Effect of Methotrexate Drug on Some Parameters of Kidney in Newborn mice

Zainab Kh. Hussain[1*], Ferial AL-Mhdawi[1], Nahla AL-Bakri[2]
[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
[2]Department of Biology, College of Education (Ibn AL- Haitham), University of Baghdad, Baghdad, Iraq.

Abstract
Thirty pregnant albino mice with average body weight of 25-30gm and 8-14 weeks old were used to investigate the toxicity of the oral administration of anticancer methotrexate on kidneys after birth. Pregnant were received (single dose) of drug at different days of gestation and were divided into three groups (10mice / group), control groups were administered distilled water, the two remaining groups were administered by 2.5 and 7.5 mg/kg body weight of methotrexate. The changes in kidneys of newborns revealed significant ($p<0.05$) decrease in kidneys weights, kidneys lengths, pelvises lengths, and glomeruli diameters. The increase in concentration of methotrexate has increasing significant effect on kidneys of embryo and newborns.

Keywords: Methotrexate, Kidney, Glomerulus, Embryo, Newborn, Mice.

تأثير عقار الميثوتركسيت على بعض معايير الكلى في الفئران حديثي الولادة

زينب خضير حسين[1*]، فريال المهداوي[1]، نهلة البكري[2]
[1]قسم علوم الحياة، كلية العلوم، جامعة بغداد، [2] قسم علوم الحياة، كلية التربية (ابن الهيثم)، جامعة بغداد، بغداد، العراق.

الخلاصة:
استخدمت (30) من إناث الفئران الحوامل بمعدل وزن (25–30) غرام وعمر (10–14) أسبوع لتحري عن سمية عقار الميثوتركسيت المضاد للسرطان. لدراسة تأثير العقار على الكلى بعد الولادة، جرعت الأمهات الحوامل عن طريق الفم بواقع جرعة واحدة من العقار في أ يام معينة من الحمل . قسمت الإناث إلى ثلاثة مجاميع(عشرة لكل مجموعة) وشملت مجموعة السيطرة جرعت بالماء المقطر ، والمجاميع الاخرى جرعت بتركيز(2.5 و 7.5ملغم /كغم وزن الجسم) من عقار الميثوتركسيت . أظهرت النتائج أن تعرض الأمهات الحوامل إلى جرع مختلفة من عقار الميثوتركسيت سبب انخفاض معنوي ($P<0.05$) في معدل أوزان الكلى للفئران حديثة الولادة (عمر يوم واحد) وكذلك حدوث انخفاض معنوي ($P<0.05$) في معدل أطوال الكلى وفي أطوال الحويض والأقطار الكبيبية لحديثي الولادة .ان الزيادة في التركيز للميثوتركسيت تؤدي الى زيادة التأثير في كلى الاجنة وحديثي الولادة

Introduction

Methotrexate used to treat cancer, psoriasis and rheumatic diseases, Methotrexate is used successfully in multiple kinds of cancers such as acute lymphoblastic leukemia [1]. Temporary remission leukemia, remission in choriocarcinoma, osteosarcoma, ovarian cancer, breast cancer,

*Email : zezulife80@yahoo.com

epidermoid cancers of the head and neck, advanced mycosis fungoides (cutaneous T cell lymphoma), and lung cancer, particularly squamous cell and small cell types [2]. Methotrexate is a synthetic analogue of dihydrofolate (DHF) and act as a potent inhibitor of the housekeeping enzyme dihydrofolate reductase (DHFR) [3]. Inhibition of DHFR blocks the conversion of DHF to tetrahydrofolate (THF). Which is an essential cofactor in the biosynthesis of purines, thymidylate, homeostatic levels of glycine, serine, homocysteine and some amino acid [4].

Methotrexate is an antimetabolite drug which means it is capable of blocking the metabolism of cells. It acts by inhibiting the metabolism of folic acid. Methotrexate is cell cycle S-phase selective, and has greater negative effects on rapidly dividing cells (such as malignant and myeloid cells), which are replicating their DNA and thus inhibits the growth and proliferation of these cells [2]. Inside the cell, MTX competes with folate and DHF for the active site of DHFR and is transported and modified by the same cellular factors. Reduced folate carrier (RFC), folate receptor (FR), and low PH transporters are used for cellular uptake of folate and MTX in mammals [5]. The affinity of DHFR for MTX is much higher than for either folic acid or DHF [6]. So that the enzyme-bound MTX leads to partial or complete depletion of reduced folate levels and in turn, the inhibition of processes involving folate derivative [7].

