

Effect of Methotrexate and Omega 3 in Some Parameters Related With WBCs and RBCs in Male Rats

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ARTICLE INFO

Received: 19 / 5 /2022

Accepted: 28 / 5 /2022

Available online: 19/7/2022

DOI: 10.37652/juaps.2015.127543

Keywords:

Methotrexate,

Omega-3,

RBCs,

WBCs.

ABSTRACT

The current study included understanding the effect of methotrexate and omega-3 in the thyroid and parathyroid gland and some of the physiological and histological and genetic variables in some of the organs of the body in the male rats. The study included 56 animals were divided into three groups, the first and second groups were divided into three secondary groups, the first secondary group was given the drug methotrexate only in concentration 0.05 , 0.125 and 0.250 mg / kg for 8 weeks while the secondary second group was given drug methotrexate in the same concentrations of the first group, as well as give it omega-3 concentration of 300 mg / kg for 8 weeks, the third group was considered as control group. After finishing of dosage, the animals killed and took blood samples and organs to do the required tests, included measuring of blood parameters, such as RBC ,PCV ,Hb ,MCV, MCH and MCHC. In addition to measure the number of white blood cell count WBC and Differential white blood cells, where the results showed the presence of a rise in both the RBC, PCV, Hb in the low and medium concentration of methotrexate while decreased in high concentration, omega-3 work to reduce the variables above in the low and medium concentration while worked on reducing it in the high concentration, moreover a variable of MCV and MCH and MCHC has not changed morally. With respect to the WBC was low in the totals for the study when giving methotrexate while rose when giving omega-3 with this druge, also neutrophil cells was low and accompanied by a rise in a monocytes in all concentrations of Methotrexate were not for omega 3 noticeable effect on these cells decreased. The results showed a decline in the lymph and acidophilus cells in the low concentration while rose in the medium and high concentration of Methotrexate. Omega-3 worked on the rise it in concentrations above, with respect to basophiles cells increased in the low and high concentration and decreased in medium and the omega-3 was not effect of them.

Introduction

The methotrexate (MTX) is a folic acid antagonist because of its chemical composition similar to the folic acid N-[4-[(2,4-diamino-6-pteridiny] methyl] methylamino] -L-glutamic acid, which contains two sets of Carboxyl Group because there Glutamic acid one installed and moving to body tissue by the private system for the transfer of folic acid, and that part of the methotrexate metabolizes in the liver to the 7-hydroxymethotrexate (1).

The (2) showed that methotrexate affects on the manufacturing of nuclear acid DNA and RNA by interfering with the bio synthesis process of Thymidine and purines. Methotrexate affect directly on the rapidly division cells and intact cells especially that found in the mucous membrane of mouth, intestine and bone marrow (3).

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Omega-3 is a type of unsaturated fat that are classified as essential fatty acids cannot be manufactured by body but it gets from food (4). The source of omega-3 fish oil such as salmon, sardines, tuna, soybeans, walnuts, raisins and linseed oils extracted, almonds and olive oil (5).Omega-3 used in the prevention of a number of diseases such as rheumatoid arthritis, ulcerative colitis, asthma, atherosclerosis, cancer, heart and blood vessels diseases (6). A large number of evidence indicates that omega-3 fatty acids have significant health benefits, including their effects as anti-inflammatory ,antioxidant and beneficial effects on blood cholesterol levels (7).

Materials and Methods

1-Animal groups:

The first group (methotrexate group only): This group includes 24 animals given the drug methotrexate after dilution with distilled water by 2 ml was given by

Interaperitoneal injection (IP) as a way (8),divided this group by drug concentration to three secondary groups (8 animals per group) as follows: -

- The first secondary group (higher concentration of methotrexate), these group injected single dose of 0.25mg/kg from drug weekly for 8 weeks (Symbolized with the letter x1).
- The second secondary group (middle concentration of methotrexate), these group injected single dose of 0.125 mg /kg from drug weekly for 8 weeks (Symbolized with the letter X2)
- The third secondary group (lower concentration of methotrexate), these group injected single dose of 0.05 mg /kg from drug weekly for 8 weeks (Symbolized with the letter X3).

