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Research Article

RADIPROTECTIVE EFFECT OF OXYTOCIN PRETREATMENT IN RATS

SHADAN MUSLIM ALJANABI[1], SHAZA AL-LAHAM[1], ADNAN M. EKHTIAR[2]

[1] Damascus University, Collage of pharmacy, Pharmacology Department,[2] Syrian Atomic Energy Commission, Biotechnology Department,
Email: shadan.m.aljanabi@gmail.com

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ABSTRACT

Since ionizing radiation causes cell dysfunction and mortality, extensive research is devoted to the development of effective radioprotective compounds that would diminish radiation injury in living organisms. Previously, it was observed that Oxytocin has an anti-inflammatory effect and high antioxidant activity. The aim of the present work was to evaluate the radioprotective effect on rats of 150µg/kg Oxytocin that , when administered for five days (twice a day) before irradiation with 9 Gy, provides 20% survival of experimental animals where in unprotected irradiated rats the said dose results in 100% lethality. We observed that rats pretreated with Oxytocin, after an initial decline, exhibited less infiltration of leukocyte due to the anti-inflammatory effect of Oxytocin. We conclude that the radioprotection provided by Oxytocin was in part mediated through anti-inflammatory and antioxidant mechanism.

Keywords: oxytocin, radioprotective, anti-inflammatory, antioxidant

INTRODUCTION

Oxytocin (OT), a nonapeptide produced in the paraventricular and supraoptical nuclei in the hypothalamus, exerts a wide spectrum of central and peripheral effects. In addition to its reproduction-related classical functions, such as stimulation of uterine smooth muscle during labor and milk ejection during lactation, OT displays a potent antistress and woundhealing effect that involves the suppression of hypothalamic pituitary- adrenal (HPA) axis [1].OT receptors have been identified not only in the uterine and myeopithelial tissues, but also in the kidney, heart, adipocytes, pancreas and thymus [2]. Based on the evidence that OT and OT receptors are located in the thymus, recent studies have focused on the anti-inflammatory, immune-modulatory and wound healing effects of OT [3]. Recently, Moosmann and Behl [4] suggested that the secretory peptides including Oxytocin have antioxidative properties in aqueous medium. These hormones scavenge free peroxyl radicals, prevent the oxidation of low-density lipoprotein, and inhibit lipid peroxidation in brain membranes. Moreover, secretory peptide hormones can scavenge reactive nitrogen species derived from nitric oxide and peroxynitrite[5]. OT receptor gene contains response elements for acute phase reactants, including IL-6, prostacylin, nitric oxide, IGF-I and growth hormone[6]. In accordance with its anti-inflammatory effects, analgesic and thermoregulatory effects of OT have also been reported [7]. The deleterious effects of ionizing radiation are mediated through direct deposition of energy to biological molecules and indirectly through generation of highly reactive free radicals [8]. Exposure to ionizing radiation induces the production of reactive oxygen species (ROS) which include superoxide, hydroxyl radicals, singlet oxygen, and hydrogen peroxide. Free radicals react with DNA, RNA, proteins, and membranes, resulting in cell dysfunction and death. Radiation sickness, also referred to as the acute radiation syndrome (ARS), is a serious illness that occurs when the entire body (or most of it) receives a high dose of radiation over a short period of time [9].

There are three types of ARS syndromes: the bone marrow or hematological syndrome, gastrointestinal and the cardiovascular/central nervous system syndromes. The bone marrow syndrome is characterized by anorexia, fever and a decrease in all blood cell types, the gastrointestinal syndrome includes diarrhea, fever, dehydration and electrolyte imbalance and the central nervous system syndrome is characterized by damage to cells that do not reproduce, such as nerve cells [9]. Reactive oxygen free radicals are produced by aerobic cell metabolic activity. The accumulation of these radicals can produce toxic changes within the cells by an uncontrolled self-enhancing process of lipid peroxidation of membranes and inner cell components resulting in a disruption of membrane lipids and other cell components. The cell defensive system consists of antioxidative free-radical scavenging molecules such as glutathione (GSH—a tripeptide consisting of glutamic acid-cysteine-glycine). GSH acts as the substrate for the enzyme glutathione peroxidase. As such it is an important component of intracellular antioxidant defense, protecting cytosolic organelles, in particular, from the damaging effects of hydroperoxides.

