Full Length Research Paper

Changes in the pharmacokinetic parameters of methotrexate by co-administration of indomethacin in rabbits

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Abstract

Methotrexate (MTX), a disease modifying antirheumatic drug (DMARD), and indomethacin, a non-steroidal anti-inflammatory drug (NSAID), are usually prescribed concomitantly for treatment of rheumatoid arthritis. The study aimed at investigating the effect of co-administration of indomethacin on the pharmacokinetics of MTX. Six female New Zealand rabbits (Group I) received MTX (10 mg/kg IV, once weekly). Six more animals (Group II) were treated concomitantly with MTX (10 mg/kg IV, once weekly) and indomethacin (4 mg/kg IV, once daily). Plasma levels of MTX were determined at 24, 36, 48, 60, 72, 96, and 120 hr after dosing during first, second, and third weeks. Concomitant administration of indomethacin significantly altered the studied pharmacokinetic parameters of MTX. The mean half life of MTX of about 13 hr in Group I gradually increased in Group II to 34.3 hr by the end of treatment. This was accompanied by 1.5-fold increase in the area under plasma concentration time curve (AUC). The total body clearance of MTX significantly decreased by 63.6%, while its mean residence time increased by about 1.43 fold during third week. In conclusion, the concurrent use of indomethacin with MTX caused significant changes in MTX pharmacokinetics and therefore care must be given in such situations.

Keywords: Methotrexate, indomethacin, drug-drug interactions.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints, with no known cure (Graves et al., 2009). Drugs able to reduce inflammation and cells activation may be used in the management of RA. In particular, nonsteroidal anti-inflammatory drugs (NSAIDs) as well as immunosuppressive agents (i.e., glucocorticoids), disease-modifying antirheumatic drugs (DMARDs), and agents that are able to block the proinflammatory cytokine tumor necrosis factor-α (anti-TNF-α) may be used (Patanè et al., 2013).

DMARDs have been found both to produce durable symptomatic remissions and to delay progression of the disease state (Nordström et al., 2006). All DMARDs possess a slow onset of action, with response to treatment usually expected within 4-6 months. The choice of a DMARD depends upon the balance between adverse effects and efficacy. Methotrexate (MTX) is among the most effective DMARDs because of its efficacy and acceptable safety profile and represents the first choice for the treatment of RA (Tian and Cronstein, 2007). Excellent correlation has been reported between MTX clearance and endogenous creatinine clearance (Nishimoto et al., 2004). As MTX is eliminated in the urine, mainly by glomerular filtration and proximal tubular transport, in a metabolically unchanged form, inhibition of renal tubular secretion has been considered as a site of

drug-drug interactions (Nozaki et al., 2007).

Methotrexate and NSAIDs are often used concomitantly in clinical practice such as RA and cancer. Such combination has been reported to increase MTX-related adverse effects (Daly et al., 1986: Singh et al., 1986). The mechanisms responsible for NSAIDs-induced increase in MTX concentrations include decreasing the glomerular filtration of MTX via reduction of renal blood flow with inhibition of prostaglandin synthesis and inhibition of MTX tubular secretion by inhibition of renal transporters (El-Sheikh et al., 2007; Brouwers and de Smet, 1994). Organic anion transporters (OAT1, OAT3, OAT4, OATK1) and reduced folate carrier 1 (RFC-1) are competitive sites between NSAIDs and MTX (Nozaki et al., 2004; Takeda et al., 2002). Moreover, NSAIDs have been recognized to modulate multidrug resistance proteins (MRP) and to interact with MTX through MRP2 and MRP4 (El-Sheikh et al., 2007; Lo¨scher and Potschka, 2005). Based on these findings, this study aimed at investigating whether co-administration of indomethacin would cause significant changes in the pharmacokinetics of MTX. Evaluation of such possible changes may lead to modification in dosing regimens of MTX/indomethacin combination.

