Role of Tempol on Redox System and Lipid Peroxidation in Gamma Irradiated Rats

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Abstract: The present study was designed to evaluate the potential efficacy of tempol a superoxide dismutase mimetic agent against redox imbalance and cytotoxicity induced by protracted exposure to gamma rays. Tempol was orally administered to Sprague Dawley male albino rats via intragastric intubation (40 mg / Kg body wt /day) for 7 days before exposure to gamma-rays and continued during the whole period of irradiation processing. Whole body γ- rays was delivered at 3 doses of 2 Gy increment every week up to total cumulative dose of (6 Gy) in 3 weeks. The results obtained showed increased level of lipid peroxides contents and xanthine oxidase (XO) activity in irradiated animal groups with concomitant depletion in the level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GSH-Px). Administration of tempol has significantly lowered the level of lipid peroxidation and enhanced the antioxidant status of irradiated animals. It could be concluded that tempol exerts a protective effect against radiation-induced cytotoxicity by modulating the extent of peroxidation and augmenting antioxidant defence system.
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1.Introduction

Exposure to ionizing radiation initiates a cascade of events that are based not only on direct DNA damage (Moulder, 2004) but also on other effects including oxidative-damage that leads to alteration of tissue physiological functions (Ropenga et al., 2005).Meanwhile, lipid peroxidation of biological membranes can cause alterations in fluidity, reductions in membrane potential, increased permeability to H^+ and other ions and eventual membrane rupture leading to release of cell and organelle contents (Geetha *et al.,* 2004). Moreover, cytotoxic – aldehydes resulting from lipid peroxidation could block macrophage action, inhibit protein synthesis, inactivate enzymes, cross-link proteins and lead to the generation of thrombin (Marnett *et al.,* 2005). In higher eukaryotes, reactive oxygen species (ROS) are generated normally during cellular-metabolism in the course of molecular oxygen reduction as well as by distinct enzyme system.ROS have been implicated in the regulation of diverse cellular functions including defence against pathogens, intracellular signaling, transcriptional activation, cell proliferation, and apoptosis (Davis *et al.,* 2003). ROS production *in- vivo* include superoxide anion radical (O^-_2), hydrogen peroxide (H_2O_2), and singlet-molecular oxygen, as well as hypochlorite, nitric oxide and peroxynitrite. H_2O and O^-_2 can interact in the presence of certain transition metal ions to yield a highly- reactive oxidizing species, the hydroxyl radical (OH^o) (Droge, 2002).The reduction-oxidation (redox) state of the cell is a consequence of the balance between the levels of oxidizing ROS and reducing equivalents.

Elevation of ROS in excess of buffering capacity and enzymatic activities designed to modulate ROS level result in potentially cytotoxic "oxidative stress" (Kohen and Nyska, 2004). The oxidation induced by ROS can result in cell membrane disintegration, membrane protein –damage and DNA-mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver-injury and cardiovascular-disease (Nishinaka *et al.,* 2003). The body possesses many defence mechanisms, as enzymes and antioxidant-nutrients, which arrests the damaging properties of ROS (Wilson *et al.,* 2001). However, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radical in the body beyond its capacity to control them, and cause irreversible oxidative damage (Clark, 2003). Therefore, antioxidants with free radical scavenging activity may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radical are implicated (McDermott, 2000).

4-Hydroxy-2, 2, 6, 6-tetramethylpiperidin-1-oxyl (Tempol) is an antioxidant drug, specifically, a superoxide dismutase mimetic agent, which is able to metabolize superoxide anions due to the presence of a reducible nitroxide group (Wilcoxn, 2008). This drug has previously been used in a mouse model of pre-eclampsia, the BPH/5 mouse. This is a model which spontaneously develops some of the hallmark features of pre-eclampsia, namely, onset of hypertension and

proteinuria (Davisson *et al.,* 2005). Administration of Tempol to BPH/5 mice was able to ameliorate placental oxidative stress and rescue fetal growth (Hoffmann *et al.,* 2008). An advantage of using Tempol, as opposed to other antioxidant therapies, is that it is able to permeate the membrane and specifically targets superoxide anions that are produced intracellularly.