In addition, GSH also acts synergistically with ascorbic acid and alphatocopherol to recycle these nutrient antioxidant vitamins to their reduced state after their interaction with reducing chemical species inside the cell [10]. Recently, anti-inflammatory, immune-modulatory and wound-healing effects of OT were reported [1]. Experimental studies have revealed that OT increases the survival of ischemic skin flaps in rats by activating growth factors or anti-inflammatory mechanisms [11]. Similarly, OT treatment displays anti-inflammatory and antifibrotic action on various tissues with inflammatory challenges[5,12].

MATERIALS AND METHODS

Animal care and handling

The animal care and handling were done according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi). Seven to eight weeks old male Wister Rats weighing 150g to 200g were selected from an inbred colony maintained under controlled conditions of temperature (25± 2°C), humidity (40 ± 2%) and light (10 h and 14 h of light and dark, respectively). The animals were provided with clean food and water *ad libitum*. Five to six animals were housed in a polypropylene cage containing clean paddy husk (procured locally) as bedding throughout the experiment.

Study design

The animals were divided into two groups as follows:
Oxytocin+ Irradiation: The animals of this group were injected with (150 µg/Kg) oxytocin (twice a day) for five days before irradiation.
Irradiation only: The animals of this group didn't receive any treatment before irradiation.

Irradiation

1 h after the last administration of Oxytocin on the fifth day, the prostrate and immobilized animals (achieved by inserting cotton plugs in the restrainer) was whole body exposed to 9 Gy of 60Co gamma radiation in a specially designed well ventilated acrylic box. A batch of 10 animals was irradiated each time. The animals of both groups were monitored daily for the development of symptoms of radiation sickness and mortality. A total of 20 animals were used for each group.

Hematological studies

Blood sample (0.5–0.8 ml) was obtained every 48 hours from anesthetized rats by cardiac puncture using a heparinized syringe

attached to a 21- gauge needle. White blood cell (WBC), platelet (PLT) and red blood cell (RBC) count were performed using a Hematology System (Humacount).

RESULTS

Radioprotective Effect of oxytocin

The radioprotective effect of oxytocin was studied in the rats treated with 150µg/kg oxytocin (twice a day) before exposure 9 Gy of gamma radiation. The animals of *irradiation only* group exhibited signs of radiation sickness within 2 days after exposure to gamma radiation. The main symptoms included reduction in the food and water intake, irritability, epilation, weight loss, emaciation, lethargy, diarrhea, and ruffling of hair. Urinary bleeding was also observed in a few animals between 1 and 2 weeks after exposure to irradiation. A few animals exhibited paralysis and difficulty in locomotion during the second week after exposure. The pretreatment of rats with 150 µ/kg oxytocin delayed or reduced the severity of radiation sickness and also delayed the onset of radiation-induced mortality when compared with the concurrent *irradiation only* group (Table 1). This delay in the onset of mortality was by 30 hours in the *oxytocin + irradiation* group when compared with the *irradiation only* group. The oxytocin pretreatment of rats reduced the 14-day mortality by 20% for 9 Gy when compared with the concurrent non-treated group (Figure 1).

Table 1: Mortality on different post-irradiation days

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Survivors %	
Irradiation only	1	3	2	1	3	1	2	1	1	2	_	2	1	_	Nil	Nil
Oxytocin+ irradiation	_	2	1	2	2	1	2	1	2	2	1	_	_	_	4/20	20%