MATERIALS AND METHODS

Animals and experimental groups

White female New Zealand rabbits weighing between 1.8 and 2.0 kg were used in the present study. Animals were kept in standard cages with free access to food and water. All procedures were performed in accordance with regulations of the National Research Council's guide for the care and use of laboratory animals. Six rabbits were assigned to group I and were given weekly IV 10 mg/kg doses of MTX (Methotrexate-Ebewe Injection®, Ebewe Pharma, Austria) for three consecutive weeks. Besides the same weekly doses of MTX, the six rabbits assigned to group II were given daily IV doses of 4 mg/kg indomethacin (Liometacen®, Nile Pharmaceutical Company, Cairo, Egypt) for the 3 week duration of the experiment.

Preparation of blood samples

From each rabbit, 2 ml blood samples were collected from the marginal ear veins in heparinized tubes at 24, 36, 48, 60, 72, 96, and, 120 hrs, after MTX dosing, during the first, second, and third week of administration. Samples were centrifuged at 4000 rpm for 15 min. Plasma was separated and stored at -20°C until analyzed for MTX by high-performance liquid chromatography (HPLC).

HPLC assay of methotrexate in plasma samples

Methotrexate concentrations in the plasma were determined by HPLC according to the reported methods (Hobl et al., 2012; Felson et al., 1990). A plasma sample of 500 µL was added to 500 µL acetonitrile, vortexed for 2 min then centrifuged at 4000 rpm for 15 min and the supernatant was separated and evaporated to dryness overnight. The dried samples were reconstituted with 100 µL of methanol. The reconstituted samples were analyzed for MTX by HPLC using a Waters 600 controller (Waters, USA) equipped with an ultra violet detector (Waters 486) set at 305 nm. The mobile phase consisted of water-acetonitrile-methanol (50:35:15), with pH adjusted to 3.75 using ortho-phosphoric acid. Separation was achieved using a reversed-phase column, Hypersil® ODS 3 µm 4.6 X 150 mm C18 at room temperature with a flow rate of 2 ml/min. With these adjusted settings, the retention for MTX was 6 min.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was utilized by standard methods. The maximum plasma concentration (C_{max}) and the time of its occurrence (t_{max}) were obtained from the concentration-time data. The area under the plasma concentration vs. time curve from 0 to last sampling time ($AUC_{0 \rightarrow t}$) using the linear trapezoidal rule was determined and then extrapolated to infinity ($AUC_{t \rightarrow \infty}$). The elimination rate constant (k_e) was estimated from the slope of the terminal phase of the MTX plasma concentration vs. time curve. The other pharmacokinetic parameters such as half life ($t_{1/2}$), and TBC/F where TBC is the total body clearance, and F is the bioavailability were also calculated using the following set of equations:

$$t_{1/2} = 0.693/K_e \qquad \text{Equation (1)}$$

$$AUC_{0 \rightarrow \infty} = \sum (C_n + C_{n+1})/2 \quad X \quad (t_{n+1} - t_n) + C^*/k_e$$
Equation (2)

Where C^*/k_e is the area of the tail end of the curve, from the last measured concentration, C^*, to infinity. The total area under the curve is the sum of all trapezoids plus the area of the tail.

$$TBC = D \ F/AUC, \ TBC/F = D/AUC \qquad \text{Equation (3)}$$
Where D is the dose administered

MRT = Mean Residence Time

The MRT defined as the average time for the residence of all the drug molecules in the body was calculated using equation (4)

$$MRT = \frac{AUMC}{AUC} \qquad \text{Equation (4)}$$

Where AUMC is the area under the first moment-time curve, and AUC is the area under the zero moment-time curve, or the area under the plasma concentration-time curve. The MRT has units of time.