Tempol can inhibit the aforementioned reaction between superoxide and NO and thus release the inactivated NO. Short- and long-term administration of tempol has been shown to increase medullary blood flow in rats by 35–50% and reduce mean arterial pressure (**Fenoy** *et al.,* 2003). More recently several studies have shown that TEMPOL also demonstrates potent anti-inflammatory activity (Cuzzocrea *et al.,* 2004).

The present study was designate to evaluate the potential efficacy of tempol, against redox imbalance state as well as lipids peroxidation induced through protracted exposure to gamma rays.

2.Material and Methods

Sprague Dawley male albino rats (110 ± 10 g) were obtained from the Egyptian Holding Company for Biological Products and Vaccines; Cairo, Egypt. Animal were maintained on a12/12h light-dark regimen.

All animals were housed in a certified animal care facility and given starter poultry pellets and water *ad libitum.* All animals received human care in compliance with institutional guidelines.

Radiation process

Irradiation processing was performed with a Canadian gamma cell-40. (^{137}Cs) at (NCCRT). Cairo, Egypt. Animals were submitted to fractionate whole body γ- irradiation; 2 Gy instalments every week at dose rate of (0.61 Gy/min) up to 6 Gy total dose (3 weeks).

Tempol treatment

Tempol was purchased from Sigma-Aldrich Co., St. Louis Mo, USA. Animals received tempol orally for 7 consecutive days before radiation-exposure and treatment was continued during the whole period of irradiation processing (3 weeks). Dissolve 4 gm of Tempol Crystal in 100 ml 0.9% sterile saline each of 0.1 ml was contain on 4o mg /Kg b.w. was administrated based on protocol described by Feng *et al.* (2001).

Experimental design

The rats were randomly distributed into 4 groups of 8 animals each.

Control group: served as controls.**Tempol group**: received orally tempol 4o mg /Kg b.w. daily before exposure to radiation.**Irradiated group (IR)**: submitted to fractionate whole body gamma-rays. **Tempol plus IR group**: administered tempol for 7 days before exposure and continued during protocol of γ- radiation exposure (3 weeks).

Samples Collection

Animals were fasted over nigh prior to sacrificing.Samples were collected one hour post-irradiation processing (2Gy, 4Gy and 6Gy).Whole blood was collected by heart puncture after light anaesthesia using heparinised syringes. Livers were dissected out, washed, weighed and homogenized in ice cold 0.1 M potassium phosphate buffer (pH7.4) to yield (W/v) 10% homogenates.

Biochemical assays

Lipid peroxide content was determined by quantifying the thiobarbituric acid reactive substance (TBARS) in plasma and tissue homogenates after the method described by Yohioka *et al.* (1979). XO activity was assayed according to the method described by Bergmeyer *et al.* (1974).SOD activity was determined according to the method of Minami and Yoshikawa (1979). GSH content was performed according to Beutler *et al.* (1963). CAT activity was determined according to the method described by Johansson and Hakan Borg (1988). Estimation of GSH-px in whole blood and liver homogenate was performed after the method of Gross *et al.* (1967). Haemoglobin content was determined after (Wintrobe, 1965) Tissues total protein content was assayed according to Lowry *et al.* (1951).

3.Results

Table 1 shows that the concentration of lipid peroxides (TBARS) was significantly increased ($P \leq 0.05$) in irradiated animals (2, 4, and 6 Gy) in examined plasma and hepatic tissues vs. to control rats. Moreover, TBARS levels in the plasma and liver of tempol treated and irradiated animals were significant decreased as compared to irradiated animals ($P \leq 0.05$).

