

Protective effect of captopril against methotrexate- induced nephrotoxicity in mice

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Abstract:

The present study was designed to investigate some histopathological changes produced by methotrexate on kidney in mice and extended to examine whether there using captopril have ability to reduce this toxicity.

Forty mice(20 male and 20 femel) were divided in to 4 groups . The first group received 10mg/kg B.W. of methotrexate I/M twice weekly. The second group received 60mg/kg B.W. of captopril and then received methotrexate. While the third group received captopril alone,While fourth group (control group) received normal saline and the study was continued for (30 days).

Animals which were treated with methotrexate only along the period of experiment showed sever pathological changes, characterized by severe tubular necrosis ,atrophy of glomeruli and dilatation of tubules.Where as oral administration of captopril significantly($p<0.01$) reduced these changes .The protective activity of captopril has been resumed via diminishing the renal histopathological changes which associated with methotrexate Toxicity.while there were insignificant differentiated ($P<0.01$) between the control group and the group recieve of captopril alone.

التأثير الواقي للكابتوبريل لتقليل السمية الكلوية المتسببة عن الميثوتريكسيت في الفئران

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الخلاصة:

صممت هذه الدراسة لمعرفة التغيرات النسيجية المرضية الناتجة من التأثير السمي للميثوتريكسيت على الكلية في الفئران ،ومعرفة تأثير الكابتوبريل لتقليل السمية.

استخدم في هذه الدراسة (40) من الفئران ،قسمت إلى أربع مجاميع. المجموعة الأولى استلمت 10ملغ/كغم من وزن الجسم ميثوتريكسيت مرتين أسبوعيا في العضل، إما المجموعة الثانية فقد استلمت كابتوبريل 60 ملغ/كغم من وزن الجسم فمويا ثم استلمت ميثوتريكسيت. أما المجموعة الثالثة فقد اعطيت كابتوبريل فقط. والمجموعة الرابعة أعطيت المحلول الملحي الفسيولوجي واعتبرت كمجموعة سيطرة. استمرت الدراسة لمدة 30 يوم .

قتلت الحيوانات وجمعت العينات ثم ثبتت بالفورمالين 10% ،كل هذه العينات قطعت وحضرت مقاطع نسيجية منها بعد ذلك صبغت بصبغة الهيماتوكسيلين-ايوزين ،فحصت جميع الشرائح تحت المجهر الضوئي حيث لاحظنا في حيوانات المجموعة الأولى وجود تغيرات نسيجية شديدة تمثلت بالتخر

الشديد لنبيبات الكلية، ضمور في الكبيبة وكذلك هناك توسع في النبيبات الملتنوية للكلية. أما حيوانات المجموعة الثانية (والتي أعطيت الكابتوبريل ثم أعطيت الميثوتريكسيت) قد لاحظنا انخفاض معنوي شديد ($P < 0.01$) في التغيرات الناتجة في المجموعة الأولى. كذلك لاحظنا ان ليس هناك فرق معنوي ($P < 0.01$) بين مجموعة السيطرة والمجموعة التي أعطيت الكابتوبريل فقط على نسيج الكلية في الفئران.

Introduction:

Methotrexate (MTX; 4-amino-10-methyl folic acid) is an anti proliferative chemotherapeutic that interrupts folate metabolism by inhibition of dihydrofolate reductase a required precursor for co- factors involved in macromolecule biosynthesis(1). It one of most successful drug in cancer chemotherapy (2). It has been used for many years in treatment of various types of cancer and autoimmune disorder such as rheumatoid arthritis and psoriasis(3). It is develop in early 1950s to treat children with acute lymphocytic leukemia and still a key component of standard chemotherapy regimen for treating leukemia and other several cancer(2,4)

In veterinary medicine, methotrexate used mostly at modest dose in combination regimen in treatment of lymphosarcoma and osteosarcoma (5).

Methotrexate is a folic acid antagonist it classified as cellular metabolism of folate , in particular it inhibits the activity of dihydrofolate reductase along with tree enzymes involved in purine and thymidine biosynthesis, the efficacy of methotrexate in treatment of cancer is widely attributed to a decrease in the production of nucleotide (2,6).

However, the efficacy of this agent often is limited by sever side

effect and toxic condition (7) , nephrotoxicity is one of adverse side effect of methotrexate (8) and can cause acute renal failure when administrate in high dose(7). However , the dose of the drug had to be totally stopped due to supervening toxicity resulting directly from the drug use and this means strategy to get maximum therapeutic response minimum toxicities still requires(9)

There is a great interest in expanding the clinical usefulness of methotrexate by developing new agent in order to reduce it is nephrotoxicity therefore admenstration of various agents with methotrexate should has been make.