Calculation of AUMC

The AUMC can be calculated utilizing the trapezoidal rule after the dividing the curve to trapezoids.

$$area\ of\ a\ trapezoid = \left[\frac{t_n c_n + t_{n+1} c_{n+1}}{2}\right] \cdot [t_{n+1} - t_n]$$

Equation (5)

$$AUMC_{t-\infty} = \left[\frac{t_{last} + c_{Plast}}{z}\right] + \left[\frac{c_{Plast}}{z^2}\right]$$

Equation (6)

$AUMC_{0-\infty} = AUMC_{0-t} + AUMC_{t-\infty}$ Equation (7)

Statistical analysis

All data are presented as mean ± SD. Unpaired t-test was used for statistical analysis of the weekly changes. Differences were considered significant at $P < 0.05$.

RESULTS

The weekly changes in the mean levels of MTX in rabbit plasma are presented in Table 1. Repeated administration of MTX resulted in small increases in its plasma concentration, especially in the late time points. However, because of the relatively long dosing interval of one week, there was practically no effect on the concentrations at the early time points of the following injections. The effect of co-administration of indomethacin on the plasma concentration of MTX is clearly apparent, even from week 1. Higher plasma concentrations of MTX are found, as compared to those of the group which did not receive indomethacin (Table 1). Such changes affected the calculated pharmacokinetic parameters of MTX as the elimination rate constant (k_e) significantly increased in rabbits treated with both drugs as compared to MTX alone (Figure 1). The $t_{1/2}$ of MTX following weekly IV doses was calculated to be about 13 hours when administered alone and did not change by repeated administration over the study period. However, co-administration of indomethacin resulted in significant gradual increases as the treatment progressed and the mean $t_{1/2}$ of MTX went up from an average of nearly 21 hours in the first week reaching more than 34 hours by the end of the experimental period (Figure 2). These changes in half life were coupled with significant decreases in total body clearance (TBC) as a result of co-administration of indomethacin (Figure 3). The mean residence time (MRT) was also affected following adjunct treatment with daily indomethacin. The slight increase observed with repeated administration of MTX was exaggerated following treatment with indomethacin (Figure 4). All changes in pharmacokinetic parameters were indicative of accumulation of MTX and were reflected in increases in the calculated areas under the concentration/time curves (Figure 5).

DISCUSSION

Drug-drug interactions involving metabolism and/or excretion processes prolong the plasma elimination half-lives leading to the accumulation of drugs in the body and potentiate pharmacological/adverse effects. Recent studies have shown that many types of transporters play important roles in the tissue uptake and/or subsequent secretion of drugs in the liver and kidney, and such transporters exhibit a broad substrate specificity with a degree of overlap, suggesting the possibility of transporter mediated drug-drug interactions with other substrates (Li et al., 2006; Shitara et al., 2005).

Methotrexate is an analog of natural folate that has been widely and successfully used in the treatment of solid tumors and leukemias as well as autoimmune diseases including rheumatoid arthritis (Majumdar and Aggarwal, 2001). When administered weekly in a low dose, MTX has been proven to significantly suppress rheumatoid arthritis progression (Refaat et al., 2013). Renal impairment and age are generally considered risk factors for developing methotrexate toxicity, but studies show conflicting results (Tahiri, et al., 2006; Bologna et al., 1997).

Methotrexate and indomethacin are usually co-prescribed for treatment of patients with rheumatoid arthritis. Such combination has been found to produce durable symptomatic remissions and to delay progression of the disease state (Nordström et al., 2006).

Renal clearance of unchanged drug is the principal mechanism of MTX elimination, through renal glomerular filtration as well as active secretion and reabsorption across the renal tubules. Active secretion of MTX utilizes the multispecific ATP-binding cassette (ABC) transporters common for a wide variety of endogenous substances, xenobiotics, and their metabolites which can alter the pharmacokinetics of MTX (Guo et al., 2007; Melter et al., 2002). Indomethacin may be a good candidate to produce such effect. In this case, it would be of importance to study the changes in the pharmacokinetics of MTX in order to guard against possibility of its accumulation.

