Ameliorating Effects of Bone Marrow Transplantation and Zinc Supplementation on Physiological and Immunological Changes in γ-Irradiated Rats

Omaima M. Ashry*, Maha G. Soliman**, Mervat A.E. Ahmed ** and Yasmine H. Abd El-Naby**

** Radiation Biology Department, National Center for Radiation Research and Technology (NCRRT), P.O. Box 29 Nasr City, Egypt.

** Faculty of Science, Al-Azhar University for Girls, Zoology Department.

(NCRRT), P.O. Box 29 Nasr City, Egypt. E mail: Omaimashry@yahoo.com

ABSTACT

Purpose: **The present study was carried out to determine the prophylactic impact of zinc sulphate administration to irradiated rats treated with bone marrow transplantation (BMT) as indicated by the hematological and immunologic response as well as oxidative stress.**

Material and methods: **Rats were injected orally with zinc sulphate, 10 mg/ Kg body wt, daily for 2 weeks before whole body 5Gy gamma irradiation and intravenous injection of bone marrow cells, one hour post irradiation.**

Results: **The results revealed a significant decrease in red blood cells (RBC), white blood cells (WBC), glutathione (GSH) and zinc superoxide dismutase (Zn /SOD), splenocyte count as well as bone marrow lymphocyte count and viability of irradiated rats. Regarding immunological data: tumor necrosis factor alpha (TNF-α) and interleukin 2 (IL-2) recorded a significant decrease while interleukin 6 (IL-6) and lipid peroxidation product (MDA) in the serum and spleen were conversely elevated. Zn supplementation before irradiation and BMT and showed significant decrease of serum and tissue MDA compared to the irradiated group. Lymphocytes, bone marrow viability percentage, splenocytes percentage, IL-2, IL-6 and GSH were significantly elevated compared to irradiated group.**

Conclusion: **Protection with Zn, enforcing significant innate response, could trigger and augment adaptive immune response by BMT which suggests its use to protect against radiation hazards.**

Key words: BMT; gamma irradiation; zinc sulphate;

Aim of the Work

This work aims to investigate the effect of boosting of immune response, by zinc administration before irradiation followed by bone marrow transplantation (BMT) on immunological recovery and oxidative stress induced by gamma irradiation.

Animals and Methods

Mature male albino rats of pure strain Rattus rattus (110-130g). Animals were randomly assigned into 6 groups:

1.Control rats received distilled water throughout the experiment(C).
2. Rats injected with BMT cells through the caudal vein (CBM).
3. Rats received orally 10mg / Kg body weight of zinc sulphate as a single daily dose for 14 successive days (CZn).
4. Rats exposed to 5Gy whole body gamma rays (R).
5.Rats exposed to 5Gy gamma rays and treated with BMT one hour after irradiation (R + BM).
6. Rats received 10mg / Kg body weight of zinc sulphate for 14 successive days before 5Gy irradiation (R+Zn).
7. Rats received orally 10mg / Kg body weight of zinc sulphate as a single daily dose for 14 successive days before irradiation and treated with BMT one hour after irradiation (R+BM+Zn). All animal groups were sacrificed after 14 days from treatment, irradiation or pre-irradiation treatment and BMT.

Irradiation

Whole body irradiation was performed using Gamma Cell – 40 (137 Cesium) biologicalirradiator manufactured by Canada Ltd, Ottawa, Ontario, Canada. located at NCRRT. Animals were irradiated at an acute single dose of 5Gy at a dose rate of 0.49Gy/min.

Bone Marrow Transplantation

Donors and recipients were chosen of the same inbred strain.Femur bones were dissected out and cleaned. The ends of the bones were chipped by a bone nibbling forceps and the marrow was blown out of the femur into isotonic solution under sterilized conditions inside a laminar flow cabinet. The marrow was collected into a sterile container surrounded by ice cubes

Animals and Methods (Cont.)

asyringe without needle in order to avoid mechanical damage to the cells. Femur marrow cells (1x 10 ^{7}) (Chen et al.2007) were injected intravenously (i.v.) Zinc sulfate from zito each rat, 1 hour after irradiationnd mixed by drawing and expelling it several times from a

Zinc Supplementation

Zinc stock solution of 80mM, which was then sterile filtered. Rats were injected with 10mg / Kg body weight (oral injection) as a single daily dose for 14 successive days before irradiation

Measured Parameters:

- Blood reduced GSH and Zn/ SOD contents
- ELIZA for IL-2 , IL-6 and TNF-α concentrations in plasma
- Dissection of spleen and a known weight of spleen was homogenized in 0.15KCl to obtain 10% tissue homogenate to estimate MDA
- Determination of viable BM cell percentage
- Determination of blood parameters

RESULTS

Table 1: Effect of BMT and Zn Supplementation on Some Blood parameters: Total Red Blood Cells, Total White Blood Cells and Lymphocytes, in Irradiated and Non-irradiated Rats.

Groups	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Lymphocytes (%)
Control	7.32±0.38	6.04±0.57	44.8±0.37
CBM	6.92±0.31	5.14±0.42	37.4±0.73 c
CZn	6.74±0.43	6.14±0.44	38.6±0.6 c
R	5±0.3 c	2.52±0.46 c	22.2±0.86 c
R + BM	6.18±0.27 r	3.52±0.45 c	31.4±0.25 c r
R + Zn	6.32±0.46 r	5.14±0.31 r	37±0.35 c r
R + BM + Zn	6.54±0.44 r	4.66±0.73 r	39.4±0.5 c r

Values are expressed as mean ± SE.c: Significant difference compared to control. r: Significant difference compared to R group.

