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Radiation and cadmium induced biochemical alterations in mouse kidney

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Background: In the present investigation radiation and cadmium induced biochemical changes in the kidney of Swiss albino mice have been studied. ***Materials and Methods:*** For this purpose, adult male Swiss albino mice (6-8 weeks old) were divided into four groups. Group I (sham-irradiated), Group II (treated with $CdCl_2$ solution 20 ppm), Group III (irradiated with 1.25, 2.5 and 5.0 Gy gamma rays), Group IV (both irradiated with 1.25, 2.5 and 5.0 Gy gamma rays and treated with $CdCl_2$ solution). The animals were autopsied after 1, 2, 4, 7, 14 and 28 days of treatment. The kidney was taken out and different biochemical parameters, such as total proteins, glycogen, cholesterol, acid phosphatase activity, alkaline phosphatase activity, DNA and RNA were estimated. ***Results:*** In irradiated animals, the values of total proteins, glycogen, acid phosphatase, alkaline phosphatase activity and RNA increased continuously up to day-7 and decreased thereafter up to day-28. The changes were dose dependent. In $CdCl_2$ treated animals, the values of glycogen and total proteins decreased during the early intervals and increased thereafter whereas the values of acid and alkaline phosphatase activity and RNA increased during early intervals and decreased thereafter. The values of cholesterol and DNA showed decrease in all the experimental groups (except group I) up to day-7 and increase thereafter up to day-28. After combined treatment also, the parameters followed the same pattern of increase and decrease, but the changes were more pronounced indicating their synergistic effect. The biochemical parameters showed highly significant values ($P<0.001$) as compared to normal ones. ***Conclusion:*** These results indicate that combined treatment of cadmium and gamma radiations causes synergistic or additive effect. **Iran. J. Radiat. Res., 2007; 5 (3): 125-130**

Keywords: *Radiation, cadmium, kidney, mice.*

INTRODUCTION

The extensive use of atomic radiations now-a-days in various branches of natural economy, biology, physiology, medicine, science and technology has made radiation injury an urgent problem attracting the attention not only of specialists in a variety of clinical disciplines but also of a vast army of theoretical scientists.

The wide variety of tissues constituting the kidney together with its importance and accessibility has made it a favorite site of study among the radiobiologist. Amongst numerous problems pertaining to the biological effects of ionizing radiation, which have been carefully investigated in recent years in many countries radiation injury to the kidney, occupies a special place, for it is adapted to filtering wastes from the blood. Proper knowledge of the response of the kidney to ionizing radiation appears as a problem of great clinical and biological importance. But perhaps in no field of radiation biology are opinions as greatly divergent as in considering the kidney to be radio-resistant.

The kidney is resistant anatomically but probably most radiosensitive physiologically from the standpoint of serious or fatal damage. Kidney is a moderately sensitive organ. The characteristic radiation response of the kidney is acute radiation nephritis which appears 6 months to one year after the completion of radiation therapy [in case of human]. The major complaints are swelling of the legs, shortness of breath, headache and vomiting.

The inhibition of kidney uptake of radiolabelled Somatostatin analogues: amino acids or gelufusine has been studied. Gelofusine significantly inhibited kidney uptake of (^{111}In-DTPA) octreotide to a level comparable to the level of inhibition by

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currently applied amino acid solutions. It was reported that amino acid infusion for kidney protection may have several side effects such as vomiting and potentially fatal hyperkalamia [1].

Excessive levels of trace metals may occur naturally as a result of geological phenomena such as ore formation, weathering of rocks and leaching which may make these metals available to the biosphere. Man releases more of these metals by burning fossil fuels, mining, smelting, and discharging industrial, agricultural and domestic waste and by deliberate environmental application of pesticides. Cadmium is a heavy metal and an industrial pollutant [2], which has aroused concern because of its marked toxicity in man through contaminations of food [3]. The biochemical and histopathological changes have also been studied in the kidney of rats exposed to cadmium. In rats exposed to 5 mg/l, first symptom of injury of the main tubules of long and short nephrons (structural damage to epithelial cells, increased urinary activities of NAG-T and NAG-B) were noted after 12 weeks of the experiment. On exposure to 50 mg Cd/l damage to the main tubules (blurred structure of tubular epithelium, atrophy of brush border and partial fragmentation of cells with release of nuclei into tubular lumen as well as increased urinary activities of NAG-T, NAG-B and ACP) was observed after 6 weeks. These findings revealed that Cd acts on the whole kidney, especially on the main tubules, even at relatively low accumulation in this organ [4].

