EFFECT OF FOLIC ACID ON SOME PHYSIOLOGICAL PARAMETERS IN FEMALE RABBITS TREATED WITH METHOTREXATE

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ABSTRACT

The present study was carried out to investigate the effect of folic acid (FA) on the hematological picture of female rabbits treated with methotrexate MTX. A total of twenty female rabbits were used in this study. They were at age 4-5 months. Their body weight ranged between 1-1,200 Kgm. All animals were kept under normal condition. its divided in to four groups and each group consist of five animals as follows (Control group :5 rabbits were received distilled water, Folic acid group : 5 rabbits were received folic acid at 0.07mg/kg body weight daily, Methotrexate group : 5 rabbits were received methtrexate (0.03 mg/kg body weight) three times a week , folic acid with Methotrexate group : 5 rabbits were received folic acid (0.07 mg/kg body weight) and methotrexate (0.03mg/kg body weight) three times a week. The drugs were given by intubation. The experiment was last for 9 weeks . Blood sample were collected after end of the experiment to study the following hematological parameters: RBCs count, Hb, PCV, RBCs indices (MCV, MCH, MCHC). The group treated with folic acid showed a significant increase P≤0.05 in RBCs count, Hb conc. and PCV% as compared with all other groups. The results of MTX group reveal a high significant decrease in their RBC count, Hb conc., PCV%. At the same time there is a significant increase in MCV, MCH and MCHC indices. The group of animals received FA with MTX showed a good prognosis with health improvement characterized by high significant changes in all studied parameters to return back to their normal values. It was concluded that folic acid is very important for erythropoiesis. MTX treatment induce megaloblastic anemia resulted from inhibition of DNA synthesis in RBCs mainly by folate deficiency. FA administration with MTX correct these changes and the animals return to normal conditions. More work is needed to study the effects of these drugs on other systems in the body.

KEY WORDS : hematological parameters, drug, folic acid, methotrexate

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تأثير حمض الفوليك على بعض المعايير الفسيولوجية في اناث الأرانب المعاملة بعقار الميثوتريكسات

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المستخلص

أجريت الدراسة الحالية لمعرفة تأثير حمض الفوليك على الصورة الدموية لإناث الأرانب المعاملة بالميثوتريكسات. اذ تم استعمال عشرين أنثى من الأرانب في هذه الدراسة تراوحت اعمارها من 4-5 شهر وباوزان بين 1-1,200 كغم. تمت تربية الحيوانات تحت ظروف طبيعية وقسمت إلى أربعة مجاميع تكونت كل مجموعة من خمسة حيوانات على النحو التالي (مجموعة السيطرة: 5 ارانب تم اعطاؤها ماءا مقطرا، مجموعة حمض الفوليك: 5 ارانب تم اعطاء حمض الفوليك بمعدل 0.07 مجم / كجم من وزن الجسم يومياً ، مجموعة الميثوتريكسات: 5 ارانب تم اعطاء الميثوتريكسات (0.03 مجم / كجم من وزن الجسم) ثلاثة مرات في الأسبوع ومجموعة حمض الفوليك والميثوتريكسات: 5 ارانب تم اعطاؤها حمض الفوليك (0.07 مجم / كجم من وزن الجسم) والميثوتريكسات (0.03 مجم / كجم من وزن الجسم) ثلاثة مرات)، استمرت التجربة لمدة 9 أسابيع، تم جمع عينات الدم بعد انتهاء التجربة لدراسة المعايير الدموية التالية: عدد كرات الدم الحمراء، تركيز خضاب الدم، حجم الخلايا المرصوصة ، حجم كريات الدم الحمراء ، معدل هيموكلوبين الكرية، معدل الحديد في داخل الكرية. أظهرت نتيجة هذه التجربة تأثيرا واضحا لحمض الفوليك على الصورة الدموية، اذ أظهرت المجموعة التي عوملت بحمض الفوليك زيادة معنوية P≤0.05 في عدد كرات الدم الحمراء، تركيز الهيموغلوبين وحجم الخلايا المرصوصة مقارنة مع جميع المجموعات الأخرى. كما أظهرت المجموعة المعاملة بالميثوتريكسيت انخفاضًا كبيرًا في عدد كرات الدم الحمراء، تركيز خضاب الدم، حجم الخلايا المرصوصة مع زيادة معنوية في حجم كريات الدم الحمراء، معدل هيموكلوبين الكرية ومعدل الحديد في داخل الكرية كما لوحظ ان المجموعة المعاملة بحمض الفوليك والميثوتريكسات شهدت تحسنا جيدا اذ ان كل المعايير المدروسة عادت الى وضعها الطبيعي عند مقارنتها مع مجموعة السيطرة . نستنتج من هذه الدراسة ان حمض الفوليك مهما جدا لتكوين كريات الدم الحمراء. وان علاج الميثوتريكسات يسبب فقر الدم الضخم الأرومات الناتج عن تثبيط تصنيع الحمض النووي في كرات الدم الحمراء بسبب نقص الفولات بشكل رئيسي. وان اعطاء حمض الفوليك مع الميثوتريكسات يؤدي الى عودة المعايير الى وضعها الطبيعي لذا فان هنالك حاجة إلى مزيد من الدراسات لدراسة آثار هذه الأدوية على اجهزة الجسم الاخرى.