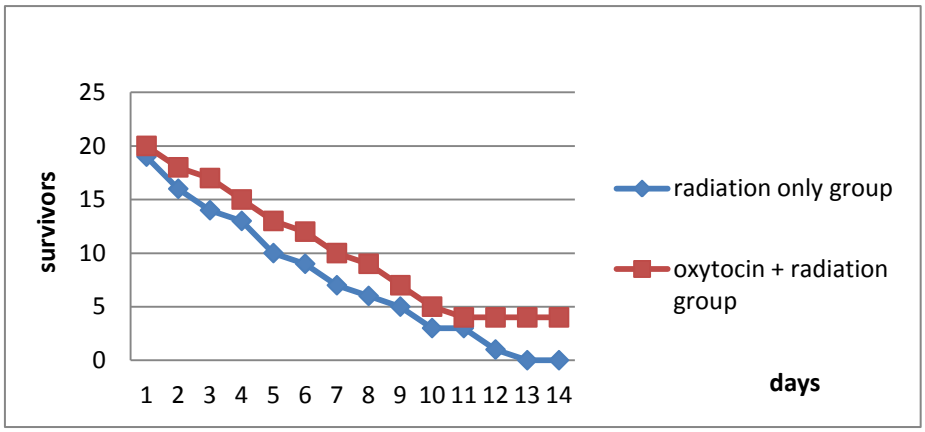

Figure 1: effect of OT on the survival of rats exposed to 9 Gy of gamma radiation. Upper curve oxytocin group 14 days survival and lower curve untreated group 14 days survival

Haematological studies

One day after irradiation, the number of WBC reduced significantly from 6.4 ×10^3/µl in control group to 4.3 ×10^3/µl in irradiated group. A significant increase was observed at day 3, at this time, the WBC count in *irradiation only* rats were 71.3% higher than day 1 , but still 33.7% lower than control group (Figure 2). The WBC count reduced again to the maximum decrease by 97.8% from day 3 (98.4% from control group). At day 9 the WBC increase significantly by 83.5% and start to back to the normal range at day 12. However, treatment with oxytocin decreased the WBC from day 1 to day 3 gradually to reach 32.2% decrease from day 1. The count started to rise again by day 6 then reach a maximum increase by day 12 (26.8% from control group). After 9 days irradiation, a sudden decrease of red blood cells was observed in both groups (*radiation only* and *oxytocin + radiation*). However the decrease in the *oxytocin + radiation* group was 41.2% less in day 9 (Figure 3). On the other hand the decrease in haemoglobin was 52.3% less in the *oxytocin + radiation* group than the *radiation only* group (Figure 4). There was no significant difference in platelets between the *irradiation only* group and the *oxytocin + radiation* group except the day 6, a 20% increase in the *oxytocin + radiation* group from the *radiation* group (Figure 5).

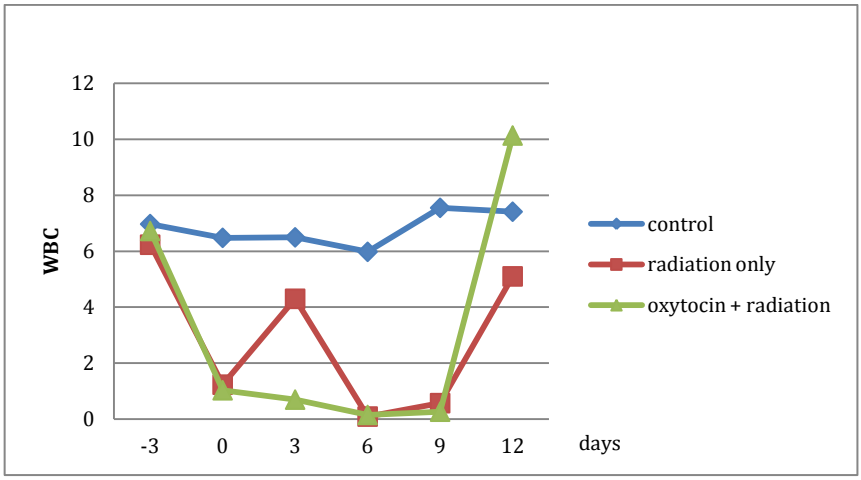

Figure 2: effect of OT on the WBC count (X 10^3 /mm^3) of rats exposed to 9 Gy of gamma radiation for 12 days

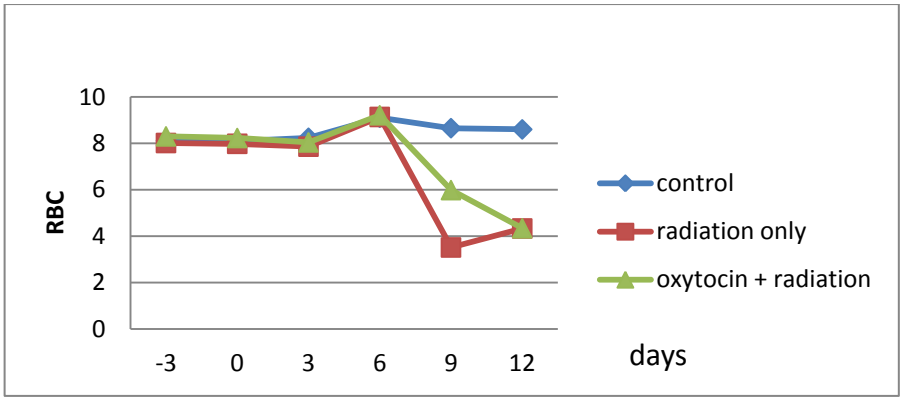

Figure 3: effect of OT on the RBC count (X 10^6 /mm^3) of rats exposed to 9 Gy of gamma radiation for 12 days

