Int J Pharm Bio Sci 2013 July; 4(3): (P) 223 - 227

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ACCELERATED RECOVERY OF 5-FLUOROURACIL-DAMAGED BONE MARROW AFTER OXYTOCIN TREATMENT

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ABSTRACT

Our preliminary data indicate that Oxytocin may be myeloprotective. We investigated whether it can modify bone marrow recovery. Oxytocin (150 µg/kg) accelerated the recovery of the number of medium size cells (MI%) and platelets (PLT) but did not affect the number of circulating red blood cells (RBC) and total count of white blood cells (WBC).Oxytocin injected subcutaneously for 5 consecutive days at doses of 150 µg/kg (twice a day) after 5-FU single dose. Glutathione GSH (anti-oxidant) has preventive effects against 5-FU-induced myelosuppression and can accelerate recovery of bone marrow. Oxytocin only increase GSH levels insignificantly ($P > 0.05$).These findings suggest that Oxytocinselectively enhances the recovery of the population of progenitor cells reduced by 5-FU in rats.

KEYWORDS: **Oxytocin, Myeloprotective, Medium size cells, 5-Fluorouracil**

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1- INTRODUCTION

Cancer chemotherapy causes severe damage to haematopoietic stem cells. Meylotoxicity is a serious risk for chemotherapy,associated with leukopenia infectious complications necessitating the use of intravenous antibiotics and hospitalization, and mayeven result in death[1].Severe anaemia that is resistant to medical treatment isalso observed in patients with malignancies and who are undergoing chemotherapy. The pathogenesis of this anaemiais multifunctional. Low levels of circulating EPO, shortened survival time of circulating red blood cells (RBCs), and a decrease in the number of immature erythroid cells in bonemarrow probably due to chemotherapy or cytokines such astumour necrosis factor (TNF), have been demonstrated tobe the causes[2].The 5-fluorouracil (5-FU) is one of the most widely used agents in the treatment of advanced colorectal cancer[3]. The primary target of this agent is thymidylate synthase(TS), which catalyzes the reductive methylation of deoxyuridine monophosphate (dUMP) by methylenetetra hydrofolate(CH2H4PteGlu), to form thymidine monophosphate (TMP). This reaction is the onlyde novo source of TMP, and is critical for proper DNA synthesis and cell proliferation. 5-Fluoro-20-deoxyuridylate(FdUMP), the active metabolite of 5-FU, inhibitsTS via formation of a covalent ternary complex with CH2H4PteGlu and the enzyme[4].Various antioxidants and freeradical scavengers have been suggested to be generalcy to protective agents of therapeutic benefit.In case of reduced or impaired defence mechanism and excess generation of free radicals that are not counterbalanced by endogenous antioxidant defence exogenously administered antioxidants have beenproven useful to overcome oxidative damage[5].

Oxytocin (OT), a thiol anti-oxidant agent is 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 labour and milk ejection during lactation, OT displays a potent antistress and wound healing effect that involves the suppression of hypothalamic pituitary- adrenal (HPA) axis [6]. 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 defence, 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 [7]. Our new studies are designed to evaluate whether Oxytocinhas beneficial effects on bone marrow preservation when administered concurrently with 5-FU.

2.MATERIALS AND METHODS

2.1. Animals 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 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. An adaptation period to these conditions of at least 1 week was allowed before the experiment.

2.2 Study design

A rat model of myelotoxic effects was made by single intraperitoneal injection of 5-FU at a dose of150 mg/kg. The first day of the experiment was defined as day 0. Oxytocin (150 µg/kg,subcutaneous injection) was injected twice a day for 5 consecutive days after one hour of the 5-FU injection. The control group received a vehicle instead of testsamples. Rats were scarified by an anesthetizati on with

overdose of ether and then femurs and whole blood were collected at indicated time points.

2.3 Hematological analysis

Blood sample (0.5–0.8 ml) was obtained every second day from anesthetized rats by cardiac puncture using an EDTA-coated syringe attached to a 21- guage needle. White blood cell (WBC), platelet (PLT) and red blood cell (RBC) count were performed using a Hematology System (Humacount).

