepatocyte size was within normal range. At 4 weeks size of hepatocyte and central vein diameter increased to 16.6 and 53.8μm respectively. At 8 weeks capsule thickened (12.5-15μm) and central vein diameter and hepatocyte size increased significantly to 18.1 and 63μm respectively. No statistically significant changes were observed in nuclear size or number of nucleoli in any group.

Discussion

Various morphometric parameters showed progressive increase with advance in Cr treatment. Dilatation of vessels is reported by other workers as well[6,10,14] and progressed with advance in treatment. This is due to congestion which could be a sequel of impaired venous drainage leading to increased deoxygenated hemoglobin in blood. Increased hepatocytic dimensions_with nuclear changes observed at 2 weeks accentuated with advance in treatment. By the 8th week large necrotic foci were observed. The effects of biochemical lesions are exhibited in the form of degenerative structural changes due to metabolic disturbances. These could be exhibited due to fatty changes, cellular swelling or hyaline degeneration ultimately leading to nuclear changes in the cell[15,16]. Intracellular Cr. reduction increased O2 extraction from blood[17]. This hypoxia causes impaired Na pump mechanism leading to Na retention and thus water in the cell, leading to cloudy swelling, vacuolation, hydropic degeneration and necrosis. The increased size of hepatocytes was probably due to this. Capsular thickening 8 weeks after treatment was due to collagen synthesis in response to chronic cell injury. After 4 weeks, AsA alone showed mild dilatation of central vein. Antioxidants occasionally become prooxidants when large amount is ingested . Dosage used in this study was median supplemental dose which also covers for the antioxidant effect[11]. Toxic effects due to AsA have not been reported due to its high excretory ability[18]. Effects of high doses of Ascorbic Acid need further exploration. Continued administration of Cr and Ascorbic Acid at 2 weeks, dilated the central vein to considerable value. These changes progressed with time and were the same as produced by Cr alone. So extracellular Cr reduction was either absent or incomplete. The reason for cellular reduction of Cr despite giving Ascorbic Acid could be insufficient dose leading to incomplete or no extracellular reduction. Pretreatment time was the same as used in other studies[19,20] but route of administration differed. This

could have played a role in the obtained results. The degree of changes after 8 weeks was less than that of comparable group receiving Cr alone. Meaning thereby, that some but not all Cr did get reduced extracellularly. The rest entered the cell and underwent reduction leading to low grade necrosis.

In this study a detailed morphometric analysis of various histological parameters of Cr induced toxicity and prevention by Ascorbic Acid was performed. In addition a baseline was formed for morphometric parameters in normal tissue comparing it with treated tissue showing the degree of damage done by Cr and its prevention by Ascorbic Acid in a time related sequence.

In conclusion the hepatotoxic effects of Cr are established even in low doses proving it to be a health hazard among Cr workers and people living in nearby areas of these industries. Further exploration is required as regards the role of AsA as a detoxicant in chronic Cr poisoning in terms of its administration route and dosage

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