الكلمات المفتاحية: المعايير الدموية، دواء، حمض الفوليك، الميثوتريكسات

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INTRODUCTION

Micronutrients include vitamins and minerals that our bodies require them in small quantities and their deficiency will produce abnormal functions of cells and organs (23) . Folic acid is a synthetic folate compound also known generically as folate or folacin, pteroylglutamic acid (PGA), is a member of the B-complex family of vitamins, and works in concert with vitamin B12 (1). Folic acid functions primarily as a methyl-group donor in transferring one carbon atom involved in many important body processes, including building blockers of DNA and RNA needed for protein synthesis (3). Therefore, rapidly growing tissues, such as those of a fetus, and rapidly regenerating cells, like red blood cells have a high need for folic acid (5). Therapeutically, folic acid is instrumental in reducing homocysteine levels and the occurrence of neural tube defects (13). It may play a key role in preventing cervical dysplasia and protecting against neoplasia in ulcerative colitis. Folic acid also shows promise as part of a nutritional protocol to treat vitiligo, and may reduce inflammation of the gingiva (20). Furthermore, certain neurological, cognitive and psychiatric presentations may be secondary to folate deficiency (11). Such presentations include peripheral neuropathy, myelopathy, restless legs syndrome, insomnia, dementia, forgetfulness, irritability, endogenous depression, organic psychosis, and schizophrenia-like syndromes (18). Green vegetables and certain (citrus) fruits are important natural dietary sources of folates (3, 10). Methotrexate and formerly known as amethopterin, is an antimetabolite and antifolate drug used in treatment of cancer and autoimmune diseases (16). It acts by inhibiting the metabolism of folic acid. Methotrexate replaced the more powerful and toxic antifolate aminopterin, and the two should not be confused with each other (12). Its mechanism of action is inhibiting the conversion of inactive folate [dihydrofolate (DHF)] to active folate [tetrahydrofolate (THF)] (7). Therefore, this study was designed to investigate the effect of folic acid on hematological picture in female rabbits. It also aimed to study the effect of folic acid deficiency due to MTX treatment on hematological picture by measuring the following parameters : Red Blood Cells (RBCs) count, Hemoglobin concentration (Hb), Packed Cell Volume (PCV), RBCs indices: MCV, MCH and MCHC.

