

Methotrexate-induced histopathological changes in the kidneys of mice

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Abstract

The present study was conducted on (40) white mice of approximately the same age (4-6 weeks) and body weight (23-25 gm) for the aim of observing the histopathological changes for kidneys due to prolonged treatment (6 months) with anticancer chemotherapeutic agent namely methotrexate. Forty mice were divided into 4 groups (10 mice of each group, 5 mice per sex). The first group (low or therapeutic dose group) was received 0.15 mg/kg. The second group (intermediate dose group) received 0.3 mg/kg. The third group (toxic dose group) received 0.45 mg/kg. The fourth dose group was a control group; it received 0.2 ml buffered normal physiological saline. All these groups injected intramuscularly, once weekly dose for 6 months. The results showed that methotrexate cause damage and severe toxicity in renal convoluted tubules and glomeruli in kidneys.

Keywords: Methotrexate, Anticancer, Kidney.

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التغيرات المرضية النسجية المحدثة بالميثوتريكسات في كلى الفئران

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الخلاصة

أستخدم في الدراسة الحالية (40) من الفئران البيضاء ذات أعمار متقاربة (4-6 أسابيع) وأوزان تتراوح بين (23-25 غرام)، وذلك من أجل معرفة التغيرات المرضية النسجية في الكلى بسبب المعالجة الطويلة (6 أشهر) بالعامل العلاجي الكيماوي المضاد للسرطان والمسمى الميثوتريكسات. (40) من الفئران قسمت إلى 4 مجاميع (10 فئران لكل مجموعة، 5 فئران لكل جنس). المجموعة الأولى (مجموعة الجرعة العلاجية) أو الجرعة الواطئة استلمت 0,15 ملغم/كغم. أما المجموعة الثانية (مجموعة الجرعة المتوسطة) استلمت 0,30 ملغم/كغم. المجموعة الثالثة (مجموعة الجرعة السمية) استلمت 0,45 ملغم/كغم. وجميع الجرعات أعطيت مرة واحدة أسبوعياً بالعضل. أظهرت النتائج إن الميثوتريكسات يمكن إن يسبب تلف وسمية شديدة في النبيبات الكلوية الملتهبة والكبيبات في الكلى.

Introduction

For the last 40 years, chemotherapy has had a role in cancer management in human and animal patients. More than 50 agents are useful in human medicine (1), but the number of the partial use in veterinary medicine is smaller.

Chemotherapy may help control generalized rapidly progressive not amenable to surgery or radiotherapy or may help increase the disease – Free interval after other initial

treatment. It may prevent spread of a neoplasm by controlling early metastases that are proliferating rapidly and have a relatively small likelihood of containing resistant cells (2).

The main problem in cancer chemotherapy is the lack of highly selectively toxic agents. Cancer cells arise from normal cells and, unlike the situations described for viral, bacterial and fungal infections, there is a paucity of obvious selective drug targets. With currently used antiproliferative

anticancer drugs, many rapidly dividing normal cells (bone marrow, gut epithelium, spermatogenic cells, lymphoid tissue, hair follicles, and fetus) are also killed (3).

Chemotherapy involves the use of one or several antineoplastic (anticancer) drugs to kill cancer cells. Methotrexate is an example of an anticancer drug that interferes with cellular reproduction and is also used in the treatment of psoriasis and certain inflammatory disease (4).

Methotrexate is a chemical agent that acts by inhibiting the enzyme dihydrofolic acid reductase, which catalyses the conversion of folic acid to its active form folinic acid, by binding to it (5).

Methotrexate is primarily cleared by the kidney (6). Methotrexate concentrates in the kidney, gallbladder and spleen as well as in the liver. Renal excretion eliminates 60 to 90% of a dose. Risk of renal damage leading to acute renal failure due to primarily to the precipitation in the kidney can be reduced by adequate oral hydration and urine alkalization (methotrexate is a weak acid and tends to precipitation at urine PH below 6.0) (7).

This study is designed to investigate on side effects of methotrexate on kidneys in white mice because methotrexate has been use in various fields of oncology for along time, and also it was use for other diseases such as psoriasis and rheumatoid arthritis.

The aim of present study is to study of side effects at toxicity of methotrexate (chemotherapeutic agent) on kidneys.

Materials and methods

Forty mice (20 males and 20 females) of 1-1.5 months old were divided into 4 equal groups (each groups consisted of 5 males and 5 females).

The animals were housed in a 6× 4× 3 m³ room in animal house of Veterinary Medicine College, Baghdad University under 12 hours light / 12 hours dark at 21 ± 4 °C and put as 5 mice in each standard plastic cages.

Treatment

Methotrexate (Trixiem)^R is a clear yellowish solution, vial of 5 mg / 2 ml for injection, (Iemery-Uppsala Sweden).