Our results revealed that injection of rabbits concomitantly with MTX and indomethacin caused a significant increase in MTX plasma concentrations at the different sampling time points compared with the rabbits injected with MTX alone. Interference of indomethacin in the process of urinary excretion of MTX can explain the observed pharmacokinetic changes. Competitive inhibition of tubular secretion of MTX by indomethacin may lead to increase in its plasma levels and prolongation of its biological half-life which were observed in the present study. These results are in line with a

Table 1. Plasma levels of methotrexate (MTX) when given alone in IV weekly doses of 10 mg/kg (Group I) and when given in combination with IV daily indomethacin (IND) doses of 4 mg/kg (Group II)

| Time (hr)	MTX plasma levels (µmol/l)					
	Group I (MTX)			Group II (MTX+IND)		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
24	101.0 ± 2.6	101.1 ± 2.2	102.0 ± 4.3	101.3 ± 7.4	109.0 ± 7.6[a]	115.5 ± 10.9[a]
36	81.1 ± 2.3	83.8 ± 4.0	85.0 ± 3.9	86.5 ± 6.5	90.0 ± 5.5[a]	93.5 ± 6.9[a]
48	56.3 ± 2.7	63.2 ± 3.2	69.5 ± 3.7	72.0 ± 12.2[a]	77.5 ± 11.0[a]	80.5 ± 9.2[a]
60	25.6 ± 1.8	37.1 ± 2.6	39.0 ± 1.8	41.5 ± 6.2[a]	52.8 ± 6.7[a]	59.5 ± 7.1[a]
72	11.9 ± 1.2	19.1 ± 2.0	22.3 ± 1.5	25.0 ± 3.9[a]	36.5 ± 4.9[a]	43.5 ± 5.6[a]
96	4.6 ± 0.5	10.8 ± 2.7	12.5 ± 1.3	14.5 ± 2.8[a]	22.0 ± 3.6[a]	33.5 ± 5.7[a]
120	1.3 ± 0.2	3.2 ± 0.5	3.4 ± 0.4	6.7 ± 0.4[a]	11.8 ± 1.7[a]	20.5 ± 1.9[a]