The second group (methotrexate and omega-3 group): this group included 24 animals were injected with a drug methotrexate (0.25, 0.125, 0.05mg/kg) for 8 weeks, as well as give it a drug omega-3 at concentration of 300 mg/kg by mouth. (Symbolized the three groups with the letter Y1, Y2, Y3).

The third group consists of eight animals represent the control group injected distilled water intraperitoneally and given distilled water orally in a single dose a week for eight weeks.

2-Tests:

The study included measuring of Total RBCs Count, Concentration of Haemoglobin estimation (9), Packed Cells Volume (PCV) (10), Mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) (11) Total WBCs count (12) and Differential count of leukocytes (13 ; 14).

3- Statistical analysis:

The Statistical Analysis System –SAS (2012) was used for the analysis of data, and to determine the significance differences between treatments according to the Completely Randomized Design-CRD, and compared the significant differences between the mean by Least Significant Differences (LSD).

Results

1- Effect of Methotrexate and Omega 3 in parameters related with RBCs

The results showed in table (1) that there is a significant difference (P <0.01) in some means of blood parameters, it is found that each of the RBC, Hb and PCV it a increasing significantly in the same direction in low and mid concentrate of methotrexate alone or with omega-3 in groups X1 X2, Y1 and Y2, while a significant decrease in the low concentration of methotrexate (X3, Y3) compared with the control group. The results showed a significant differences at the level of the probability (P <0.01) between the means of the MTX alone and between the means of the MTX and omega-3, where found a significant decrease in this parameters in low and mid

concentration of MTX in groups X1 X2, Y1 and Y2, while increased a significant in high concentration (in groups X3, Y3) when giving omega-3 with methotrexate. With regard to the other parameters, which include all of the MCV, MCH and MCHC it was observed that the differences in the means of these parameters did not lived up to level of significance.

Table 1. The effect of methotrexate and omega-3 in parameters related with RBCs

Treatment	Mean±SE					
	RBC 10 ⁹ /ml	Hb (gm/dL)	PCV (%)	MCV fl	MCH pg	MCHC (gm/dL)
Control	5.10 ± 0.1e	14.31 ± 0.1d	42.94 ± 0.1e	83.3 ± 0.1a	29.3 ± 0.1a	33.29 ± 0.1a
X1	5.29 ± 0.1a	14.6 ± 0.1a	43.81 ± 0.1a	83.2 ± 0.1a	29.3 ± 0.1a	33.22 ± 0.1a
X2	5.19 ± 0.1c	14.29 ± 0.1c	42.2 ± 0.1c	83.2 ± 0.1a	29.3 ± 0.1a	33.22 ± 0.1a
X3	5.18 ± 0.1g	13.26 ± 0.1f	42.1 ± 0.1g	83.1 ± 0.1a	29.3 ± 0.1a	33.22 ± 0.1a
Y1	5.22 ± 0.1b	14.40 ± 0.1b	43.27 ± 0.1b	83.2 ± 0.1a	29.3 ± 0.1a	33.22 ± 0.1a
Y2	5.17 ± 0.1d	14.22 ± 0.1d	42.2 ± 0.1d	83.3 ± 0.1a	29.3 ± 0.1a	33.29 ± 0.1a
Y3	5.10 ± 0.1f	13.9 ± 0.1e	41.22 ± 0.1f	84.99 ± 0.1a	29.3 ± 0.1a	33.22 ± 0.1a
LSD	0.1 **y	0.2 **z	0.0 **x	0.4 NSy	0.1 NSy	0.2 NSy

**P<0.01(NS) :non significant. X1= 0.05 MTX, X2= 0.125 MTX, X3= 0.250 MTX, Y1= 0.05 MTX+ 300 Omega 3, Y2= 0.125 MTX+300 Omega 3, Y3= 0.250 MTX+ 300 Omega 3

2-Effect of Methotrexate and Omega 3 in parameters related with WBCs

The results showed in the table (2) there is a significant difference (P <0.01) in the number of WBC and kinds of it, was observed a significant decrease in the total number of WBC in all concentrations groups of drug, also noted a significant decrease in neutrophil ratio in the group X2 ,X3,Y2 and Y3 except in the group Y1 as observed non-significant increase, this increase accompanied with the rise in the ratio of lymphocytes in mid and high concentration of the

drug, while observed a significant increase in percentage of monocytes in all concentrations groups of methotrexate, accompanied by a significant increase in eosinophil in the high concentration in the group X3, either with respect to the basophil it was a significant increased in the group X1 and Y3 while not significantly decreased in group X3.

