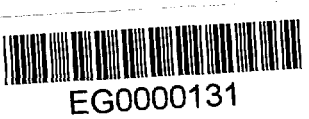
International Conference on HAZARDOUS WASTE : Sources, Effects and Management
12-16 December 1998, Cairo-Egypt



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Treatment of Radiation Induced Biological Changes by Bone Marrow Transplantation

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ABSTRACT

Preventing the propagation of radiation induced oxidative damage has been a subject of considerable investigations. The ultimate goal of the present study is to use bone marrow cells to ameliorate or to treat the radiation sickness. Transplantation of bone marrow cell has shown promising results in the present experimental radiation treatment. In this report, suspension of bone marrow cells was injected into rats 12 h. after exposure to 4.5 Gy whole body gamma irradiation. Significant results were recorded on the successful control of the radiation induced disorders in a number of biochemical parameters including certain enzymatic and nonenzymatic antioxidants (superoxide dismutase & glutathione) and certain parameters related to kidney function including creatinine, urea as well as ATPase activity in blood serum, urine and kidney tissue.

Keywords: Antioxidants / kidney / bone morrow / radiation.

INTRODUCTION

Since the last few decades, there has been a steadily increasing expansion in nuclear and radiation technologies in industry, medicine, energy development and in scientific research. This has been paralleled by a substantial increase in nuclear and radiation installations worldwide. Inspite of the significant contribution of nuclear and radiation technologies to national, regional and international development, yet the problem of radioactive waste management is still imposing serious threat to the welfare of man and his environments.

Human exposure to high doses of penetrating radiation in the general range 1-10 Gy, is known to be potentially lethal within 60 days[1]. There are many historical accounts to support this observation based on critical accidents, reactor failures, misuse of isotopes, and faulty therapeutic practices as well as accumulation of radioactive wastes. These wastes

could exert their effects as external or/and internal radiation sources which increases the hazardous impact to human being.

Besides their direct action, ionizaing radiations interact with biological systems through free radicals generated by water radiolysis[2]. Superoxide and hydroxyl radicals have been identified as the oxygen species primarily involved in cellular damage[3].

The use of chemical drugs for treatment and protection of normal tissues in patients undergoing radiotherapy or chemotherapy or in victims of accidental nuclear exposure is somewhat limited by the drug's toxicity[4]. Therefore, biological treatment of radiation induced damage is promising and safe agent and has been a subject of considerable investigation. It has been anticipated that a successful role played by bone marrow transplants against deleterious effect sof radiation exposure would certainly contribute to efforts made worldwide aiming at effective treatment of radiation victims.

The aim of the present work was twofold. Firstly, assessment of radiation induced disorders in certain sensitive parameters in whole body gamma irradiated rats. Secondly, assessment of the curative effect of transplantation of freshly prepared viable bone marrow cells against the acute manifestation of radiation syndrome.

MATERIALS AND METHODS

Male albino rats weighing 120-140 g were used in the present study and categorized as follows:

Group 1: Untreated animals which served as normal control.

Group 2: The animals were injected intrapertionealy with 3 ml of bone marrow cell suspension which contained $80x10^6$ viable cells/ml. Bone marrow cells were prepared from tibia of the same stain.

Group 3: The animal were exposed to 4.5 Gy whole body gamma radiation. The dose rate was 1.123 Gy/min.

Group 4: The animals were irradiated as group 3 then treated with bone marrow cells as described in group 2. Transplantation was performed 12 h after irradiation.

Irradiation was carried out using the Gamma cell 40, a caesium 137 irradiation unit belonging to NCRRT, Egypt. Animals were sacrificed after 2, 4 and 12 days post irradiation. Six animals were used in each time in each group.

Serum samples were prepared through blood centrifugation at 3000 rpm. Kidney samples were removed, weighed, cooled and homogenized for biochemical determinations. Kits from Bio-Merieux, Frnace, were used for the colorimetric estimation of serum and urine

parameters which included proteins, alkaline phosphatase, urea, creatinine. On the other hand, glutathione in kidney homogenates was estimated as described[5]. The activity of superoxide dismutase was determined using nitroblue tetrazolium and phenazine methosulfate[6]. The activity of total ATPase activity was estimated in kidney as described previously[7]. Kidney alkaline phosphatase activity was determined as described[8]. Serum sodium and potassium concentrations were estimated by flame photometer. Student's t-test was applied to calculate the significance differences.