MATERIALS AND METHODS

Animals: A total of twenty female rabbits were used in this study. They were at age 4-5 months. Their body weight ranged between 1-1,200 Kgm. All animals were kept in the same suitable environmental conditions of 25-27 c◦ ,and photoperiod of 12 hours daily . The animals were housed in plastic cages of 90×60×30 cm in diameter. These cages were cleaned once a week. The food (pellets) and water (tap water) was given freely. The animals kept at least 2 weeks for adaptation before starting the study.

Experimental Design

A total of 20 female rabbits were divided into 4 groups equally as follow:-

1- **Control (C) group** :5 rabbits were received distilled water .

2- **Folic acid (F) group** : 5 rabbits were received folic acid at 0.07mg/kg body weight daily (22).

3- **Methotrexate (M) group** : 5 rabbits were received methtrexate (0.03 mg/kg body weight) three times a week (19).

4- **Folic acid and Methotrexate (FM) group** : 5 rabbits were received folic acid (0.07 mg/kg body weight) daily and methotrexate (0.03mg/kg body weight) three times a week . The drugs were given by intubation. The experiment was last for 9 weeks .

Blood collection

Blood samples were obtained via cardiac puncture technique from each animal using disposable syringe 5 ml with needles 22G. the taken samples were kept in anticoagulants tubes in order to measure by using hematology coulter to get complete blood picture (CBC).

Dosage and preparation

Dose of folic acid: The dose of folic acid was used 0.07 mg/kg body weight according to (22) . One tablet of folic acid (5mg) dissolved in 714 ml of distilled water to obtain 0.07mg/kg BW. of folic acid in each ml (0.007mg/100g BW/ml).

Dose of Methotrexate **:** The MTX dose was used as 0.03mg/kg BW. by dissolving one

tablet of MTX (2.5mg) in 833ml of distilled to obtain 0.003mg/kg BW in each one ml (0.003mg/100g BW /ml) (19).

Statistical Analysis

Differences between groups and between times were determined by using the least significant difference (LSD), using significant level of P≤0.05.

RESULTS AND DISCUSSION

Table 1. shows Effect of folic acid on some blood parameters (Red Blood Cells, Hemoglobin Concentration, Packed Cell Volume, Mean Corpuscular Volume MCV, Mean Corpuscular Hemoglobin MCH, Mean Corpuscular Hemoglobin Concentration MCHC) in female rabbits treated experimentally with methotrexate

group / parameter	Group (C) Control Group	Group (M) Methotrexate (0.03mg/kg) three time per week	Group (F) Folic acid (0.07mg/kg) daily	Group (FM) Methotrexate+Folic acid (0.03mg/kg three time per week)+(0.07mg/kg daily)
Red Blood Cells (RBCs)	9. 83 ± 0.3 A	4. 22 ± 0.2 B	11. 1± 0.2 A	9. 31± 0.6 A
Hemoglobin concentration (Hb mg/dl)	16.63±0.3 A	8.35±0.6 B	17.82±0.2 A	15.77±0.3 A
Packed Cell Volume (PCV%)	50.4±1.2 A	26.3±1.3 B	53.4±1.4 A	48.7±1.5 A
MCV (Fl)	60.47±2.3 B	83.53±2.1 A	61.22±2.2 B	62.24±2.2 B
MCH (Pico)	19.88±0.2 B	28.43±0.6 A	19.87±0.3 B	19.66±0.5 B
MCHC (g/dl)	33.2±0.2 A	33.5±0.3 A	33.1±0.2 A	33.7±0.4 A

Values represent mean ±SE. Different capital letter indicate significant differences (P≤0.05) between groups Red Blood Cells count