Table 2 shows that the activity of XO was significantly increased ($P < 0.05$) in irradiated animals (2, 4, and 6 Gy) in examined plasma and hepatic tissues as compared to control groups. However, XO activity in plasma and liver of tempol treated and irradiated groups were significantly decreased as compared to irradiated animals ($P < 0.05$) especially at (4 and 6 Gy).

Table 1: Effect of tempol administration on thiobarbituric acid reactive substance (TBARS) concentration in rats gamma-irradiated.

Tissues and Dose of irradiation		Animal groups			
		Control	Tempol	IR	Tempol+IR
Blood µmol/dl	2 Gy	0.88±0.07	0.90±0.05	1.11±0.08[a,b]	0.96±0.14[a,b,c]
	4 Gy	0.91±0.09	0.89±0.06	1.92±0.05[a,b]	1.58±0.12[a,b]
	6 Gy	1.08±0.07	0.98±0.09	2.36±0.11[a,b]	1.70±0.03[a,b,c]
Liver nmol/g wet tissue	2 Gy	260.21±4.5	258.61±8.81	325.25±12.22[a,b]	261.45±11.80[a,b,c,]
	4 Gy	265.13±7.2	247.83±4.71	403.75±14.01[a,b]	307.15±12.60[a,b,c]
	6 Gy	268.17±7.7	265.63±7.33	436.81±12.72[a,b]	318.66±7.83[a,b,c]

Each value represents the mean of 8 records±SE.
a- Significant difference versus corresponding control group at $P \leq 0.05$.
b- Significant difference versus corresponding tempol group at $P \leq 0.05$.
c- Significant difference versus corresponding γ- irradiated group at $P \leq 0.05$.

Table 2: Effect of tempol administration on xanthine oxidase (XO) activity levels in rats gamma- irradiated.

Tissues and Dose of irradiation		Animal groups			
		Control	Tempol	IR	Tempol+IR
Plasma U/ml	2 Gy	0.80±0.04	0.81±0.09	1.29±0.21[a,b]	1.14±0.11[a,b]
	4 Gy	0.75±0.03	0.74±0.07	1.69±0.17[a,b]	1.18±0.08[a,b,c]
	6 Gy	0.71±0.06	0.70±0.04	2.01±0.15[a,b]	1.52±0.11[a,b,c]
Liver U/mg Proteins	2 Gy	10.12±1.00	9.77±0.50	16.70±0.33[a,b]	14.25±0.66[a,b]
	4 Gy	9.77±0.65	9.23±0.70	18.77±1.40[a,b]	13.11±0.81[a,b,c]
	6 Gy	8.35±0.80	9.17±0.73	20.10±2.00[a,b]	14.00±1.21[a,b,c]

Legend as in table 1

The examined antioxidant enzymatic activities SOD, CAT and GSH-px are illustrated in Tables 3, 4, 5. The experimental results pointed to significant change ($P \leq 0.05$) in the activites of SOD, CAT and GSH-Px in blood and liver tissues of rats subjected to fractionated γ-irradiation (2, 4 and 6 Gy) as compared to control groups ($P \leq 0.05$).

Table 3: Effect of tempol administration on the activity of superoide dismutase (SOD) in rats gamma- irradiated.

Tissues and Dose of irradiation		Animal groups			
		Control	Tempol	IR	Tempol+IR
Blood U/g Hb	2 Gy	400.65±6.71	390.6±5.50	407.15±7.72	403.00±12.12
	4 Gy	440.01±7.62	220.0±12.21	263.00±13.0[a,b]	325.75±15.10[a,b,c]
	6 Gy	425.00±5.65	415.77±10.14	254.00±9.00[a,b]	314.50±14.61[a,b,c]
Liver U/mg Proteins	2 Gy	7.10±0.22	7.05±0.50	4.12±0.37[a,b]	5.23±0.53[a,b]
	4 Gy	7.0±0.20	7.30±0.53	3.66±0.42[a,b]	4.93±0.62[a,b]
	6 Gy	8.60±0.83	9.12±1.07	2.82±0.29[a,b]	5.48±0.55[a,b,c]

Legend as in Table

The results obtained showed remarkable improvement in SOD, CAT and GSH-Px activities levels in rat's administered prolonged tempol before and during irradiation exposure ($P \leq 0.05$) as compared with corresponding data from irradiated rats (Tables 3,4 and 5).