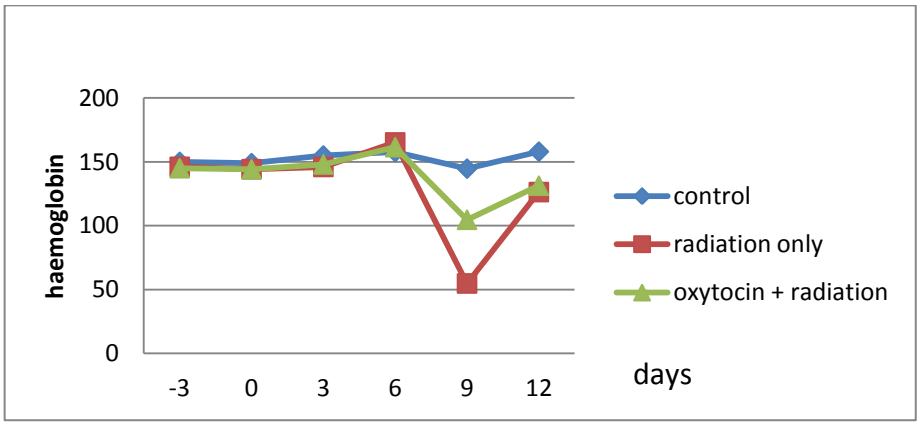

Figure 4: effect of OT on the Haemoglobin (mg/dL) of rats exposed to 9 Gy of gamma radiation for 12 days

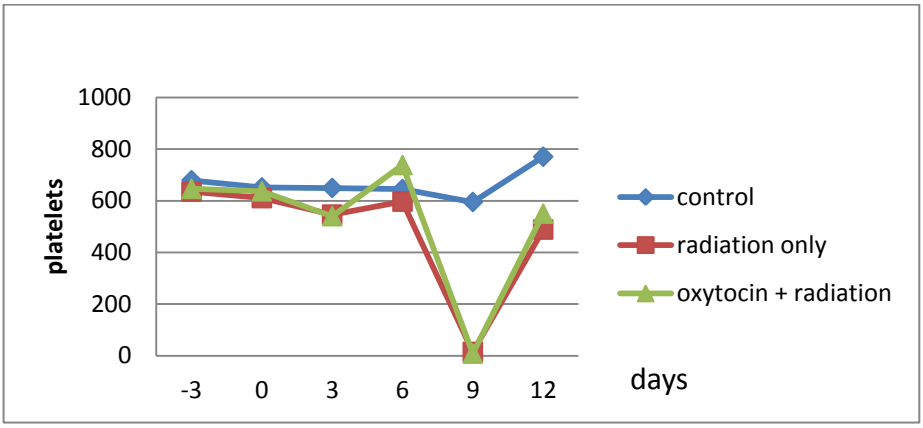

Figure 5: effect of OT on the Platelets count (X 10^3 /mL) of rats exposed to 9 Gy of gamma radiation for 12 days