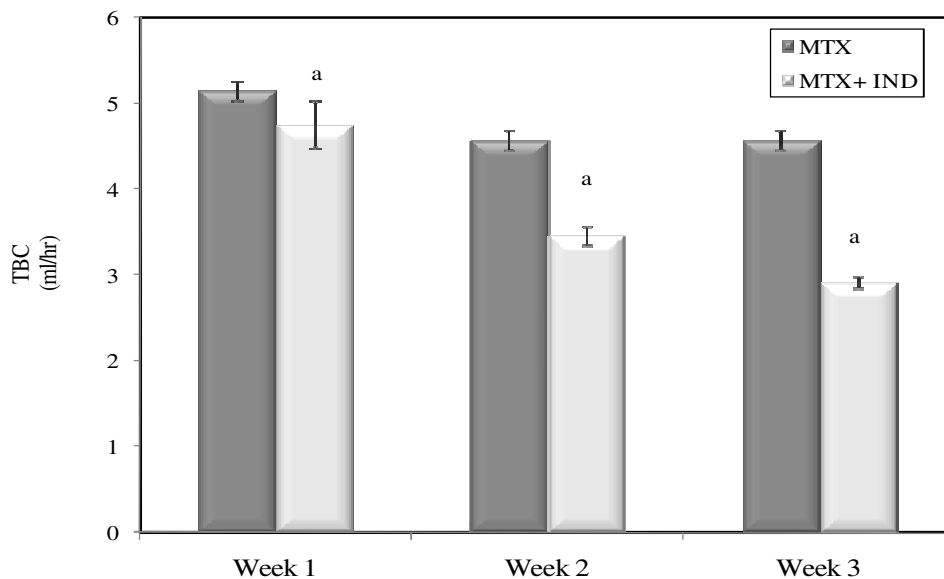Data presented as mean ± SD of 6 observations, [a]$P < 0.05$ vs. group I
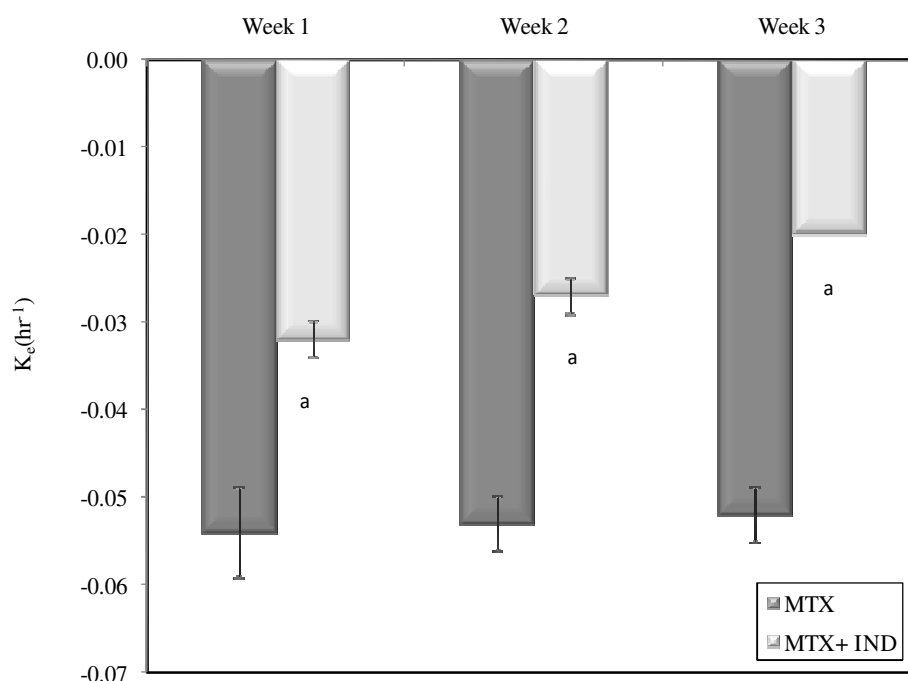
Figure 1. The elimination rate constant (k_e) of methotrexate (MTX) during first, second and third week of administration of MTX alone in IV weekly doses of 10 mg/kg and in combination with IV daily indomethacin (IND) doses of 4 mg/kg. Data presented as mean ± SD of 6 observations, [a]$P < 0.05$ vs. MTX alone.