Each 5 mg / 2 ml was diluted with 333 ml physiological normal saline and the mixture was injected intramuscularly to animals. Animals were injected once weekly and for six months, untreated controls received equivalent amount of physiological normal saline.

Experimental Design

Forty mice divided into for 4 equal groups: First group is a control group received only normal saline at a dose of 0.2 ml intramuscularly once weekly. Second group as a therapeutic (low dose) received 0.15 mg/ kg,

intramuscularly once weekly diluted with 0.2 ml saline. Third group as intermediate dose received 0.30 mg/ kg, intramuscularly once weekly diluted with 0.2 ml saline. Fourth group received a high dose (toxic dose) 0.45 mg/ kg, intramuscularly once weekly.

For histopathology, pieces of 1-2cm from kidneys were taken kept in 10% neutral buffered formalin for fixation, processed routinely in histokinette, cut at 5Mm thickness by microtome (Jung 4291, West Germany) and stained with Haematoxylin and Eosin stain then examined under light microscope (8).

Results

Macroscopic changes

The kidneys were congested in all treated groups and enlarged in toxic dose group more than in low and intermediate dose groups.

Microscopic changes

Control group

There were no significant microscopic changes in control untreated animals. The kidneys of all these group are normal in shape and size.

Therapeutic doses animals

The kidneys contained an area of cortical tubules basophilia with infiltration of lymphocytes and congested glomeruli also there were cortical areas of dilated tubules (Figure 1).

Intermediate doses animals

The kidneys were showed pericapsular aggregate of lymphocytes (perirenal adipose tissue) (Figure 2) with cortical areas of dilated tubules more than the previous group (Figure 3).

High (Toxic) dose animals

The kidneys showed severe dilatation of cortical tubules (Figure 4), with areas of cortical tubular basophilia, also there was congestion and infiltration of lymphocytes (Figure 5), and there was degenerated cortical tubules characterized by exfoliated epithelial cells in the lumen with nuclear and debris and peritubular inflammatory cells (Figure 6).

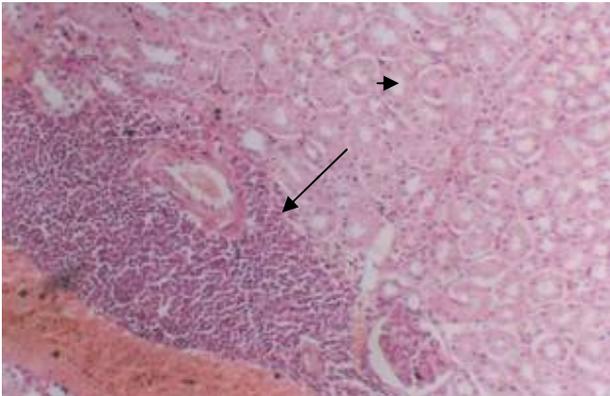


Figure (1): Kidney (therapeutic dose group), there is sever infiltration of lymphocytes cells (arrow) with an area of dilated cortical tubules (head of arrow) (X 50 H&E).

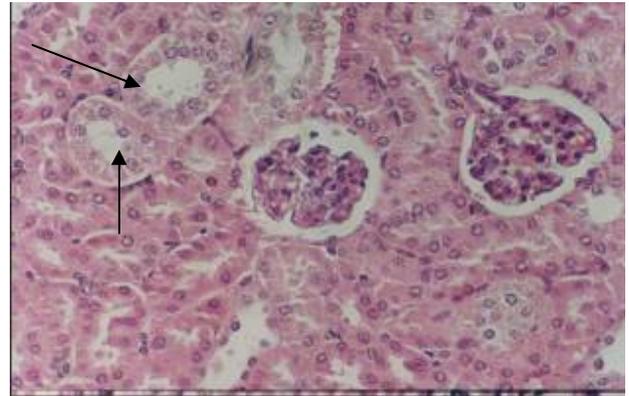


Figure (4): Kidney (toxic dose group) note an area of cortical tubular basophilia (arrows) (X 50 H&E).

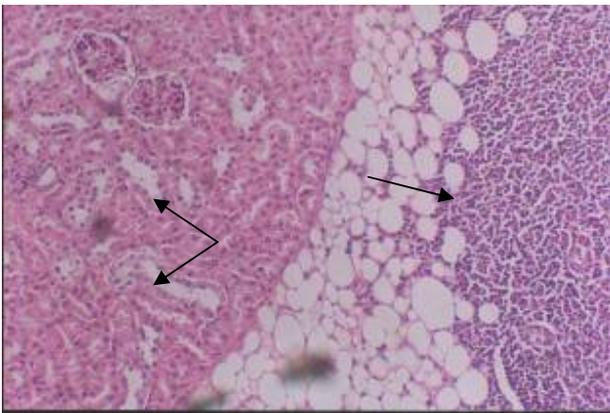


Figure (2): Kidney (intermediate dose group), pericapsular aggregate of lymphocytes (perirenal adipose tissue) (arrow), also there is cortical dilated tubules (X 50 H&E), (double arrow).

