



Effect of Folic Acid on Some Biochemical Parameters in Female Rabbits Treated Experimentally with Methotrexate

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THE present study was carried out to investigate the effect of folic acid (FA) on the some biochemical parameters of female rabbit's treated and non-treated with methotrexate (MTX). Twenty female rabbits were divided in to four groups, control group 5 rabbits were received distilled water, (FA) group rabbits were received folic acid at 0.07mg/kg body weight orally, Methotrexate group : 5 rabbits were received methtrexate (0.03 mg/kg body weight orally) three times a week and (FA) with (MTX) group : 5 rabbits were received folic acid (0.07 mg/kg body weight orally) daily and (MTX) (0.03mg/kg body weight orally) three times a week. The drugs were given by intubation. The experiment was last for 9 weeks. Blood sample were collected after nine weeks of the experiment to study the following biochemical parameters bilirubin in serum, unbound Iron Binding Capacity (UIBC), Total Iron Binding Capacity (µg/dl), Total Serum Iron and Ferritin concentration in serum. The results of MTX group reveal high significant decrease ($P \geq 0.05$) bilirubin conc. At the same time there is a significant increase in total SI, TIBC, UIBC in this group. 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 (FA) is administration with MTX very important to 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.

Keywords: Folic acid, biochemical parameters, female rabbits, methotrexate.

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 [1]. (FA) 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 [2]. (FA) 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

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regenerating cells, like red blood cells have a high need for (FA) [4]. Therapeutically, folic acid is instrumental in reducing homocysteine levels and the occurrence of neural tube defects [5]. 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 [6]. Furthermore, certain neurological, cognitive [7] and psychiatric presentations may be secondary to folate deficiency [8]. Such presentations include peripheral neuropathy, myelopathy, restless legs syndrome, insomnia, dementia, forgetfulness, irritability, endogenous depression, organic psychosis, and schizophrenia-like syndromes [9]. Green vegetables and certain (citrus) fruits are important natural dietary sources of folates [10]. MTX and formerly known as amethopterin, is an antimetabolite and antifolate drug used in treatment of cancer and autoimmune diseases [11]. It acts by inhibiting the metabolism of (FA). MTX replaced the more powerful and toxic antifolate aminopterin, and the two should not be confused with each other, Its mechanism of action is inhibiting the conversion of inactive folate [dihydrofolate (DHF)] to active folate [tetrahydrofolate (THF)] [12]. Therefore, this study was designed to investigate the effect of folic acid on biochemical parameters in female rabbits. It also aimed to study the effect of folic acid deficiency due to MTX treatment on studied biochemical parameters.

Materials

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 Kg. 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.

Methods

Experimental Design

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

1. Control (C) group: Five rabbits were received distilled water.

2. Folic acid (F) group: Five rabbits were received folic acid at 0.07mg/kg body weight daily [13].
3. Methotrexate (M) group: Five rabbits were received methotrexate (0.03 mg/kg body weight) three times a week [13].
4. Folic acid and Methotrexate (FM) group: Five rabbits were received (FA) (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. These samples were centrifuged at 2500 round/minute (rpm) for 15 minute and then serum sample were stored in freezer at -18°C until they were used for ferritin, Tiron, TIBC and bilirubine tests.

Dosage and Preparation

Dose of (FA)

The dose of folic acid was used according to Woo [13]. One tablet of (FA) (5mg) dissolved in 714ml of distilled water to obtain 0.07mg/kg BW of folic acid in each ml (0.007mg/100g BW/ml).

Dose of (MTX)

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/kgBW in each one ml (0.003mg/100gBW/ml) [14].

Biochemical parameters

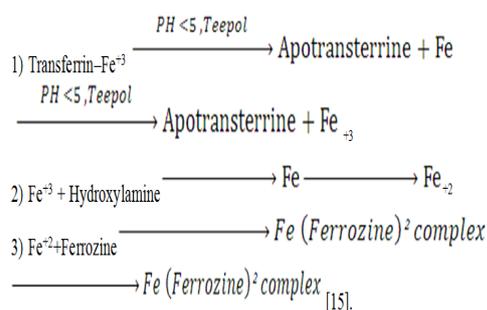
Serum ferritin concentration

For the quantitative determination for ferritin concentration in blood, ferritin enzyme immunoassay test kit was used. This test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The concentration of ferritin is directly proportional to the color intensity of the test sample [15].