Angiotensin –converting enzyme inhibitors (ACEI) are widely prescribed for treatment of hypertension and congestive heart failure . they also delay the progression of chronic renal failure and diabetic nephropathy(10) .Captopril an ACEI containing sulfhydryl (-SH) group is widely use for such disorder (11).

The aim of the present study is to examine the role of captoprile in protection of methotrexate induced nephrotoxicity in white mice besides the histopathological changes in kidneys which occur due to using of methotrexate with high dose..

Materials and methods :**Animals:**

The present study was conducted on (40)white mice(20 male and 20 femal) of approximately the same age (6weeks) and body weight(25-30 gm).

The animals were housed in 6x4x3m³ room in animals house of vet.medicine collage / Al-Qadisiya university under 12 hours light/12 hours dark at 28±2c and put as 5mice in each standard plastic cages and this study continued for 30 days.

Materials :

Methotrexate Ebewe(50mg in 5ml,EBEWE pharma GES.M.B.H.nfg.Kg) and captophen 25mg (IBN Hyyan pharma –Homs-syria) were dissolved in normal saline and give 10 mg /kg B.W. I/M as toxic dose (12) and 60mg/kg B.W orally.(13) respectively.

Experimental design:

Mice were randomly divided in to 4 equal groups ten animals in each group:

First group :Administered I/M with methotrexate at dose 10mg/kg twice weekly .

Second group :Administered captopril at dose 60mg/gm orally and treated with methotrexate at dose 10mg/kg I/M.

Third group: Administered orally60mg/kg B.W of captopril only.

Forth group: treated with normal saline only and serve as a control group I/M.

Methods :

For histopathology ,pieces of 1-2cm³ from kidneys taken then kept in normal buffered formalin for fixation , processed routinely in histokinette , cut at 5 Mm thickness by microtome (jony 4291, west Germany) and stained with Haematoxylin &Eosin stain then examined under light microscope(14).The lesions were generally scored as light(+), mild(++), and severe(+++) , according to slightly modified (15).Scorings done according to following criteria: degeneration in renal tubules, normal (0), mild degeneration(1) ,moderate degeneration(2), severe degeneration (3).Tubular necrosis, glomerular atrophy and cystic dilatation were also enumerated in ablined manner.

Statistical analysis :

Data are given as mean ±SEM. Histopathology scores were analyzed by ANOVA and T-test to know significant differentiated between the values on a level (P<0.01).

Results& discussion:

The present study demonstrated that methotrexate considered as nephrotoxic to renal system in mice in high doses due to histopathological changes which showed in renal tissue.

The changes were sever and clear in renal tissue characterized by degenerative change in glomeruli and tubular epithelium (Figure 1),These degenerative changes were more frequent and severe(2.8±0.133)

in first group (methotrexate group) compared with second group (captopril and methotrexate group) (1.9 ± 0.233), or control group (0.0 ± 0.000) (table 1). It was reported by (16) they was reported that in high dose of methotrexate can induce nephrotoxicity.

There are tubular necrosis of the kidney (figure 2), these changes observed in the kidneys and these injury was significantly increase (2.6 ± 0.163) in first group (methotrexate group) compared with second group (captopril and methotrexate group) (1.8 ± 0.200) in which there were mild tubular necrosis (figure 3), or control group (0.0 ± 0.000) (table 1) And these reported by (17 & 18) they was reported that methotrexate has lethal effect on renal tubular cells due to direct damage of tubular epithelium or by precipitate of methotrexate in these tubules and cause damage. Also there are atrophy of glomeruli (figure 4), these changes was more severe in methotrexate group (2.8 ± 0.200) compared with second group (methotrexate and captopril group) (1.7 ± 0.260) or control group (0.0 ± 0.000) (table 1) In which there were mild glomerular atrophy (figure 5) and that agreed with (19) they suggested that there was degeneration of glomerular structure, glomerular crowding a were showed due to long time treatment of methotrexate.

In present study the result showed there was cystic dilatation of renal convoluted tubules with sever

hemorrhage and congestion in methotrexate group (2.9 ± 0.233) (figure 6) while in second group (methotrexate and captopril group) the severity is mild (1.8 ± 0.249) or control group (0.000 ± 0.000) (table 1) and that agreed with (20) they suggest when methotrexate level increase in the body that would cause sever renal toxicity and result in accumulation of methotrexate crystals in the nephron which resulting in dilatation of renal tubules.