Early effects of cadmium on the structure and function of the kidney were studied in an experimental model using rats intoxicated with Cd at the level of 5 and 50 mg Cd/l drinking water. The effect of Cd was evaluated histopathologically and biochemically. Damage to the cellular structures was assessed on the basis of histoenzymatic analyses of the activity and localization of indicator enzymes (Succinate dehydrogenase, glucose-6-phosphatase, Mg^{2+} dependent adenosine triphosphatase and acid phosphatase). The histochemical observations indicated that Cd causes damage to the organization and function of the nephron. The cytotoxic action of Cd occurred mainly in the tubules and partially also in the glumeruli [5].

Combined action of ionizing radiation and other agents is of potentially great importance, because there are many occasions when interactions might occur in our environment. The combined effect of chemicals and radiation has mostly been studied in unborn babies because of their higher sensitivity to these toxicants. The general aspects of the interaction between radiation and chemicals during prenatal development were summarized [6].

A lot of work has been done to see the effect of radiation and cadmium given separately on the animal tissues but there are rarely any reports about the effect of radiation and cadmium administered simultaneously. Therefore in the present investigation an attempt has been made to assess biochemical changes induced by radiation and cadmium alone or in combination in the kidney of Swiss albino mice.

MATERIALS AND METHODS

Animals

Healthy male Swiss albino mice (6-8 weeks old) were procured from CCS Agricultural University, Hissar and maintained at 20-25°C. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*.

Source of radiation

Cobalt gamma radiotherapy source (Theratron, AECL, Canada) was used to irradiate the animals. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan), India. The animals were irradiated at the dose rate of 0.97 Gy/min.

Cadmium chloride treatment

The aqueous solution of the cadmium chloride (SDS chemicals, India) was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water, thus giving a concentration of 20 ppm and then administered orally in drinking water.

Plan of experimentation

For the present experiment the animals were divided into four groups. Group I included sham irradiated animals and served as normal. The animals of Group II were treated with cadmium chloride (20ppm) throughout the experiment. The cadmium chloride was administered simultaneously with gamma rays exposure and continued up to the last autopsy interval (i.e. day-28). The mice of Group III were irradiated with 1.25, 2.5 and 5.0 Gy of gamma rays and divided into sub groups IIIa, IIIb and IIIc respectively. The animals of Group IV were treated with cadmium chloride and also exposed to 1.25, 2.5 or 5.0 Gy of gamma rays and divided into sub groups IVa, IVb and IVc respectively.

Autopsy of animals

Five animals were autopsied by cervical dislocation from every set of experiment at each post-treatment interval of 1, 2, 4, 7, 14 and 28 days. The weight of the animals was recorded and their kidney was removed. The biochemical parameters studied were: Total proteins; glycogen; cholesterol; acid and alkaline phosphatase activity; DNA and RNA [7-11].

RESULTS AND DISCUSSION

In the present study, the total protein content of the mouse kidney showed an increasing trend in the gamma rays exposed group III and combined treatment group IV. The value of total proteins increased in both the groups on day-1 and continued up to day-7. Thereafter, it declined from day-14 to day-28. Similarly in cadmium chloride treated group II, the value decreased up to day-7 and then increased on day-14 and continued so up to day-28 (Fiture 1). This observation indicated that the amount of total proteins is adversely affected by cadmium. A significant increase in the number of ribosome may occur due to their increased mobilization from ER and this leads to the increased protein synthesis [12]. The irradiation may bring about increased synthesis of m-RNA and ribonucleoprotein, which in turn would synthesize more proteins. The irradiation may have increased the stability of polysomes already present. This may be either due to some factors, which maintain polysome integrity or due to decreased activity of enzymes involved in the catabolism of polysome [13].

There was a reduction in the value of glycogen in cadmium chloride treated animals (group II) reaching at a minimum on day-7 followed by an increase on day-14 and continued so up to day-28 (Figure 2). Depletion in glycogen has been noticed with a number of chemicals present in the environment [14]. In the present study, this decrease in the glycogen content of kidney as a result of cadmium chloride treatment finds support from the observation of other workers [15-16]. On the contrary, an increase in the value of liver glycogen in rats with 450 R exposures for the first 6-days was observed which may be due to gluconeogenesis [17].