Materials and Methods

Animal Breeding:

Animals approximately were (10-14) weeks old and their weights were around 25-30 gm, the animals were housed in standard plastic cage measuring 40×25×15 cm in animal house of Biology Department of College of Science in University of Baghdad under 12 hours light /12 hours dark at 21 ± 4 ^{o}c, 4mice (3females:1male) were placed in each cage with wood shaving bedding, free access to food and water *ad libitum* was allowed all the time [8].

Animal mating:

After one week adaptation, females' mice were mated with healthy male mice. Mating was determined by checking vaginal plug. The vaginal plug was regarded as zero day of pregnancy, pregnant mice in experimental groups were fed separately (one per cage) [9].

Treatment:

Methotrexate was obtained from (EBEWE pharms. Austria), was used by oral administering of 0,01mg and rout of gavage with two concentrations (2.5 and 7.5) mg/kg of methotrexate of body weight of mice by aid of special needle [10].

Experimental Design

Thirty pregnant mice were divided into three groups:

group1: Ten pregnant mice were given distilled water and served as control till the day of birth.

group2: Ten pregnant mice were given a dosage (2.5) mg/kg body weight of MTX.

group3: Ten pregnant mice were given a dosage (7.5) mg/kg body weight of MTX.

Each pregnant in these subgroups administered by MTX in a single dose given once which started at the 6th day of gestation, then waiting to birth, kidneys of newborns (one day) examined and this was repeated for every days of gestation from 7th till 15th day.

Animals killing and Specimens collection:

New borns were euthanized with an overdose of ether an aesthesia, newborns placed on dissecting plate then the kidneys were removed, the kidney weight and length were measured , then the kidney fixed in Bouins fluid for 12-24 hours then washed several times with ethanol 70% and kept until use [11]. For histopathology, processed routinely in histokinette, the thickness of slices was at seven micrometer [12] and stained with haematoxylin and eosin stain then examed under light microscop. Photographs were taken by digital camera. Glomerular diameter and pelvis length were assessed in each kidney using previously calibrated micrometer (Ocular micrometer, stage micrometer). The diameters of 25 glomeruli were measured in five fields (5 glomerular per field).

Statistical analysis:

All data are expressed as mean ± standard error (M±SE) were calculated for final parameters, the statistical analysis system SAS (2004) program was used for data analysis (the effect of treatment in study traits). Least significant different –LSD test was used to the significant comparison among means. Groups were compared with each other on the level 0, 05 to detect the significant of the drug concentration effect on the newborns kidney[13]

Results and Discussions

The results of methotrexate effect on the kidneys of newborns revealed in treated groups and increasing in toxicity in animales treated with high dose more than in animales treated with low dose.

Control group

There are no significant microscopic changes in control untreated animales. The kidnyes of all these group are normal.

Low dose animale

The treated groups with (2.5 mg/kg) of MTX showed significant ($p<0.05$) decrease in kidneys weights, kidneys lengths, kidneys pelvises lengths and glomeruli diameters values, figures-1,2,3 and 4.

High dose animales

The treated groups with (7.5 mg/kg) of MTX showed significant ($p<0.05$) decrease in kidneys weights, kidneys lengths, kidneys pelvises lengths and glomeruli diameters values, figures-1,2,3 and 4.