The mechanism of methotrexate nephrotoxicity is not fully understood and damage to glomeruli and tubules because of methotrexate induced free radicals production causing oxidative stress on the kidney tissue and administration of anti oxidative as adjuvant therapy may be promising in alleviating the renal side effect of methotrexate (21) and administration of captopril protect methotrexate nephrotoxicity, reducing these toxicities occur due to activity of captopril which ameliorate or protect the kidney from toxic effect of methotrexate because of captopril has anti oxidative properties and free radicals scavenging this agreed with (13) they reported that captopril are possess anti oxidative could have a protective effect against cisplatin-induced nephrotoxicity. More over, our result are consistent with (22) they revealed the promising protective effect of captopril against doxorubicin-induced nephrotoxicity.

However , microscopical examination from kidney tissue that treated with captopril alone appeared similar to control group

and there were no significant differentiated ($P>0.01$) between the tow groups.

Table (1):Mean of pathological lesion of kidneys

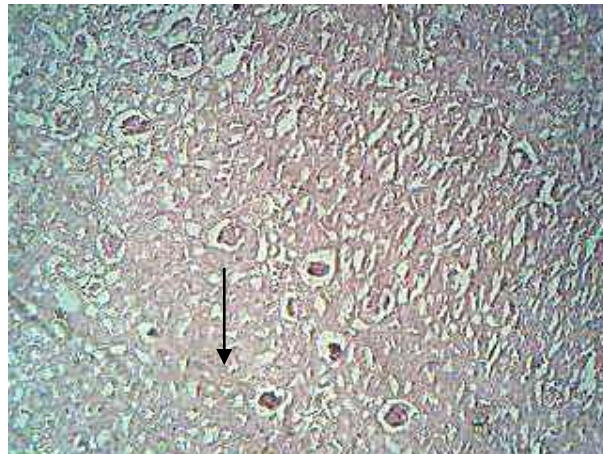
Groups Lesion	G1(methotrexate group)	G2(methotrexate and captopril group)	G3(captopril group)	Control group
Degeneration in renal tubules	2.8±0.133c	1.9±0.233b	0.0±0.000a	0.0±0.000a
Tubular necrosis	2.6±0.163c	1.8±0.200b	0.0±0.000a	0.0±0.000a
Glomerular atrophy	2.8±0.200c	1.7±0.260b	0.00±0.000a	0.0±0.000a
Dilatation of tubules and congestion	2.9±0.233c	1.8±0.249b	0.0±0.000a	0.0±0.000a

* Different small figure represent significant differentiated($P<0.01$) between groups horizontally .

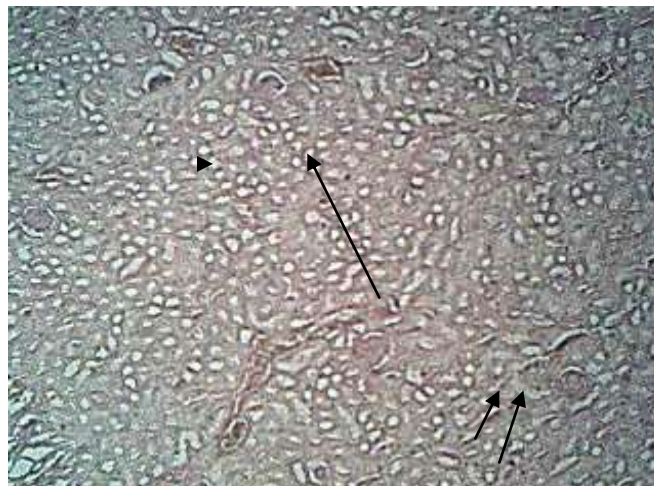
** Similar small figure represent insignificant differentiated($P<0.01$) between groups horizontally .



Figure(1):Histopathological section of kidney treated with Methotrexate show sever degeneration in renal tissue(arrow).(X 100H&E)



Figure(2): Histopathological section of kidney treated with Methotrexate show tubular necrosis of renal tubules(X 50 H&E)



Figure(3): Histopathological section of kidney treated with methotrexate and captopril show mild tubular necrosis (arrow) and The glomeruli are normal(double arrow) and there is no congestion or hemorrhage.X50 H&E.



Figure(4): Histopathological section of kidney treated with Methotrexate show there are atrophic changes of glomeruli(arrow).x50(H&E)



Figure(5): Histopathological section of kidney treated with methotrexate and captopril show mild atrophy of glomeruli (arrow) Also there is less dilatation of renal tubules (double arrow) . (X100H&E).



Figure(6): Histopathological section of kidney treated with Methotrexate show there are cystic dilatation of renal tubules(double arrow) also There are congestion in kidney tissue (arrow) .(X100 H&E).

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