The present investigation exhibited a decrease in the level of cholesterol after the treatment with cadmium chloride (Group II). The value declined up to day-7 and increased thereafter up to day-28 without reaching to the normal. A decrease in the value of cholesterol was also observed up to day-7 in

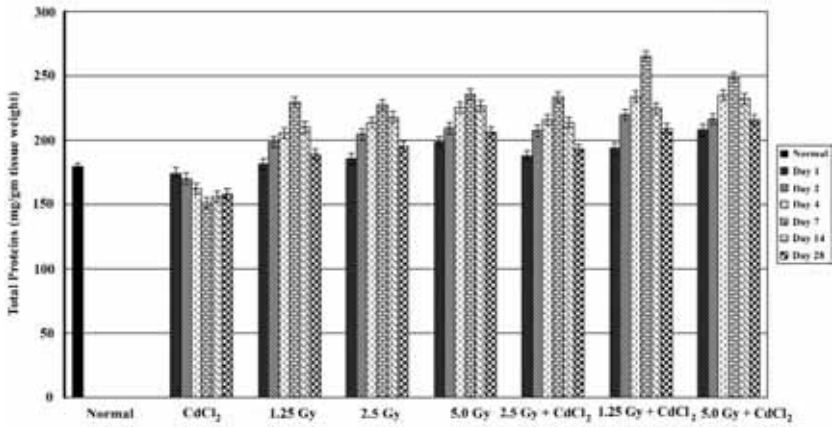

Figure 1. Variation in the values of total proteins (mg/gm tissue weight) in the kidney of mice in various experimental groups.

the groups III and IV. Thereafter, the value increased on day-14 and continued so up to day-28 but still could not to reach to the normal (Figure 3). Decrease in the cholesterol level after irradiation has also been reported [18-21].

In the present investigation the value of acid phosphatase activity increased on day-1 which continued up to day-7 significantly ($P<0.001$) in the groups II, III and IV and then decreased on day-14 significantly ($P<0.001$), and continued so up to day-28, but still the difference in the value was significant ($P<0.001$) as compared to normal group (Figure 4). The increase was found severe after the combined treatment with radiation and cadmium chloride showing synergistic effect. The increased acid phosphatase activity seems to be characteristic of tissue damage by radiation [22]. Lysosomal hydrolyses are thought to contribute to the degradation of damaged cells, hence facilitate their replacement with normal tissue [22]. Acid phosphatase is a lysosomal enzyme and is a non-specific phosphomonoestrase. It helps in the autolysis of cells after death. It hydrolyses various phosphate esters and liberates phosphates. Heavy metals induce cellular damage in the tissue that in turn releases lysosomal enzymes, thereby increasing the acid phosphatase activity [23]. The cellular damage might cause rupture of lysosomes and hence acid phosphatase activity increases due to heavy metal toxicity.

Alkaline phosphatase is a zinc containing enzyme present in many tissues. It hydrolyses phosphorylcholine, so that choline can be transported across the bile canalicular membrane. In the present study, group II, III and IV showed an increase in the value of alkaline phosphatase activity up to day-7 which then declined on day-14 and so continued up to day-28 (Figure 5). The increased value of alkaline phosphatase activity is in good agreement

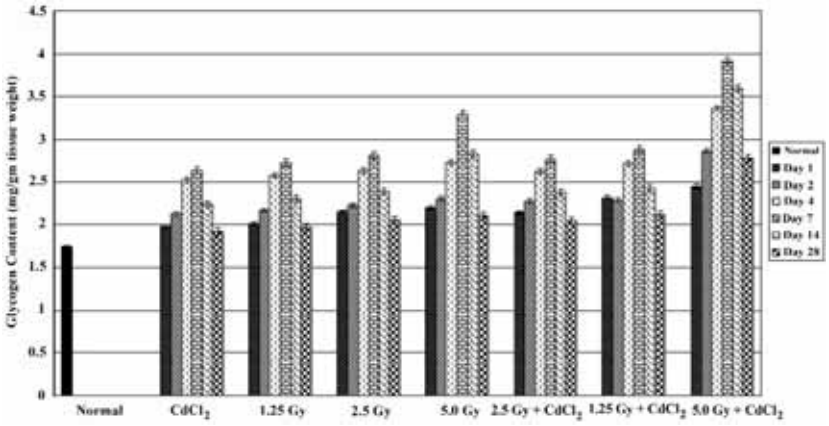

Figure 2. Variation in the values of glycogen (mg/gm tissue weight) in the kidney of mice in various experimental group.