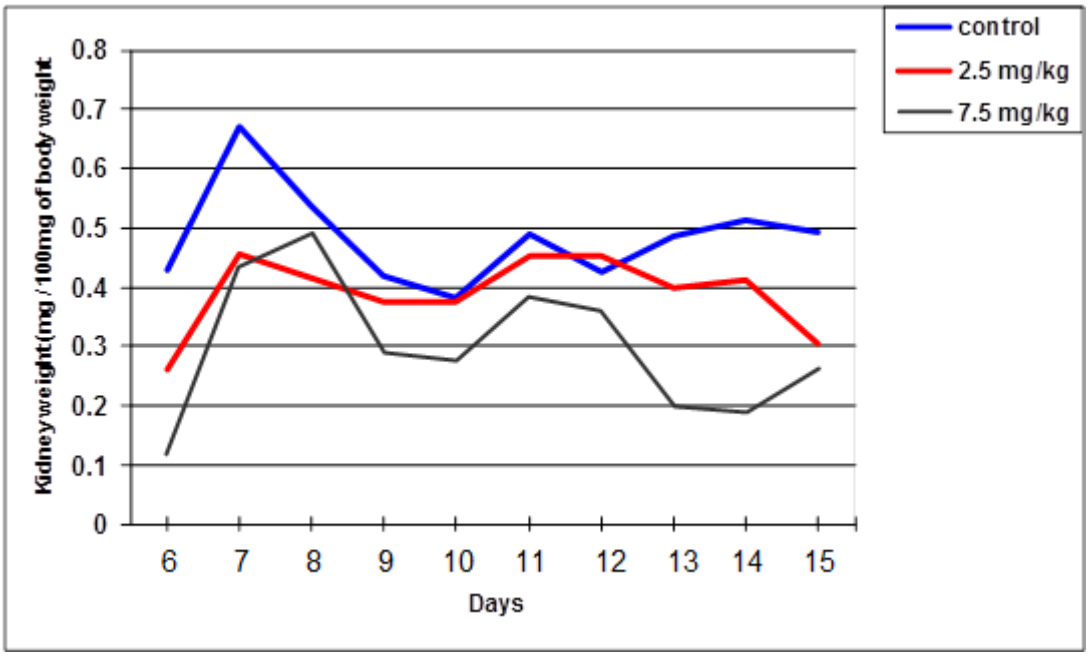

Figure 1- Effect of (2.5 and 7.5) mg/kg of MTX on kidneys weights (mg/100mg) of body weight of newborns aged one day.

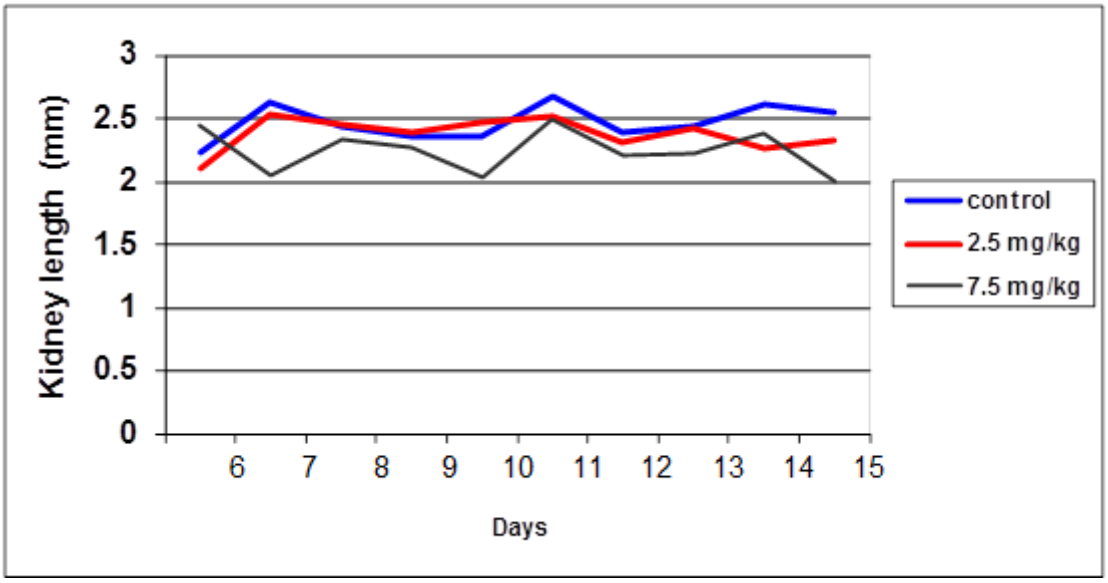

Figure 2- Effect of (2.5 and 7.5) mg/kg of MTX on kidneys lengths (mm) of newborns aged one day.