The results showed a significant differences at the level of the probability (P <0.01) between the means of the MTX alone and between the means of the MTX and omega-3, where found a significant increase in the number of WBC when giving omega-3 with methotrexate for all concentration, It was also noted omega 3 not effect in the percentage of neutrophil, monocytes and basophil, and found a significant decrease in the percentage of lymphocytes and eosinophil when giving omega-3 with high concentration MTX.

Table 1. The effect of methotrexate and omega-3 in parameters related with WBCs

Treatment	Mean±SE					
	WBC	neutrophil (%)	lymphocytes (%)	monocytes (%)	eosinophil (%)	basophil (%)
Control	4.22 ±0.01a	21.33 ±0.01a	22.00 ±0.01c	2.00 ±0.01c	1.55 ±0.01bcd	1.55 ±0.01cd
X1	4.09 ±0.01c	21.00 ±0.01a	22.00 ±0.01c	4.00 ±0.01b	1.00 ±0.01d	1.00 ±0.01ab
X2	4.11 ±0.01e	21.00 ±0.01b	20.33 ±0.01b	0.00 ±0.01ab	2.55 ±0.01bc	1.33 ±0.01d
X3	3.11 ±0.01g	22.00 ±0.01c	22.00 ±0.01c	0.00 ±0.01a	4.00 ±0.01a	1.00 ±0.01abc
Y1	4.09 ±0.01b	21.00 ±0.01a	22.00 ±0.01c	4.00 ±0.01b	1.00 ±0.01cd	1.00 ±0.01bcd
Y2	4.09 ±0.01d	21.00 ±0.01b	22.33 ±0.01ab	4.33 ±0.01b	2.00 ±0.01b	1.55 ±0.01cd
Y3	4.83 ±0.01f	22.00 ±0.01c	20.00 ±0.01d	0.00 ±0.01a	2.00 ±0.01b	1.00 ±0.01a
LSD	0.01**	0.01**	0.01**	0.01**	0.01**	0.01*

(**P<0.01) * ((P<0.05 X1= 0.05 MTX, X2= 0.125 MTX, X3= 0.250 MTX, Y1= 0.05 MTX+ 300 Omega 3,

Y2= 0.125 MTX+300 Omega 3, Y3= 0.250 MTX+ 300 Omega 3
Discussion

The results showed a significant difference (P <0.01) in the blood parameters related with RBCs, that observed the PCV, RBC and Hb increased in the low and mid concentration of the methotrexate and decreased at the high concentration of it, due to the testosterone hormone that works to stimulate the manufacturing process of Hemoglobin and RBCs (15). The decrease was due to an increase in water intake due to the fish food found in omega-3 and this leads to the hemodilution and as a result getting a decrease in these parameters (16), and decrease due to anemia which is considered one of side effects associated with chemotherapy (17), or may be due to hemolysis because of the harm resulting from the oxidation process and increased free radicals (18) or a disruption in the bone marrow which is the main supporter organ of the blood (19). The (20) showing that the reason for the decrease in RBCs back to ulceration in the gastrointestinal tract, which causes bleeding through it and this ulceration is one of the side effects of methotrexate. In addition, the red blood cells and Hemoglobin affected by feeding and decreases with low feeding (21) Because of the lack of food proteins consumption, which led to a lack of blood proteins and that one of the reasons leading to the lack of Hemoglobin and other blood components (22). The parameters of blood decreases with the increase in thyroid hormones, as explained it (23) that hyperthyroidism causes a state of anemia as a result of a decrease in the amount of iron needed to form Hemoglobin. The decrease in blood parameters was due to the affected bone marrow by methotrexate and reduced its ability to manufacture blood cells, (24) showed the negative effect of chemotherapy in the bone marrow and thus reduced its ability to manufacture cells naturally. The reason for not affected the MCV,MCH and MCHC by methotrexate because they are not associated accurately with the increase or decrease in the number of red blood cells because it have some independence (25).