DISCUSSION

Exposure to ionizing radiation directly damages hematopoietic stem cells and alters the capacity of bone marrow stromal elements to support and/or maintain haematopoiesis *in vivo* and *in vitro*. Exposure to ionizing radiation induces dose dependent declines in circulating hematopoietic cells not only through reducing bone marrow cell production, but also by redistribution and apoptosis of mature form elements of the blood cells [13]. The exposure of animals to gamma rays resulted in radiation-induced sickness and mortality. In this study, a five days administration of oxytocin, an antioxidant drug, before gamma irradiation subcutaneously, significantly reduced mortality induced by irradiation. It is well known that oxidant injury is initiated by free radicals and reactive oxygen molecules, which are generated by activated neutrophils, monocytes and mesangial cells during inflammatory processes [14]. Clinical studies in patients with diabetic foot showed the improving effects of OT on wound healing [3]. Oxytocin may affect several mediators involved in the pathogenesis of inflammation. These include: (i) stimulation of nitric oxide release, which inhibits adhesion and aggregation of neutrophils [15]; (ii) decreased release of IL-6 and increased release of prostacyclin, leading to inhibition of platelet aggregation [16]; (iii) influencing plasma levels of IGF-I and growth hormone and ultimately stimulating phagocyte migration and cytokine production [17]. Moreover, oxytocin was shown to increase corticosterone levels acutely in rats [18] and therefore it is likely that the anti-inflammatory action of oxytocin may be caused by a rise in corticosterone, which is capable of inhibiting neutrophil extravasation in response to different stimuli [6]. The anti-inflammatory properties of OT have been observed in the early stages of research on OT [19], and its role in the modulation of immune and inflammatory responses is supported by the fact that the entire OT system, OT and functional OTR, are expressed in the thymus network [20]. Furthermore, the OTR gene contains acute phase reactant and interleukin response elements [19]. Several works have

documented OT's anti-inflammatory action in humans and animal models. Petersson *et al.* [21] noted that OT decreased carrageenan-induced edema and neutrophil recruitment. The anti-inflammatory effects of OT have been demonstrated in experimentally-induced ulcer and colitis in rat and guinea pig models [22]. OT protects against sepsis-evoked multiple organ damage, supporting the potency of its anti-oxidative action in injured tissues [12] Furthermore, OT has been shown to improve the anti-oxidative stress of colonic tissue and ameliorate oxidative colonic injury *via* a neutrophil-dependent mechanism [5]. In our study, we showed that WBC increased in irradiated rats while administration of OT decreased WBC in irradiated rats. OT also improves skin damage and oxidant gastric injury in rats exposed to high temperature insult. Subcutaneous OT administration reverses burn-induced increases in malonedialdehyde and myeloperoxydase as an index of tissue neutrophil infiltration, and reduces oxidant gastric injury [23]. OT treatment before or immediately after hepatic ischemia-reperfusion significantly reverses transaminase and TNFα elevation in the circulation [24]. OT administration to hamsters blocks stress-induced increases in cortisol levels and facilitates wound-healing [1].

Interestingly, social interactions of animals during OT treatment promote additional benefit most likely by the release of other factors that may facilitate the effect of endogenous OT [25]. Several subsets of T cells, such as CD4+ and CD8+, express OTR mRNA, indicating an important role of the OT system in the response of these immune cells [20]. T cell infiltration is accompanied by monocyte/macrophage infiltration [26]. Therefore, by primarily affecting T cells, OT can also limit monocyte/macrophage infiltration, as reported in our recent study [27]. On the other hand, the presence of OTR in monocytes and macrophages suggests that these cells are direct targets of OT in inflammation [28]. OT binding to OTR on pre-T cells elicits rapid phosphorylation of focal adhesion-related kinases [29]. This could also play a major role in the promotion of "immunological synapses" between immature T lymphocytes and antigen-presenting cells (e.g., macrophages) [19]. Formation of lipid peroxides in the cells exposed to γ-radiation is one of markers of membrane damage. Lipid radicals are formed by the reaction of hydroxyl radicals generated by ionizing radiation with polyunsaturated fatty acids, which can subsequently react with oxygen to form lipid peroxyl radicals, which then go on to damage the cell. Thus, scavenging free radicals and inhibiting lipid peroxidation is a key target in developing successful radioprotection strategies [30]. It is known that the survival time for radiated animals can be lengthened with the inhibition of free radical generation or acceleration of the removal of free radicals, enhancement of DNA repair, replenishment of dead hematopoietic cells, and stimulation of immune cell formation or activity. Thus, the elimination of the free radical species can act as a target in the search for radioprotective compounds. To date, several natural products have been shown to protect cells against radiation-induced damage by virtue of their antioxidant properties [31]. The present study showed that oxytocin, a thiol anti-oxidant agent, effectively protected cells against radiation-induced damage via a mechanism involving the regulation of cellular antioxidant enzyme activity, by inhibition of oxidative stress and free radical scavenging and via an anti-inflammatory mechanism.

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