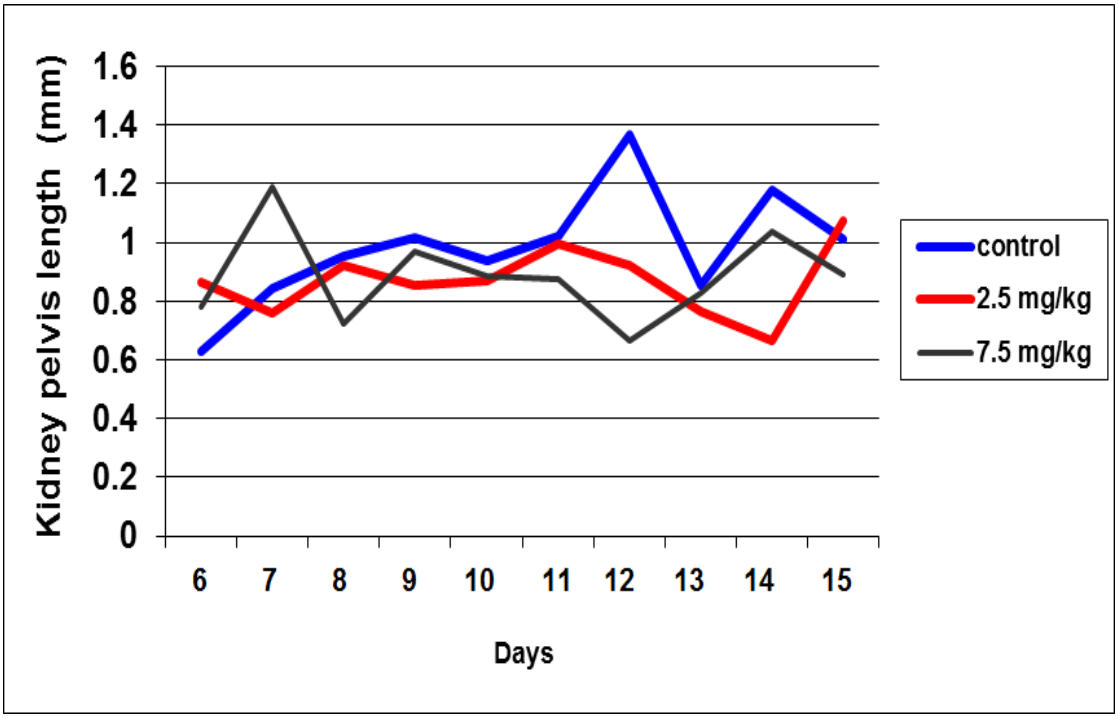

Figure 3- Effect of (2.5 and 7.5) mg/kg of MTX on kidneys pelvises lengths (mm) of newborns aged one day.