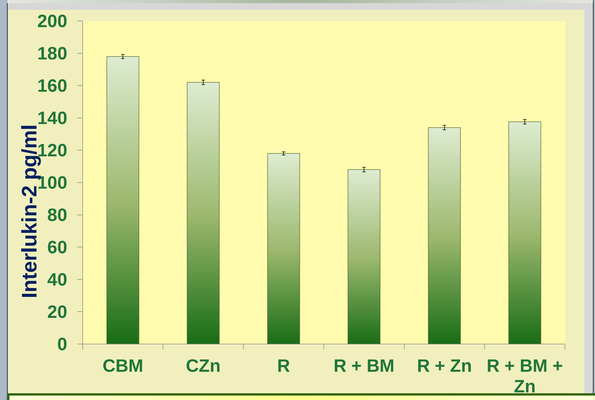

Figure 1: Effect of BMT and Zn Supplementation on Interlukin-2 in Irradiated and Non-irradiated Rats.

RESULTS (Cont.)

Table 2: Effect of BMT and Zn Supplementation on Bone M arrow percentage, Viability Percentage and Splenocyte Percentage in Irradiated and Non-irradiated Rats.

Groups	Bone marrow lymphocyte count (%)	Viable bone marrow count (%)	Splenocyte (%)
Control	20.4±0.5	66.4±0.5	77±0.7
CBM	21±0.7	65±0.7	74.6±1.2
CZn	20±0.54	64.4±0.81	77.2±0.86
R	13.4±0.5 c	41.2±0.58 c	44.4±1.91 c
R + BM	16.16±0.60 c r	50.4±0.51 c r	52.4±0.81 c r
R + Zn	13.33±0.80 c r	52.6±0.4 c r	52.8±0.86 c r
R + BM + Zn	18±0.31 c r	67.8±0.37 c r	57±0.54 c r

Values are expressed as mean ± SE.
c: Significant difference compared to control. r: Significant difference compared to R group.

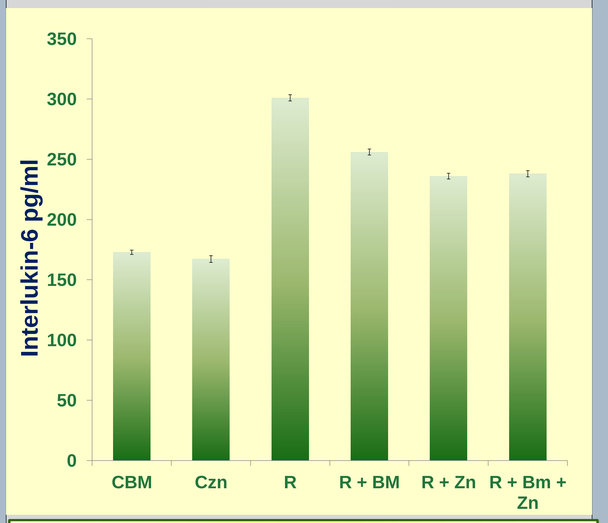

Figure 2: Effect of BMT and Zn Supplementation on Interlukin-6 in Irradiated and Non-irradiated Rats.

RESULTS (Cont.)

Table 3: Effect of BMT and Zn Supplementation on Bone M arrow percentage, Viability Percentage and Splenocyte Percentage in Irradiated and Non-irradiated Rats.

Groups	GSH (mg/ml)	MDA (μ mol/ml)	MDA (n mol/g tissue)	Zn/SOD (μg/ml)
Control	31.2±0.58	37±0.7	40.8±0.73	4.62±0.39
CBM	29.8±0.58	40±1 c	50±1 c	4.24±0.2
CZn	30.8±0.37	36.6±1.12	46.6±1.12 c	4.26±0.34
R	10.8±0.58 c	62.2±0.86 c	70±1 c	2.56±0.18 c
R + BM	16.2±0.86 c r	46.2±1.15 c r	53.2±1.46 c r	3.26±0.23 c
R + Zn	18.4±0.51 c r	47.4±1.2 c r	57.6±1.02 c r	3.5±0.4 c
R + BM + Zn	23.2±0.66 c r	43.8±1.59 c r	53.8±1.59 c r	3.6±0.41 c r

Values are expressed as mean ± SE.
c: Significant difference compared to control. r: Significant difference compared to R group.

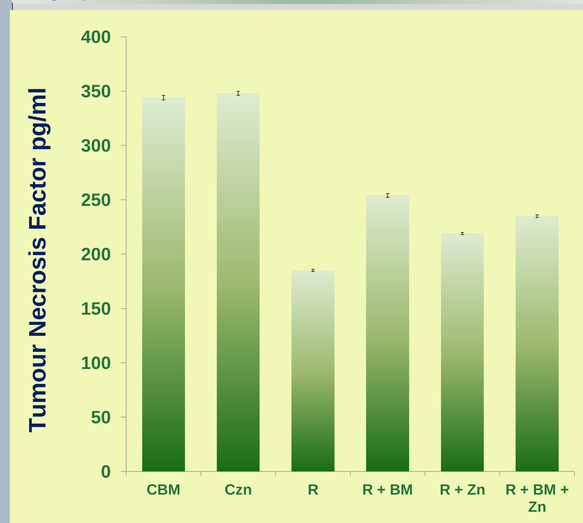

Figure 3 : Effect of BMT and Zn Supplementation on TNF-ά in Irradiated and Non-irradiated Rats.

Conclusion

The present findings confirmed protective potential of zinc sulphate administration against the severity of radiation induced disturbance and in enforcing allogenic bone marrow transplantation and the immune response. Zinc should be further evaluated for its radioprotective potential in a clinical setting..