Table 4: Effect of tempol administration on the activity of catalase (CAT) in rats Gamma–irradiate

Tissues and Dose of irradiation		Animal groups			
		Control	Tempol	IR	Tempol+IR
Blood U/g Hb	2 Gy	22.85±0.54	21.75±0.55	15.56±1.05[a,b]	18.43±1.00[a,b]
	4 Gy	25.11±1.03	24.82±0.82	12.33±0.54[a,b]	16.99±1.02[a,b,c]
	6 Gy	24.15±0.18	23.77±1.18	12.08±0.51[a,b]	15.82±0.77[a,b,c]
Liver U/mg Protein	2 Gy	2.32±0.14	2.10±0.06	1.55±0.06[a,b]	1.98±0.05[a,b]
	4 Gy	2.45±0.15	2.33±0.10	1.24±0.05[a,b]	1.71±0.07[a,b,c]
	6 Gy	2.35±0.12	2.39±0.12	1.11±0.01[a,b]	1.64±0.08[a,b,c]

Legend as in Table 1

Table 5: Effect of tempol administration on the activity of glutathione peroxidise (GPX-Px) in rats gamma-irradiated.

Tissues and Dose of irradiation		Animal groups			
		Control	Tempol	IR	Tempol+IR
Plasma U/ml	2 Gy	80.20±2.41	82.06±2.22	82.10±3.11	85.20±4.20
	4 Gy	85.10±4.31	88.00±3.21	59.35±3.03[a,b]	66.75±4.25[a,b]
	6 Gy	90.60±4.22	89.38±3.21	56.31±2.50[a,b]	69.15±3.38[a,b,c]
Liver U/mg Protein	2 Gy	12.60±1.05	11.05±1.06	11.92±0.81	12.17±1.20
	4 Gy	13.57±1.21	12.35±0.77	7.77±0.80[a,b]	11.31±0.54[a,b,c]
	6 Gy	14.49±0.78	12.82±0.61	6.85±0.52[a,b]	10.30±0.61[a,b,c]

Legend as in Table 1

The concentration of GSH in blood and liver of rats exposed to fractionated γ- rays (2, 4 and 6 Gy) with or without tempol administration are present in Table 6. There were no significant changes in blood and liver GSH levels between different groups of animals after exposure to 4 Gy of Gamma –rays. However, there were marked significant declines ($P \leq 0.05$) in blood and liver samples at (4 and 6 Gy) as compared to control groups. The prolonged administration of tempol before and exposure to fractionated gamma –irradiation induced a significant amelioration in GSH contents in blood and liver comparing with irradiated animals ($P \leq 0.05$).

Table 6: Effect of tempol administration on the concentrations of reduced glutathione (GSH) in rats gamma-irradiate

Tissues and Dose of irradiation		Animal groups			
		Control	Tempol	IR	Tempol+IR
Blood mg/dl Packed cells	2 Gy	50.50±1.11	48.32±1.42	47.13±1.21	51.11±1.40
	4 Gy	50.18±1. 42	51.18±1.33	35.19±0.42	42.22±2.12
	6 Gy	46.80±1.32	48.50±1.70	32.81±1.32	39.85±2.15
Liver mg/g wet tissue	2 Gy	32.10±1.33	31.50±1.82	30.20±1.22	33.50±1.11
	4 Gy	30.25±2.10	32.30±1.25	22.80±1.16	23.52±0.05
	6 Gy	31.30±2.08	34.00±1.52	17.70±1.12	23.25±1.13