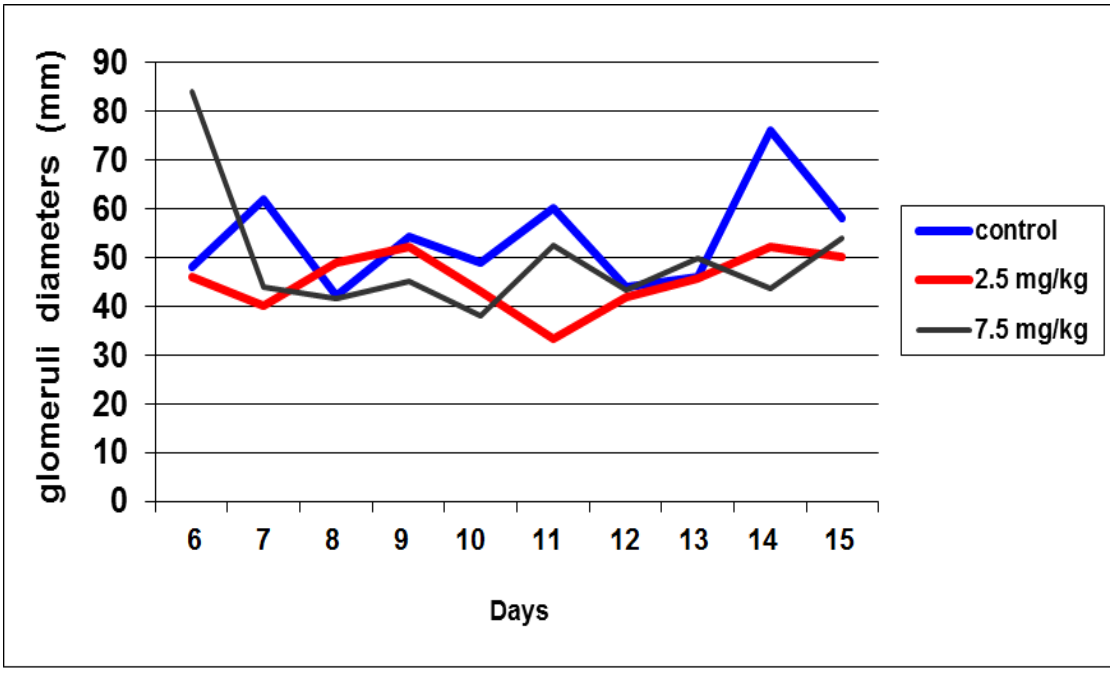

Figure 4- Effect of (2.5 and 7.5) mg/kg of MTX on glomeruli diameters (µm) of newborns aged one day.

The most critical period for teratogenic effects occur during the embryonic period . In the fetal period, effect of drug exposure can cause fetotoxic effects such as growth restriction, change in size or functioning of certain organs, or development and behavioral abnormalities [14].

In animal studies, significant kidneys weights loss were shown in newborn aged one day after birth, the oral administration of pregnant with MTX (2.5, 7.5) mg/kg at (6-15) day of gestation in compared with the control group in Figure 1. The reason of kidneys weights loss, may be due to the fact that MTX blocks folic acid dependent steps in the synthesis of purin and pyrimidines [15]. This result may suggest that this concentration may limit the intracellular supply of reduce folates (tetrahydrofolate)

through inhibition of dihydrofolate reductase (DHER) and therefore is consider to induce cytotoxic effects with resultant inhibition of new DNA [16].

The administration of pregnant by MTX (2,5 and 7,5 mg/kg) in single dose at (6-15) days of gestation resulted in treatment of related decrease in kidneys lengths of newborns . in Figure 2. The reason behind kidneys lengths loss, is perhaps due to that MTX drug which is classified as an anti metabolite drug which means it is capable of blocking the metabolism of cells [17]. Anti metabolites drug are chemically related to naturally occurring compounds which interfere with the cellular metabolism especially processes involved in synthesis of DNA, they include some of the most widely prescribed cytotoxic agents [18].

The administration of MTX in concentration (2,5 and 7,5 mg/kg) in single dose at (6-15) days of gestation resulted in treatment related decrease in kidney pelvis length of newborn mice in Figure 3. MTX is an anti folate drug which inhibits cell division by interfering with DNA replication [19].

[20] Pointed that the MTX prevents auto-repair damage in DNA, in addition to leading to the damage of DNA molecule as a result showed that interpreted leads to the accumulation of DNA fraction in single thread within cell and that this effect occurs as a result because the drug inhibits the act of DNA polymerase is necessary for repair system by excision repair, and when thymidin and hypoxanthine were added to the cells treated with MTX in vitro, it was observed that the accumulation of DNA fraction stopped, and this proves that the role of MTX leads to a depletion in nucleotide and therefore affects the process of repairing damage that occurs by exogenous agents.

The administration of MTX in concentration (2,5 and 7,5 mg/kg) in single dose at (6-15) days of gestation resulted in treatment related to decrease in means glomeruli diameters of kidney of newborn mice in Figure 4. MTX blocks cell proliferation in the S- shape of the cell cycle during which DNA replication.

The effect of MTX is produced for it is easy to uptake the cell-mediated, and thus easily lead to inhibition the effectiveness of the dihydrofolate reductase enzyme is the key to multiply in the cell [21]. MTX acts as an alkylating agent which leads to prevent the division of cells by effecting the single thread in the same DNA or between the two different bands as well as to inhibit the DNA synthesis, and the metabolic effectively results in MTX leading to chromosomal fractions [19].