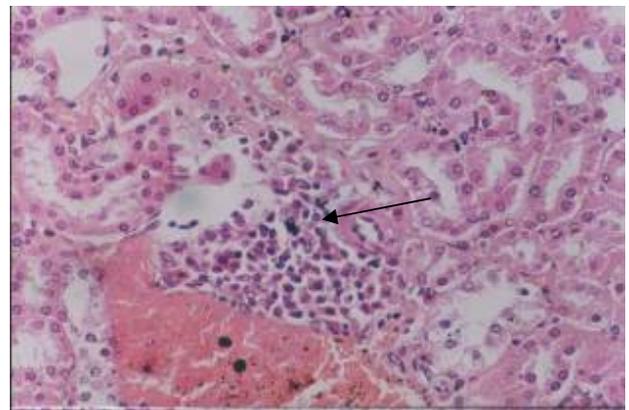


Figure (5): Kidney (toxic dose group) note perivascular aggregation of lymphocyte in the cortex (arrow) (X 100 H&E).

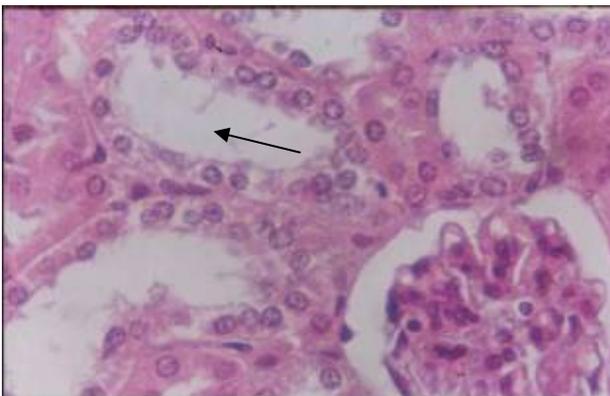


Figure (3): Kidney (intermediate dose group) there is cortical area of dilated tubules (arrows) (X 200 H&E).

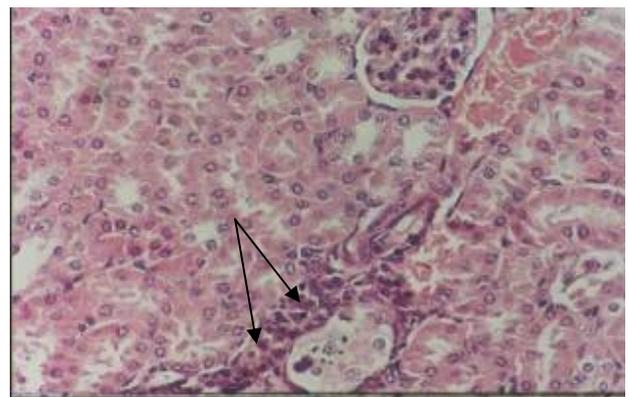


Figure (6): Kidney (toxic dose group) note degenerated cortical tubules characterized by exfoliated epithelial cells in the lumen with nuclear and debris and peritubular inflammatory cells (arrows) (X 100 H&E).

Discussion

Cancer occurs when cells grow too rapidly and in an uncontrolled way. For cancer cells to grow, new DNA needs to be made. Methotrexate is a drug that is used to treat certain cancers. With methotrexate, cancer cells can not make DNA, this kills cancer cells (9). However, methotrexate can also be harmful to other normal cells and organs in the body, this harmful effect is called methotrexate toxicity, using methotrexate for long period and the longer methotrexate stays in the body, can increase the risk of toxicity (10).

The kidneys were appeared affected in all treated animals especially in toxic dose group animals. The kidneys showed marked cortical dilatation of tubules, the dilatation was cystic in toxic group, that the kidney, the main site of excretion of methotrexate (11), when methotrexate level increase in the body, that would cause severe renal toxicity and result in accumulation of methotrexate crystals in the nephron and that lead to dilatation of nephrous tubules, these results agreed with (12) which indicated that methotrexate crystals accumulated in the nephrous tubules. Also the dilatation of nephrous tubules of renal cortex occur due to thickening of basement membranes of tubules and deposition of hyaline as acidic substance and this lead to dilatation of nephrous tubules and suggested that there was tubular dysfunction (13) and (14). Also, the results of the present study showed tubular basophilia, the tubular basophilia is a form of regenerated tubules preceded by tubular degeneration which caused by treatment of methotrexate, these results agreed with (12) that indicated the toxic accumulation of methotrexate in a patients may be associated with renal failure (15).

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