Total Serum Iron

The Fe⁺³ bound to serum ferritin once dissociated in a weak-acid medium by teepol and guanidium chloride then is reduced by hydroxylamine to Fe⁺². The ferrous ion forming a colored complex with ferrozine proportional to the concentration of iron present in the sample [16].

This measurement takes place by colorimetric method by the following steps:



Total Iron Binding Capacity ($\mu\text{g/dl}$)

Serum iron (Fe^{+3}) is bound to transferrin. The amount of iron that serum transferrin can bind when completely saturated with an excess of (Fe^{+3}) is the total iron binding capacity (TIBC). A special kit is used for measuring TIBC by colorimetric method at $560 \pm 20 \text{ nm}$ [16].

Unbound Iron Binding Capacity (UIBC)

UIBC was estimated by numeric differences between TIBC & SI is the amount of Iron Binding Capacity remaining on the transferrin or the Unbound Iron Binding Capacity [17].

Serum Bilirubin

Bilirubin meter and non-heparinized Microhematocrit tubes were used for this determination. Standardization by distilled water at the beginning and after each test was done. The capillary tube containing the sample after centrifugation can be inserted on the serum gauge and then the optical density for each sample was recorded within seconds [18].

Results and Discussion

Ferritin, SI, TIBC and UIBC in serum

Table (1) ferritin, SI, TIBC and UIBC and bilirubin concentration in female rabbits after nine weeks of treatment. This table shows that ferritin level decreases significantly in MTX group as compared with control and FM groups due to folate deficiency. MTX inhibits the conversion of inactive dihydrofolate to active form tetrahydrofolate by inhibition of THFR enzyme and acts by blocking DNA and RNA synthesis causing intestinal mucositis [19]. This may be due to malabsorption of iron from the GIT that results as a side effect of MTX treatment [20].

TABLE 1. Ferritin, total serum iron (SI), total iron binding capacity (TIBC) and unbound iron-binding capacity (UIBC) and bilirubin concentration for control (C), methotrexate (M), folic acid (F), and methotrexate+folic acid (FM) groups after nine weeks of the experiment in female rabbits .

Parameter	Group (C) Control group	Group (M) MTX (0.03 mg/kg) three time a week	Group (F) FA (0.07 mg/kg) daily	Group (FM) MTX + FA (0.03 mg/kg three time a week) + (0.07mg/kg daily)
Ferritin (ng/ml)	10.2 \pm 0.09 A	8.1 \pm 0.09 C	7.2 \pm 0.06 D	9.2 \pm 0.09 B
SI (mg/dl)	433.6 \pm 6.01 D	683.8 \pm 12.33 A	489.4 \pm 13.12 C	523.6 \pm 8.92 B
TIBC (mg/dl)	937.6 \pm 13.3 B	1467.0 \pm 12.3 A	561.2 \pm 17.1 D	735.2 \pm 13.2
UIBC (mg/dl)	475.6 \pm 5.81 B	765.2 \pm 17.81 A	54.8 \pm 2.66 D	302.4 \pm 11.49 C
Bilirubin (mg/dl)	0.51 \pm 0.01 A	0.33 \pm 0.01 D	0.36 \pm 0.04 C	0.44 \pm 0.01 B

Values represent mean \pm SE.

Different capital letters indicate significant differences ($P \leq 0.05$) between groups. Control group, MTX, FA and MTX + FA

A highest significant value when compared with other groups.

B less significant value than A.

C less significant value than B.

D lowest significant value when compared with other groups.

The decrease in ferritin level in FA group as compared with other groups may be due to high consumption of iron in RBCs and Hb formation. At the meantime, the amount of iron in the diet (pelet) may be inadequate to compensate the animals' need for RBCs formation. It had been reported that patients with low serum ferritin had iron deficiency but normal Hb and PCV usually suffer from fatigue that reversed by iron treatment [21]. In rabbits treated with FA+MTX ferritin level increased significantly as compared with M and FA groups and decreased significantly as compared with control but the values approached to that of control. This may be due to the effect of (FA) on GIT to enhance the absorption of iron which leads to increase the formation of RBCs and Hb because dietary iron is not enough [22].

Depending on this fact when ferritin decreased in the body, the absorption of free iron increased from GIT as possible as leading to increase serum iron. The high erythropoiesis process in F group as a result of erythropoietin hormone stimulation needs more amount of iron which is probably inadequate in diet [22].