The results showed a significant increase in lymphocytes ratio in the mid and high concentration of methotrexate compared with the control group, while monocytes was significantly increased in all concentrations of methotrexate, either neutrophil cells was significantly decreased in the mid and high concentration of MTX, and observed significant increase of eosinophil in these concentrations, Basal cells was significantly increased at low concentration and higher also decreased the total number of white blood cells in all concentrations of methotrexate. The reason for the high lymphocytes ratio and low neutrophil ratio return to systemic effect of methotrexate (26), or increase due to damage in a spleen tissue therefore the lymphocytes increases to

removal damage in tissue and remove damaged cells, also monocytes increases response to the damage in the liver tissue resulting through hyperthyroidism where cause the infiltration of these cells from the liver (27), or due to the affected bone marrow by methotrexate (28).

The results showed significant differences between the groups took methotrexate and omega-3 together and between groups took methotrexate alone, this difference in white blood cells values return to the role of omega-3 antioxidant and inflammation and have a role in increase or decrease of these cells by stimulating anti-inflammatory cells (29), As well as omega-3 plays an important role in increasing the lymphocytes and neutrophils decrease by enhancing the immune and stimulate the immune system (30 , 31).

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تأثير عقاري الميثوتركسيت والاوميغا 3 في بعض المتغيرات الدموية المتعلقة بكريات الدم الحمر وخلايا الدم البيض في ذكور الجرذان

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الخلاصة

تهدف الدراسة الحالية معرفة تأثير عقاري الميثوتركسيت والاوميغا 3 في بعض المتغيرات الدموية المتعلقة بكريات الدم الحمر وخلايا الدم البيض في ذكور الجرذان. شملت الدراسة ٥٦ حيوان قسمت على ثلاث مجاميع وزعت المجموعة الاولى والثانية الى ثلاث مجاميع ثانوية، اعطيت المجموعة الاولى عقار الميثوتركسيت فقط ووزعت الى ثلاث مجاميع ثانوية حسب التراكيز (٠.٠٥ و ٠.١٢٥ و ٠.٢٥٠) ملغم/كغم لمدة ٨ اسابيع واعطيت المجموعة الثانية عقار الميثوتركسيت بنفس تراكيز المجموعة الاولى فضلاً عن اعطائها الاوميغا 3 بتركيز 3٠٠ ملغم/كغم لمدة ٨ اسابيع اما المجموعة الثالثة فمثلت مجموعة السيطرة التي لم تجرع بالعقاقير، وبعد انتهاء مدة التجريب شرحت الحيوانات وأخذت عينات الدم لعمل الفحوصات اللازمة. شملت الدراسة قياس المتغيرات الدموية مثل عدد كريات الدم الحمر RBC وحجم الخلايا الدم المرصوصه PCV وتركيز الهيموكلوبين Hb ومتوسط حجم كرية الدم الحمراء MCV فضلاً عن متوسط الهيموكلوبين في كرية الدم الحمراء MCH ومتوسط تركيز الهيموكلوبين في كرية الدم الحمراء MCHC وعدد خلايا الدم البيض WBC والعد التفرقي لخلايا الدم البيض، اذ بينت النتائج وجود ارتفاع في كل من Hb, PCV, RBC في مجموعتي التركيز الواطئ والمتوسط من الميثوتركسيت بينما انخفضت في التركيز العالي منه، وعملت الاوميغا 3 على تخفيض المتغيرات اعلاه في التركيز الواطئ والمتوسط بينما عملت على ارتفاعها في التركيز العالي، اما متغير الـ MCV وMCH وMCHC لم تتغير معنوياً، وفيما يتعلق بـ WBC فقد انخفضت في مجاميع الدراسة عند اعطاء الميثوتركسيت بينما ارتفعت عند اعطاء الاوميغا 3 معه، كما انخفضت الخلايا العدلة رافقها ارتفاع في الخلايا وحيدة النواة في جميع التراكيز ولم يكن للاوميغا 3 تأثير ملحوظ على هذه الخلايا واطهرت النتائج انخفاض نسبة الخلايا اللمفية والحمضة في التركيز الواطئ بينما ارتفعت في التركيز المتوسط والعالي وعملت الاوميغا 3 على ارتفاعها في التركيزين اعلاه، وفيما يخص الخلايا القعدة فقد ارتفعت نسبتها في التركيز الواطئ والعالي وانخفضت في التركيز المتوسط ولم يكن للاوميغا 3 تأثير ملحوظ عليها.