RESULTS

Effect of Whole Body γ-Irradiation

Table 1 shows serum and urine parameters after 2, 4 and 12 days after irradiation. Rats which were exposed to 4.5 Gy γ-radiation had significantly lower values of protein in serum when compared with control value. On the other hand, protein in urine of irradiated rats was significantly increased. In addition, there were significant increases in alkaline phosphatase activity, urea, creatinine, sodium and potassium concentration in serum of irradiated rats 2, 4 and 12 days after exposure. Moreover, urea and creatinine levels showed significant decreases in urine of the same irradiated rats (Table 1).

Table 2 shows that alkaline phosphatase Activity was increased significantly in kidneys of irradiated rats 2 and 4 days after radiation exposure and showed insignificant change on the 12th day post irradiation. In addition, a significant inhibition of total ATPase activity in kidney was recorded 2, 4 and 12 days after irradiation. The antioxidants, which include glutathione and superoxide dismutase, were significantly decreased in kidney 2 & 4 days post exposure to 4.5 Gy irradiation and showed insignificant change from the control level 12 days of exposure.

Effect of Bone Marrow Transplantation

Tables 1 & 2 indicate that in normal animals, the intrapertioneal injection of bone marrow cells did not produce any significant changes in all studied parameters except at 2nd day after injection in serum protein which increased significantly then showed similar value as control during the next experimentation periods.

Transplantation of bone marrow cells 12 h after 4.5 γ-irradiation produced an increase in serum total protein as compared to irradiated rats but still lower than that of unirradiated rats (table 1). On the other hand, protein in urine was normalized 4 and 12 days post bone marrow transplantation into irradiated rats. In addition, the activity of alkaline phosphatase in serum, levels of urea and creatinine in both serum and urine as well as sodium and potassium concentrations in serum were normalized and showed similar values as the unirradiated control rats (table 1).

The antioxidant system represented by glutathione content and superoxide dismutase activity in kidney were increase in irradiated-bone marrow transplanted rats as compared with irradiated group and showed similar values as the control unirradiated rats (table 2). The activities of ATPase and alkaline phosphatase were also normalized after bone marrow transplantation as compared with unirradiated control (table 2).

DISCUSSION

Effect of Acute Radiation Exposure

The present study showed that irradiation resulted in a significant decrease in total protein level in serum. Our results are in accordance with recent studies[9] that a decrease in serum total protein level post exposure to 7.5 Gy γ-radiation. The decrease in total protein might be attributed to slow rate in synthesis of all protein fractions after irradiation[10]. However, the present results suggest a loss of protein from kidney of irradiated rats. This was confirmed by the significant increased protein level in urine 2, 4, 12 days after irradiation (Table 1), suggesting kidney injury.

Moreover, the increased alkaline phosphatase activity in both serum and kidney of irradiated rats as observed in the present study suggested also kidney injury and this is concordant with those of other studies carried on guinea pigs[11] and on rats[9]. These reports described increases in alkaline phosphatase as well as transaminases activities in irradiated animals. The increase in the alkaline phosphatase activity might be attributed to the damaging effect of radiation on organs including kidney and liver[12].

The radiation induced kidney injury is confirmed in the present study. Significant increases were recorded in both urea and creatinine levels in serum of irradiated rats 2, 4 & 12 days post exposure. This was accompanied with significant decrease in their levels in urine indicating weakened elimination of urea and creatinine from circulation. The present results are in accordance with recent studies[13,14] that γ-irradiation caused renal dysfunction which was reflected by increased concentration of urea and creatinine in blood.