2.4 Blood

Rats were anesthetized with ether, and blood samples were collected by intraventricular exsanguination on day 9 of 5-FU administration.Blood samples were centrifuged (2000 g) for 5 minutes and the supernatant plasma samples were placed in microtubes and held in (-80 °C) until further processing.Plasma (200 µL/tube) were deproteinized with the 5%5-Sulfosalicylic Acid Solution, centrifuged to remove the precipitated protein, and then assayed for glutathione (GSH).

2.5 Glutathione assay

The measurement of GSH uses a kinetic assay in which catalytic amounts (nmoles) of GSH cause a continuous reduction of 5,5`-dithiobis (2-nitrobenzoic acid) (DTNB)to TNB and the GSSG formed is recycled byglutathi one reductase and NADPH. The GSSG present will also react to give a positive value in this reaction. Samples are prepared in 96-well plates and absorbance at 415 nm with a reference wavelength of 595 is determined every 60 s for 5 min.The rate of increase in absorbance is directly proportional to the amount of glutathione reductase in the sample. Activity in an unknown sample is determined from a standard curve [8]

2.6 Statistical analysis

The mean of the cell numbers and the glutathione concentration were considered as a single data point for analysis of results from at least three or four independent experiments. All data are expressed as mean ± S.E.M. Statistical significance was determined by Dunnett's multiple test after one-way analysis of variance (ANOVA)with comparison to a controlgro up, and the differences were considered significant if $P<0.05$.

RESULT

After administration of 5-FU, the numbers of circulating RBC, WBC and PLT were reduced on day9 by 26.6, 50.4 and 90%, respectively. The haemoglobin concentration were significantly decreased by 26% in 5-FU only group. In 24 hours of 5-FU administration the MI% (medium size cells percentage) was significantly decreased by 51%.The results are presented in Table 1. Subcutaneously injected Oxytocin at dose of 150µg/kg (twice a day) after 5-FU injection had no influence on the most observed parameters ($P>0.05$).

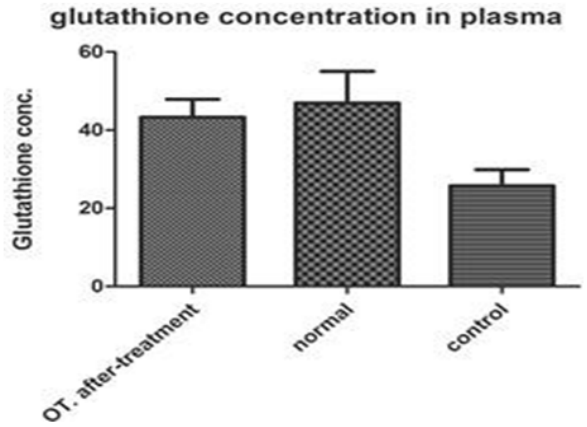

Figure 1

Effect of Oxytocin on GSH levels in plasma Values represent mean ± S.E.M. of the data from three independent experiments. Results considered significantly different if P< 0.05

Table 1

Effects of oxytocin (150µg/kg twice a day) on RBC, WBC and PLT numbers in peripheral blood after 5-FU administration Values represent mean ± S.E.M. of the data from three independent experiments