Legend as in Table 1

4.Discussion

Radiation-induced oxidative stress and injury to endothelial cells might contribute to several disease; one of the most frequent pathologies to appear after whole-body irradiation (Williams *et al.,* 2004). Meanwhile, lipide peroxidation of biological membranes can cause alterations in fluidity, reductions in membrane potential, increased permeability to H^+ and other ions and eventual membrane rupture leading to release of cell and organelle contents (Geetha *et al.,* 2004). Recently, there has been an increasing interest in the protective function of antioxidant, which are candidates for the treatment of atherosclerosis, cancer chemoprevention and extending lifespan (Valentao *et al.,* 2002). In the present study, experimental animals receiving 40 mg/kg body wt tempol, for 4 weeks showed no significant changes in the redox system (Tables 1,2,3,4,5 and 6). To counteract the effects of oxidative stress,cells have developed two important defence mechanism: a thiol reducing buffer consisting of small protein with redox-active sulfhydryl moieties e.g., GSH and thioredoxin (TRX) and enzymatic systems (e.g SOD, CAT, nad GSH-Px) (Nakamura *et al.,* 1999).

In the present study, bloods and liver TBARS content exhibit a pronounced significant increase in animals exposed to γ- irradiation (Table 1) compared to control groups. The recorded increases in TBARS contents could be explained on the basis that ionizing radiation induced lipid peroxidation through the generation of ROS which attack the polyunsaturated fatty acid constituents of the cell membrane and other cell biomolecules initiates a self perpetuating chain reaction that yields a wide range of cytotoxic products such as TBARS (Gutteridge, 1995). Lipid peroxides, can participate in redox Reactions, the nature and magnitude of which often determines whether Peroxidative injury is exacerbated or prevented (Girotti, 1998). in addition, lipid hydroperoxides or related peroxidative intermediates by- products may trigger signal transduction pathways calling for either greater cytoprotection through up- regulation of detoxifying and antioxidant enzymes or deliberate al. termination to apoptotic or necrotic death (Suzuki *et al.,* 1997).

O^-_2+ the product of a one- electron reduction of oxygen, is the precursor of most ROS and a mediator in oxidative chain reaction. *In vivo*, O^-_2 is produced both enzymatically and nonenzymatically.

Enzymatic sources include NADPH oxidases located on the cell membrane of polymorphonuclear cells. Macrophages and endothelial cells (Babior *et al.,* 2002). The proteolytic conversion of xanthine dehydrogenase (XD) to XO provide anther enzymatic source of both O^{2-} and H_2O_2 (and therefore constitutes a source of hydroxyl radical) and has been proposed to mediate deleterious processes (Turrens, 2003).

XO, a member of molybdo-flavoenzymes family, has long been recognized mainly for its role as the rate-limiting enzyme in nucleic acid degradation (Garattini *et al.,* 2003). XO catalyzes the oxidation of hypoxanthine /Xanthine to urate with concomitant generation of O^-_2 (Berry and Hare, 2004). Plasma XO level seems to be a sensitive marker of liver damage (Zima *et al.,* 2000). Its higher activity mayhave an impact on hepatocellular injury (Schimpl *et al.,* 2002). The results of the present study showed that exposure of rats to ionizing radiation significantly increase the specific activity of XO in plasma and liver tissues as compared to control rats, (Table 2). This implies a relation between increases in XO activities and endothelial injuries secondary to increased oxidative damages (Zoccali *et al.,* 2007).

SOD catalyse the dismutation of the highly reactive O^-_2 to O_2 and to the less reactive species H_2O_2 (Matsumoto and Fridovich, 2003).Numerous studies have shown the importance of SOD in protecting cells against oxidative stress (Huang, 1997). Thus the decrease in the activity of SOD observed in the present study could be due to a feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation.

CAT, which acts as preventative antioxidant plays an important role in protection against the deleterious effects of lipid peroxidation (Pigeolot *et al.,* 1990).In the present study, the inhibition of CAT activity is suggestive of enhanced synthesis of O_2. during the exposure to gamma rays since O^-_2 is a powerful inhibitor of CAT (Ashakumary and Vijayammal, 1996).