In another study[15], it was reported that arginase activity in liver was increased after irradiation. Since arginase is responsible for the last stage in urea biosynthesis[16], the increase in its activity after irradiation might provide an explanation for the increased urea concentration in plasma. Moreover, radiation exposure has been proved to cause increments

in the activities of both glutamate dehydrogenase and carbamoyl phosphate synthetase enzymes[17]. These observations suggest that the Ornithin-Krebs cycle might be activated from its beginning after irradiation.

In addition, the present results indicated significant increases in the concentrations of both sodium and potassium in serum of irradiated rats. These results are in agreement with recent findings[18] that suggested development of a condition similar to hypertension. The increased Na & K levels in serum might be attributed to kidney dysfunction which lead to electrolyte retention[19].

Biological membranes have been postulated as a cellular target of radiation damage[20]. Reduction of antioxidants is accompanied by increased lipid peroxidation of membranes and is followed by inactivation of membrane-bound enzymes[21]. The present results confirm this idea and demonstrated inhibition of renal ATPase and decreases in both glutathione content and SOD activity in renal tissue of irradiated rats.

The production of substantial amounts of superoxide radical and hydrogen peroxide argues that γ-irradiation could have a direct deleterious effect on renal cells and their constituents. Several authors described some of the molecular mechanisms causing cell death. One of the first events was a substantial drop in both Na/K ATPase activity and the level of glutathione (GSH)[22]. The two events could be explained as a direct action of superoxide. The direct damage of Na/K ATPase caused by superoxide was first observed in eye lens[23]. The drop in GSH level could be explained by direct action of superoxide on glutathione peroxidase, GSH itself and catalase. It is well documented that micromolar concentrations of superoxide radical could cause the inhibition of these enzymes[24]. If the concentration of these enzymes and GSH decrease markedly, the ensuing elevation in H_2O_2 levels will cause an inhibition of SOD activity[25]. The direct action of superoxide radical on GSH in the absence of antioxidant enzymes can cause a chain reaction, facilitating the formation of oxidized glutahtione[26] and mixed disulfides. Finally, significant lipid peroxidation accompanied by degradation of membrane and the cellular function and structure may favour apoptosis[3].

Effect of Transplantation of Bone Marrow On Radiation Effects

In the present study, administration of viable bone marrow cells 12 h after irradiation, produced marked restoration of radiation induced changes in total proteins, urea, creatinine, Na, K, activities of alkaline phosphatase and ATPase as well as the antioxidants including GSH and SOD activity. These finding support previous results [27] that shielding of femur during irradiation was followed by early recovery from radiation sickness and associated with elevated $LD_{50/30}$ of irradiated mice which received bone marrow cells after exposure. It was also reported that rats exposed to 8 Gy whole body gamma radiation, bone marrow

transfusion markedly diminished the severity of the general manifestation of acute radiation syndrome[28].

It is anticipated that viable bone marrow cells contain sufficient amounts of enzymatic, and non-enzymatic antioxidants including SOD, catalase, glutathione peroxidase and glutathione, and probably vitamins C and E. The administration of substantial amounts of these viable cells might reinforce the antioxidant capacity of cells and tissues by activating antioxidant recycling mechanism of the renal cells which can restore the balance between oxidant process and the antioxidant defense resulting in a curative effect[29]. This is supported by normalization of superoxide dismutase activity and glutathione content in renal cells of irradiated - bone marrow transplanted rats (table 2).

It can be concluded that post-irradiation treatment with bone marrow cells seems to exert a potential curative role against the deleterious biological effects of radiation overexposure. Such findings seem to be of particular interest to physicians dealing with radiotherapy and radiodiagnosis, to radiobiologist dealing with mechanisms of radiation illness and repair as well as to health physicists dealing with protective measures against hazards of occupational and accidental radiation exposure.

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Table 1. Tested parameters in serum and urine of control and different treated groups.