References

1. Aytac, S.; Yetgin, S.; and Tavil, B. **2006.** Acute and long-term neurologic complication in children with acute lymphoblastic leukemia. *Turk J. Pediatr*., 48(1), pp:1-7.
2. Natekar, P. E. **2007**. Methotrexate induce gross malformations in chick embryos. *J. Hum. Ecol*., 21(3), pp:223-226.
3. Miahara, M. ; Katsume, A. and Takeda, Y. **1992**. Eeffect of Methotrexate treatment on the onset of autoimmune kidney disease in lupus mice .*Chem.Pharm.Bull*.,40(8), pp:2177-2181.
4. Kompis, I. M; Islam, K. and Then, R. L. **2005**. DNA and RNA Synthesis: Anti folate .*Chem. Rev.*, 105, pp:593-620.
5. Berzezinska, A.; Winska, P. and Balinska, M. **2000**. Cellular aspect of folate and anti folate membrane transport. *Acta Biochim.* Pol., 47, pp:735-739.
6. Appleman, J. R.;Prendergast, N.; Delcamp, T. J. ; Freisheim, J. H.; and Blakley, R. L. **1998**. Kinetics of the formation and isomerization of methotrexate complexes of recombinant human dihydrofolate reductase. *J Biol Chem*., 263, pp:10404-10413.
7. Affleck, J. G.; and Walker, V. K. **2008**. A role for Drosophila in understanding drug-induced cytotoxicity and teratogensis. *Cytotechnology*, 57, pp:1-9.
8. Chelab, K. G. and Majeed, S. KH. **2009**. Methotrexate-induce histological changes in the kidneys of mice. *Iraqi Journal of Veterinary Science*.,23(2), pp:219-222 .
9. Yaping, J. ; Shuhua, X. ; Xin, L. ; Chunwei, L. ; Gexin, L. ; Yuanyuan, X. ; Chunqing. Q.; Yuhong, N. and Guifan, S. **2006** . Arsenic speciation transported through the placenta from mother mice to their newborn pups. *Environmental Research*, 101, pp:349-355.
10. Stephen, M.; Philipa, F. S. and Sternberg, S.S. **1971**. The cytotoxicity of methotrexate in mouse small intestine in relation to inhibition of folic acid Reductase and of DNA synthesis. *Cancer Research*,31, pp:2047-2046.
11. Kaliste,E. **2007**. *The welfare of laboratory animals*. Springer, Netherlands, pp:111-112.
12. Kiernan, J. A. **2000**. *Histological and histochemical methods (theory and practice)*.3^{ed} edition. Butter Worth Heinemann, Oxford.

13. Ukaelii, S. A. and Shaeb, S. M. **1998**. *Statistically Analysis by used SPSS program*. Al-Shoroq hous for publishers and advertisement Amaan, Jordan.
14. King, T. L. and Brucker, M.C. **2009**. *Pharmacology for women's health*. Jones and Bartlelt Publishers LLC., U.S.A.
15. Tishler, M.; Caspi, D. ; Fishel, B. and Yaron, M. **1988**. The effects of leucovorin (folinic acid) on methotrexate therapy in rheumatoid arthritis patients. *Arthritis Rheum*., 31, pp:906-908.
16. Sano, H. ; Kubota, M. ; Kasai, Y. ; Hashimoto, H. ; Shimizu, T. ; Adachi, S. and Mikawa, H. **1991**. Increased methotrexate-induce DNA strand breaks and cytotoxicity following mutational loss of thymidine kinase. *Int. J. Cancer*, 48,pp:92-95.
17. Watanabe, S.; Sato, S.; Nagase, S.; Shimosato, K. and Ohkuma, S. **1999**. Effect of methotrexat and cyclophosphamide on polyamine levels in various tissue of rats. *J. Drug. Target*, 7(3) pp:197-205.
18. Jeon, K. W. **1994**. *International review of cytology*. Academic Press INC., London.Pp:108.
19. Crook,T.; Souhami, R. and Mlean, F. **1996**. Cytotoxicity: DNA croos-linking and single strand breaks induced by activated cyclophosphamide and acrolein in human leukemia cells. *CanceRes*.,46,pp:5029 -5034.
20. Boechers, A. H.; Kennedy, K. A. and Straw, J. A. **1990**. Inhibition of DNA excision repair by methotrexate in chinese hamster ovary cells following exposure to ultraviolet irradiation or ethyl methane sulfonate. *Cancer Res.*,50, pp:1786-1789.
21. Huennekens, F. M. **1994**. *The methotrexate story: A Paradigm for development of cancer chemotherapeutic agents*. Academic Press INC.