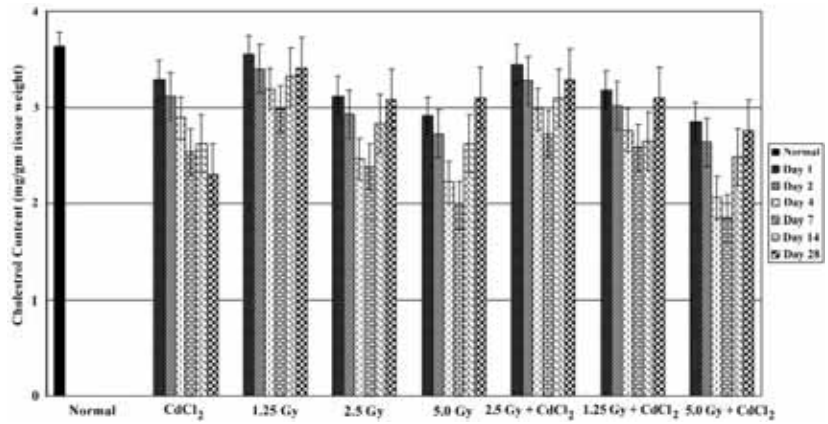

Figure 3. Variation in the cholesterol content (mg/gm tissue weight) in the kidney of mice in various experimental groups.

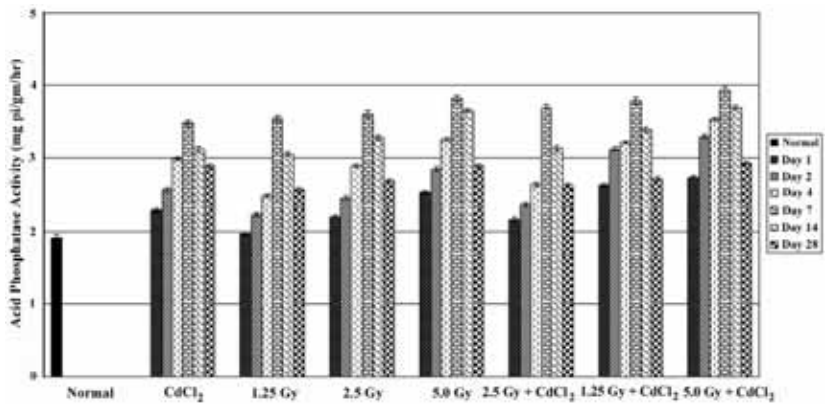

Figure 4. Variation in the values of acid phosphatase activity (mg pi/gm/hr.) in the kidney of mice in various experimental groups.

with the observation of some other workers [24, 25]. This increased activity might be due to increased phosphorylation or tissue damage caused by cadmium or gamma radiation.

The DNA content decreased in all the groups. The decrease was found dose dependent. The DNA content decreased up to day-7, and then it increased on day-14 and continued so up to day-28 without reaching to the normal (Figure 6). After combined treatment synergis-tic changes were observed. The depletion in the DNA content of a tissue *in vivo* is due to reduction in or absence of the essential factors controlling the DNA synthesis [26]. These factors are the substrates (Four deoxyribonucleoside triphosphates), enzymes (Poly-merase), template activity of deoxyribonucleoproteins activators (Mg^{++} and other divalent ions).

The concentration of RNA increased in all the groups. The RNA content increased on day-1 and continued so significantly ($p<0.001$) up to day-7. Thereafter, it declined on day-14 and continued so up to day-28 without reaching the normal value (Figure 7). The difference in the value was significant ($p<0.001$) as compared to the normal. The increase was found dose dependent. After combined treatment more severe increase was observed which may be due to the synergistic effect. Causes of this increase in the cellular RNA may be due to:

1. Ability of DNA to transcribe RNA is not affected quantitatively [27-29] but the length of the chain of RNA molecules reduces [30].