	Control	Bone marrow treated group			Irradiated group			Irradiated and bone marrow treated group		
		2 days	4 days	12 days	2 days	4 days	12 days	2 days	4 days	12 days
Serum total protein	6.24	7.19*	6.83	6.76	4.99*	5.55*	5.39*	5.10*	5.70*	5.76*†
g/100 ml	±0.13	±0.08	±0.59	±0.29	±0.09	±0.19	±0.12	±0.24	±0.22	±0.15
Urine total protein	0.32	0.36	0.35	0.35	1.20*	1.12*	1.15*	0.49*†	0.33†	0.32†
g/100 ml	±0.007	±0.03	±0.04	±0.05	±0.029	±0.011	±0.12	±0.13	±0.01	±0.01
Serum alkaline phosphatase	137.56	138.18	138.51	137.28	220.6*	300.48 *	487.52*	140.28†	135.40†	137.96†
KAU/100 ml	±1.17	±2.55	±2.25	±4.90	±6.78	±7.99	±3.94	±7.80	±4.76	±3.94±
Serum urea	19.78	21.62	20.06	19.44	38.41*	37.93*	31.45*	33.07†	20.76†	19.65†
mg/100 ml	±0.77	±1.68	±1.58	±1.12	±3.09	±2.49	±1.59	±2.22	±1.29	±0.43
Urine urea	235.9	236.2	237.3	235.8	114.68*	114.1*	212.9*	234.5†	235.9†	238.3†
mg/100 ml	±0.76	±2.59	±2.84	±2.15	±0.52	±0.66	±1.60	±2.76	±2.74	±1.89
Serum creatinine	0.56	0.52	0.53	0.53	2.00*	2.88*	1.61*	0.58†	0.57†	0.56†
mg/100 ml	±0.02	±0.08	±0.05	±0.02	±0.09	±0.24	±0.04	±0.04	±0.02	±0.03
Urine creatinine	1.82	2.00	1.72	1.79	0.49*	0.60*	0.87*	1.75†	1.79†	1.83†
mg/100 ml	±0.09	±0.07	±0.05	±0.05	±0.005	±0.01	±0.01	±0.04	±0.06	±0.03
Serum Na^+	333.7	340.8	335.8	335.4	415.3*	431.6*	441.7*	343.7*†	340.1†	336.5†
mg/100 ml	±3.2	±4.5	±3.1	±4.3	±5.0	±5.6	±4.3	±6.3	±4.8	±3.1
Serum K^+	31.41	35.76	34.42	33.88	56.48*	85.87*	54.99*	33.89†	33.58†	32.04†
mg/100 ml	±1.61	±4.26	±2.94	±1.81	±4.67	2.87	±4.14	±2.50	±2.40	±2.99

Values are meant ±SE of sex animals

* Significance compared to control non-irradiated P>0.05

† Significance compared to control irradiated rats P>0.05.

Table 2. Tested parameters in kidney of control and different treated groups.

	Control	Bone marrow treated group			Irradiated group			Irradiated and bone marrow treated group		
		2 days	4 days	12 days	2 days	4 days	12 days	2 days	4 days	12 days
Glutathione	0.32	0.35	0.34	0.33	0.21*	0.25*	0.28	0.33†	0.33†	0.35†
mg/g FT	±0.03	±0.02	±0.01	±0.01	±0.02	±0.02	±0.02	±0.01	±0.02	±0.02
Superoxide dismutase	34.76	33.84	34.05	35.60	22.53*	25.00*	30.70	35.69†	36.80†	35.92†
% Inhibition/mg FT	±2.78	±2.13	±4.32	±3.19	±2.89	±3.10	±3.25	±4.15	±3.97	±3.76
ATPase	57.84	56.69	58.84	61.36	22.04*	28.18*	34.33*	53.14†	56.87†	60.25†
µmol Pi/min/g FT	±0.75	±1.35	±1.94	±4.27	±0.54	±1.32	±2.13	±4.76	±1.58	±9.18
Alkaline phosphatase	10.22	8.80	9.52	10.92	16.09*	15.06*	12.49	12.36†	11.35†	10.15
KAU/g FT	±1.49	±1.16	±1.30	±1.19	±1.68	±1.27	±1.19	±1.52	±1.40	±0.16

Values are meant ±SE of sex animals

* Significance compared to control non-irradiated P>0.05

† Significance compared to control irradiated rats P>0.05..

F.T fresh tissue