day	treatment	RBC (x10^6 / mm^3)	WBC (x10^3 /mm^3)	*LY%*	*GR%*	*MI%*	PLT (x 10^3/ml)	HGB	n
1	Normal	8.10 ± 0.27	6.48 ± 0.35	72 ± 1.9	20 ± 1.5	8 ± 0.93	780 ± 45	160 ± 2.7	10
	5-FU alone	9.08 ± 0.24	4.67 ± 0.39	64 ± 3.6	32 ± 3.4	3.9 ± 0.37	554 ± 28	150 ± 1.4	10
	5-FU + Oxytocin	9.4 ± 0.23	5.4 ± 0.84	62 ± 7.7	32 ± 7.5	6.3 ± 0.71	560 ± 53	160 ± 3.4	10
3	5-FU alone	9.03 ± 0.20	6.1 ± 1.3	67 ± 2.9	26 ± 2.5	7 ± 2.4	370 ± 60	150 ± 2.8	10
	5-FU + Oxytocin	8.8 ± 0.58	3.27 ± 0.44	66 ± 2.0	24 ± 1.7	9.6 ± 2.8	370 ± 77	150 ± 8.5	10
6	5-FU alone	7.77 ± 0.19	3.6 ± 0.46	90 ± 1.3	4 ± 0.38	6.1 ± 1.0	132 ± 12	130 ± 3.9	9
	5-FU + Oxytocin	7.6 ± 0.6	4.17 ± 0.89	85 ± 2.7	7.6 ± 1.6	7.5 ± 2.0	150 ± 6.3	130 ± 7.5	10
9	5-FU alone	6.35 ± 0.46	3.6 ± 0.45	77 ± 2.9	14 ± 3.2	8.2 ± 0.99	59 ± 12	110 ± 6.5	8
	5-FU + Oxytocin	7.6 ± 0.60	6.41 ± 0.74	85 ± 2.7	7.6 ± 1.2	11 ± 3.7	157 ± 7	130 ± 7.5	9

However, there was a significant increase (P< 0.05) of MI% in *Oxytocin+ 5-FU* group by 38%.The values of other haematological parameters (GRA%,HGB, LY% and RBC)were not influenced(P>0.05) after Oxytocin treatment. Administration of (150µg/kg) Oxytocin after 5-FU treatment increased the platelet count in blood by 66% (P> 0.05) at day 9.There was a good effect of Oxytocin (150µg/kg twice a day) after-treatment on the plasma GSH levels by 45% (P> 0.05).

DISCUSSION

To examine further action of Oxytocin, we investigated the ability of Oxytocin to decrease the myelotoxic effect of 5-FU in this study. Oxytocin injected subcutaneously for 5 consecutive days at doses of 150 µg/kg (twice a day) after 5-FU single dose, improved the decrease in medium size cells caused by 5-FU. Petruška et al.found the similar results in study with of quercetin (flavonoid with antioxidant capacity) and acute dose of T-2 toxin (an immunodepressive) on selected haematological parameters of rabbit's blood. Authors observed significant increase of MI% after chronic application of quercetin[9]. The increase in medium size cells (a subgroup of the WBC that consists primarily of monocytes and progenitor cells) could refer to an ability of Oxytocin to release bone marrow progenitor cells. The administration of chemotherapeutic agents alone or in combination with hematopoietic growth factor scan result in a transient expansion and mobilization ofprogenitor cells into the peripheral circulation.In addition, the increases in peripheral blood progenitor cells of CFU-GM and BFU-E progenitor cells occurs subsequent to recovery of bone marrow progenitor cells but precedes or occurs simultaneously with the recovery of peripheral blood cell numbers.Therefore, changesin the number of progenitor cells in the peripheral blood may reflect hematopoietic recovery and may also reflect the ability of cytokines to expand the progenitor cells pool size[10].

Oxytocin after-treatment also increased platelets count in the blood by 66%, an effect may be due to Oxytocin ability to induce a significant increase in relative renin secretion [11]. This stimulation of renin-agiotens in system directly induces erythropoietin which increases platelets production[12].It was reported that glutathione showed preventive effects against 5-FU-induced leukopenia, thrombocytopenia and erythrocytopenia, and this anti-oxidant accelerated recovery from them after cessation of 5-FU treatment [13]. But Oxytocin after-treatment only increased GSH levels insignificantly (Fig.1) which can refer to a different mechanism of Oxytocin to increase peripheral blood progenitor cells.It was also shown that oxytocin protects cells against 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 [14]. Further studies can be done on Oxytocinmeylo suppression protective effect after chemotherapy in different doses.

CONCLUSION

Oxytocin accelerates myelosuppression after 5-Fluorouracil treatment and increases mature blood cells, as well as progenitor cells in blood. This indicates an important role of Oxytocin in stimulation of haematopoiesis.

ACKNOWLEDGEMENT

We thank Dr.EmadAbd El-Mohsen Mohamed, Ain Shams University, Cairo, Dr.Majd Aljamali and A. Dakak, Damascus University,for providing the necessary facilities and help, and reviewing this study.

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