The total SI in FM group decreased significantly as compared with MTX group and increased significantly as compared with C and F groups. This could be explained according to the role of FA. For the TIBC which is the test request in iron deficiency anemia is evaluated, by adding the iron to blood sample for saturating the transferrin and the amount of iron bounded represents TIBC [22]. The TIBC increased highly significant in MTX group as compared with the other groups due to serum iron deficiency that resulting from malabsorption of iron from GIT as side effect of MTX treatment [21]. The increase in TIBC occurs to compensate the deficiency in iron or in the β -globulin protein "transferrin" that transports the iron.

TIBC – Tiron = unbound iron binding capacity (UBIBC), which is the amount of iron excreted from the body without uses which highly increased in MTX group and decreased in F group due to the highly consumption of iron for the active erythropoiesis that stimulated by folic acid in F group. The small value of UBIBC in F group indicates that the deficiency in this group results from the consumption of iron for the erythropoiesis, and the deficiency in M group resulting from excretion of iron in high amount by the body due to no absorption of iron by the GIT, or no use by the body for erythropoiesis [17].

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In addition, there is a significant decrease in bilirubin concentration of M group as compared with other groups and this may be due to decrease in the RBCs number and their Hb content which in turn lead to decreased destruction of fragile and abnormal megaloblastic erythroid precursor, this is coincided by bone marrow examination of the same group. Also, in FA group there is a significant increase in bilirubin concentration as compared with M group and a significant decrease as compared with C and FM groups. This could be explained by the effect of FA on RBCs which lead to produce healthy RBCs characterized by high resistant cell membrane [17]. It was concluded from this study that folic acid administration with MTX is very important to 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.

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تأثير حامض الفوليك على بعض المعايير الكيموحيوية في إناث الأرانب المعالجة تجريبياً بالميتوثريكسات

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أجريت الدراسة الحالية لمعرفة تأثير حامض الفوليك على بعض المعايير الكيموحيوية لإناث الأرانب المعالجة وغير المعالجة بالميتوثريكسات MTX. إذ تم استخدام عشرون أنثى من الأرانب في هذه الدراسة تراوحت اعمارهم من ٤-٥ أشهر واوزانهم بين ١,٢٠٠-١,٢٠٠ كجم. تم تربيتها تحت ظروف بيئية مناسبة ٢٥-٢٧ درجة مئوية. وتم تقسيمها إلى أربعة مجموعات وكل مجموعة تتكون من خمسة حيوانات على النحو التالي (مجموعة السيطرة : تكونت من ٥ ارناب وتم اعطاؤها الماء المقطر عن طريق الفم، مجموعة حامض الفوليك : ٥ أرناب تم تجريعها حامض الفوليك بمعدل ٠,٠٧ مجم / كجم من وزن الجسم عن طريق الفم يومياً ، مجموعة الميتوثريكسات: ٥ أرناب تم تجرعها الميتوثريكسات (٠,٠٣ مجم / كجم من وزن الجسم عن طريق الفم) ثلاث مرات في الأسبوع ومجموعة حامض الفوليك والميتوثريكسات: ٥ أرناب تم تجريعها حامض الفوليك (٠,٠٧ مجم / كجم من وزن الجسم عن طريق الفم) والميتوثريكسات (٠,٠٣ مجم / كجم من وزن الجسم عن طريق الفم) ثلاث مرات يومياً ، استمرت التجربة لمدة ٩ أسابيع وتم جمع عينات الدم بعد تسعة أسابيع من التجربة لدراسة المعايير الكيموحيوية التالية (البيليروبين في مصل الدم ، قابلية الحديد غير المرتبط (UIBC) ، والقدرة الكلية لارتباط الحديد (TIBC). ، تركيز الحديد والحديدوز الكلي في مصل الدم) أظهرت النتائج ان مجموعة MTX سجلت انخفاضا معنوياً ($P \leq 0.05$) في تركيز البيليروبين ، وفي نفس الوقت هناك ارتفاعاً معنوياً في SI ، TIBC ، UIBC في نفس المجموعة عند مقارنتها مع مجموعة السيطرة. كما أظهرت المجموعة التي جرعت بحامض الفوليك مع MTX تحسن الحالة الصحية وعودة القيم المدروسة الى وضعها الطبيعي . نستنتج من هذه الدراسة ان تناول حامض الفوليك لوحده او مع MTX مهم جداً لتحسين الوظائف الحيوية للجسم وهناك حاجة إلى مزيد من الدراسات لمعرفة آثار هذه الأدوية على أنظمة الجسم الأخرى.

الكلمات المفتاحية : حامض الفوليك ، اناث الارانب ، الميتوثريكسات .