GSH-Px has a well-established role in protecting cells against oxidative injury. GSH-PX utilizes GSH as a substrate to catalyse the reduction of organic hydroperoxides and H_2O_2 (Ray and Husain, 2002) Therefore the excess H_2O_2 and lipid peroxides generated during radiation exposure are efficiently scavenged by GSH-Px activity. In the present study, the depression of this enzyme activity reflects perturbations in normal oxidative mechanisms during irradiation exposure.

CAT and GSH-Px which act as preventive antioxidants, SOD, a chain breaking antioxidant, play an important role in protection against the deleterious effect of lipid peroxidation.

Depletion of the activities of SOD, CAT and GSH-Px in the blood and liver tissues of irradiated rats may be due to the increased utilization of these antioxidants to counter lipid peroxidation (Kalpana and Menon, 2005).

GSH is the most abundant non protein sulfhydryl- containing compound and constitutes the largest component of the endogenous thiol buffer (Holmgren *et al.,* 2007). Assessment of GSH in biological samples is essential for evaluation of the redox homeostasis and detoxification status of cells in relation to its protective role against oxidative and free radical-mediated cell injury (Rossi *et al.,* 2005).The present study, exhibit a significant depletion of GSH concentration (Table 6) in blood and liver tissues in animals exposed to protracted γ-radiation mostly pronounced at 4 Gy and 6 Gy, compared to control groups. While exposure to 2 Gy did not show noticeable change, which might be due to adaptive protective responses against oxidative damage through redox signalling to maintain redox homeostasis (Droge, 2002).

GSH has diverse cellular functions in addition to its antioxidant properties including enzymatic conjugation through the glutathione S-transferase family of proteins and nonenzymatic conjugation to cytotoxic compounds. It is kept in its reduced state by the NADPH-dependent enzyme, glutathione disulfide reductase. Moreover, GSH may react with H_2O_2 ang lipid peroxides by the action of GSH-Px to reduce their toxicity (Davis *et al.,* 2001). In the present study, the decreased level of GSH contents in rats exposed to 4 and 6 Gy might be due to enhanced utilization during detoxification process.

In the present study, the action of tempol as a synthetic antioxidant on the redox system and lipid peroxidation and responsiveness to some parameters is evaluated.Tempol can penetrate cell membranes and reacts with both intracellular and extracellular oxygen-free radicals **(**Beckman and Koppenol, 2007). These properties make tempol attractive for treatment of some diseases associated with oxidative stress (Simonsen *et al.,* 2009).

Administration of tempol at doses of 40 mg/kg/day ameliorated the changes induced by irradiation exposure supporting the hypothesis that tempol products are effective antioxidative agents. Tempol by scavenging or neutralizing free radicals (Adeagbo *et al.,* 2006); interacting with oxidative cascade and preventing its outcome; quenching singlet oxygen it making it less available for oxidative reaction (Hoffmann *et al.,* 2008): inhibiting oxidative enzymes like cytochrome P450 and inhibits peroxidation of membrane lipids and maintains cell membrane integrity and their function **(**Cuzzocrea *et al.,* 2006). Furthermore**,** Preti *et al.* (2005) found that**,** conversion of XD to XO is reduced by tempol to the

basal level in vitro study. They suggested that, the major inhibitory mechanism of tempol on increases in XO enzyme activities is through direct inactivation at the protein level. Therefore, tempol may stabilize the cell membrane and significantly reduced the extent of lipid peroxidation in the blood and liver induced by oxidative stress-released under influences of gamma radiation.

The development of procedures to ameliorate undesirable ROS production may be one of the central issues in research on aging and oxidative stress-related diseases in the near future. Tempol are widely used to ameliorate excessive oxidative stress; despite scientific proof of their efficacy is scare **(**Chen *et al.,* 2007). However, the antioxidant and cytoprotective activities induced by tempol offers a great advantage for therapeutic purposes, and could become a part of daily use supplement.

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