2. Increase in the nuclear RNA polymerase activity may contribute to the post-irradiation increase in the cellular RNA [31].

3. After irradiation with higher doses secretion of gonadotropin is increased [32]. This increased gonadotropin secretion may

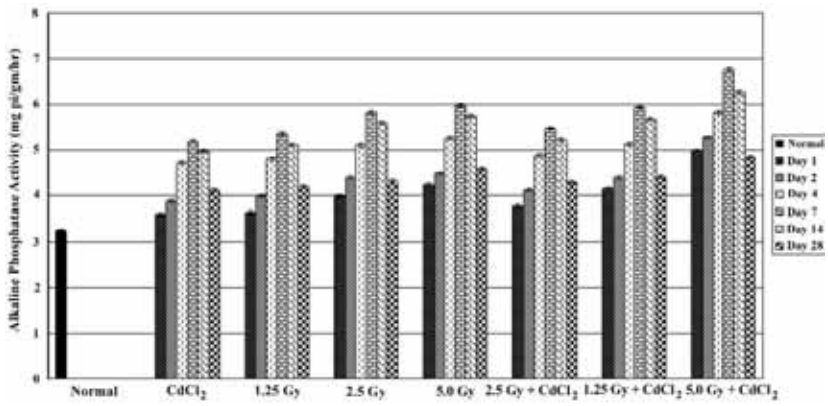

Figure 5. Variation in the values of alkaline phosphatase activity (mg pi/gm/hr.) in the kidney of mice in various experimental groups.

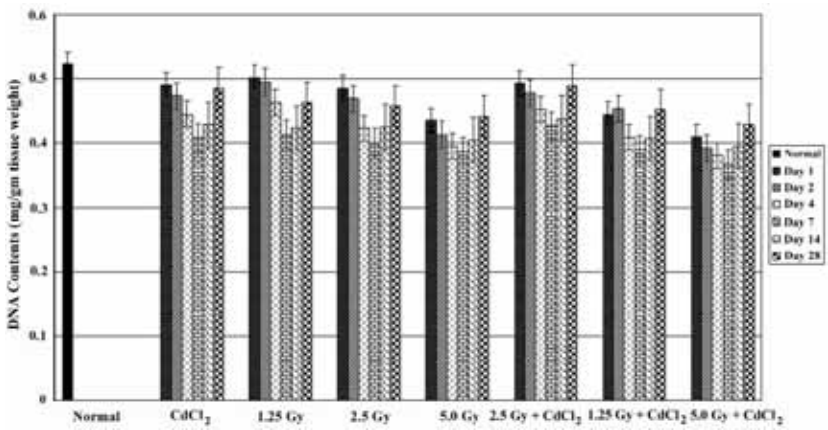

Figure 6. Variation in the values of DNA content (mg/gm tissue weight) in the kidney of mice in various experimental groups.

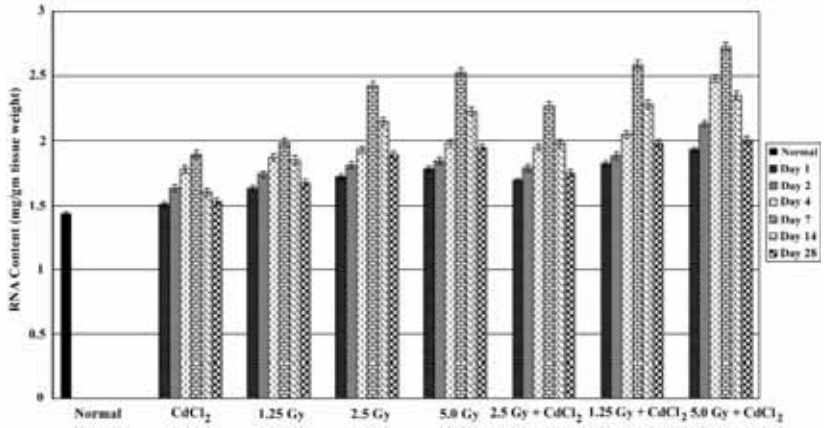

Figure 7. Variation in the values of RNA content (mg/gm tissue weight) in the kidney of mice in various experimental groups.

accelerate the RNA synthesis [33].

Thus it can be deduced that radiation and cadmium exert a synergistic effect on the mice kidney when administered together.

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