Table 1 shows the result of RBCs count after nine weeks of treatment. The Table shows that RBC_s count increases significantly in the group which treated with FA after nine weeks of the treatment as compared with the control and other groups, while the group which treated with MTX induce significant decrease in RBCs count as compared with the control and other groups after nine weeks of treatment. Animals who received folic acid with MTX showed a significant elevation in RBCs number after nine weeks of the treatment as compared with the group who received MTX alone (P≤ 0.05). These changes could be explained by the important role of folic acid. It is necessary for the normal formation of RBC_s and the synthesis of DNA which is the genetic material of the cell (18). Folate enzymes act as gen encoders, enhancing DNA synthesis and promoting cell differentiation and division (21). Moreover, folic acid protects the integrity of DNA during cell division and promotes normal differentiation and morphology of cell. Folic acid and vitamin B_{12} are important for maturation of RBC_S and both of them are essential for the synthesis of DNA because it is required for the formation of thymidine triphosphate which is one of the essential blocks of building DNA (14). So any deficiency in FA leads to decrease of RBC_S count. On the other hand, the administration of folic acid lead to inhibition of THFR enzyme which converts the DHF (inactive form) of FA to (active form) THF (7). Moreover, MTX induced a decrease in DNA biosynthesis (22). FA requires many other micronutrients eg. Mg, Zinc, Fe and Copper as well as members of it own family (B6, B12, B2, Betaine, Choline and Inositol) to work more effectively in promoting and maintaining normal cell differentiation, replication and repair (14). One of MTX side effect is gastrointestinal abnormalities (15). So inhibition of these micronutrients absorption will affect RBCs synthesis (6) and differentiation which inturn reduces their number. Moreover Methotrexate –induced histopathological changes in the kidneys so it may be reduced the production of erythropoietin (9). Folic acid supplementation with MTX reduced MTX adverse effects and

the number of RBCs increased significantly (17).

Hemoglobin concentration

In Table 1 animal received folic acid showed significant elevation ($p \leq 0.05$) in Hb concentration after nine weeks of the treatment as compared with all groups. This increase is coincided with the increase of RBC_s number and PCV% in the same group. This is mainly due to the effect of FA which is concentrated within RBC_S (red cell folate) and bound with glutamates and iron to form hemoglobin (21). Reserves are stored in the liver but become exhausted quickly (within three days). Both methyl- THF and methylene – THF play a critical role in methylation and donation of methyl groups (single carbon fragments ….CH3) in the synthesis of amino acids, DNA and RNA nucleoproteins. Each hemoglobin molecule is composed of four globin chains, each of them binds to heme group (8). Any deficiency in folate leads to decrease in DNA, RNA , and protein synthesis i.e. globin which reduces hemoglobin formation in group treated with MTX and follow the same pattern of RBCs number and PCV%. The values tend to return to normal in rats treated with FA+MTX.

Packed cell volume

Table 1 shows that PCV٪ increased significantly in rats treated with FA as compared with the control and other groups after nine weeks of the treatment. Because PCV represents the percent of blood cells to plasma, thus this increase is mainly related to the increase of RBC_S number due to FA treatment which enters in the synthesis of RBC_S (8). At the meantime , MTX induced a significant reduction in PCV٪ as compared to control and other groups which is mainly due to FA deficiency induced by MTX (7). Animals received FA with MTX had similar PCV% to that of control group. This is coincided with RBCs count and Hb concentration, because folate supplementation had ability to reduce the toxic effects of MTX (17). FA maintaind the constituent of blood cells at normal level as it interfered with the replication of RBC_s and Hb formation.

RBCs indices: Table 1 shows a significant increase in MCV, MCH and MCHC for MTX group after nine weeks of the treatment as compared with other groups. This increase is coincided with the increase of RBCs size at the same group (macrocytic anemia). These RBCs contain more Hb than normal to compensate the deficiency in RBCs number. At the meantime, these indices increased significantly in animals received FA with MTX as compared with FA and control groups and decreased significantly as compared with MTX group. MCH is usually influenced by MCV. For example, smaller erythrocytes contain less Hb, and therefore, they have a decreased MCH. Hypochromia (i.e. low MCHC) occurs in some cases of iron deficiency (4). We conclude from this study that folic acid plays crucial role in the repair of harmful effects of methotrexate

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