recent study by Elmorsi et al. (2013), investigating the effect of the same combination of drugs in mice plasma and tumor tissue. They suggested that the decrease in MTX clearance if administered after NSAIDs may be largely due to the decrease of MTX transport outside cells by efflux transporters human multidrug resistance protein (MRP) resulting from NSAIDs use. Moreover, a previous study by Maeda et al. (2008), evaluating the effect of NSAIDs on the pharmacokinetics of MTX demonstrated that MTX concentrations in rat serum were significantly increased in a NSAID concentration-dependent manner. They concluded that the major mechanism of such interaction involves inhibition of organic anion transporter 3 (OAT3) via which MTX and most NSAIDs are excreted. However, inhibition of cyclooxygenase-1 with consequent reduction in
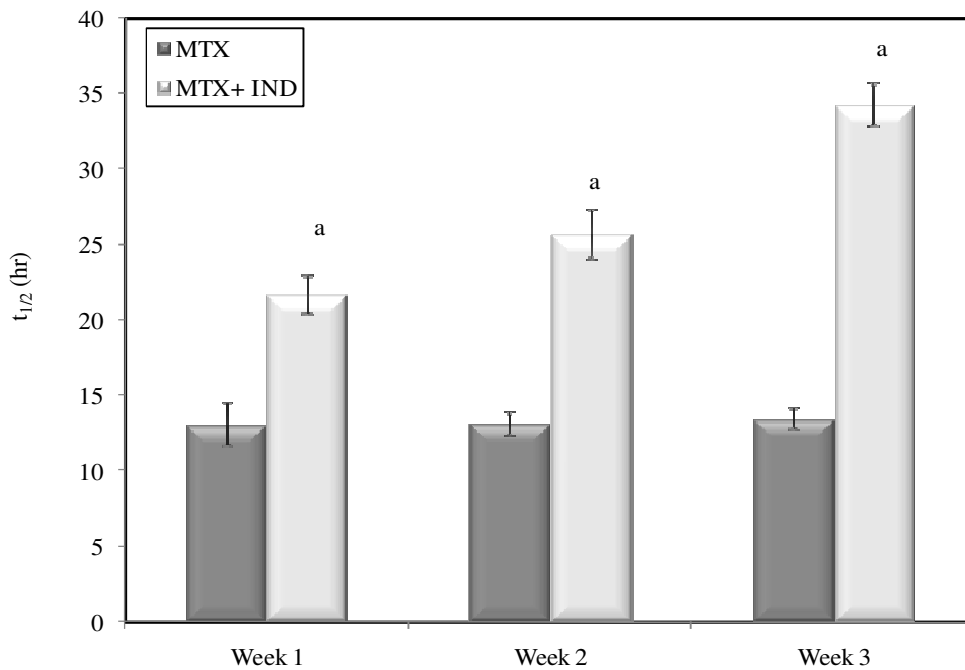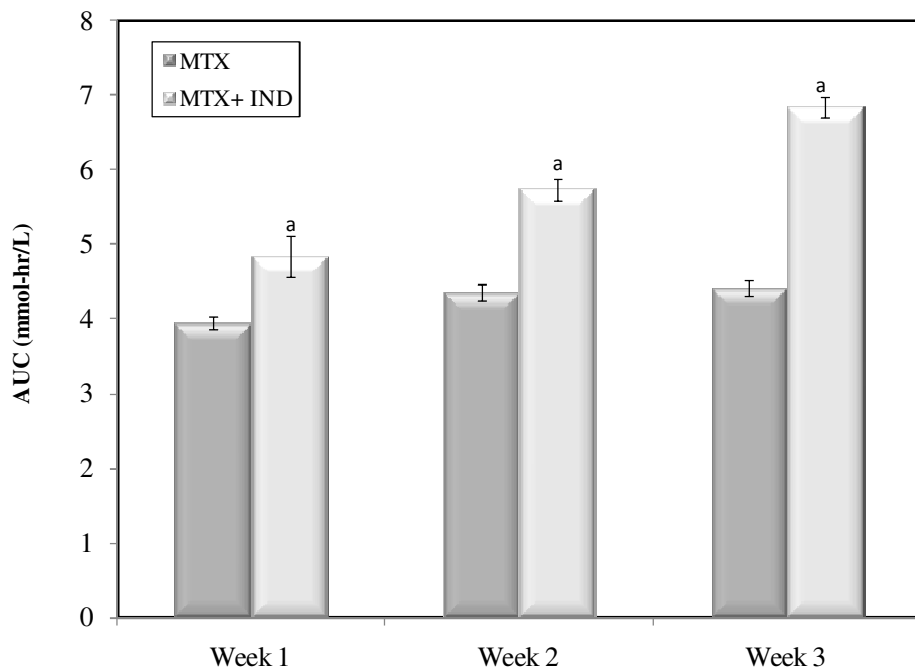
Figure 2. The half life ($t_{1/2}$) of methotrexate (MTX) during first, second and third week of administration of MTX alone in IV weekly doses of 10 mg/kg and in combination with IV daily indomethacin (IND) doses of 4 mg/kg. Data presented as mean ± SD of 6 observations, [a]$P < 0.05$ vs. MTX alone.

Figure 3. The total body clearance (TBC) of methotrexate (MTX) during first, second and third week of administration of MTX alone in IV weekly doses of 10 mg/kg and in combination with IV daily indomethacin (IND) doses of 4 mg/kg. Data presented as mean ± SD of 6 observations, [a]$P < 0.05$ vs. MTX alone.
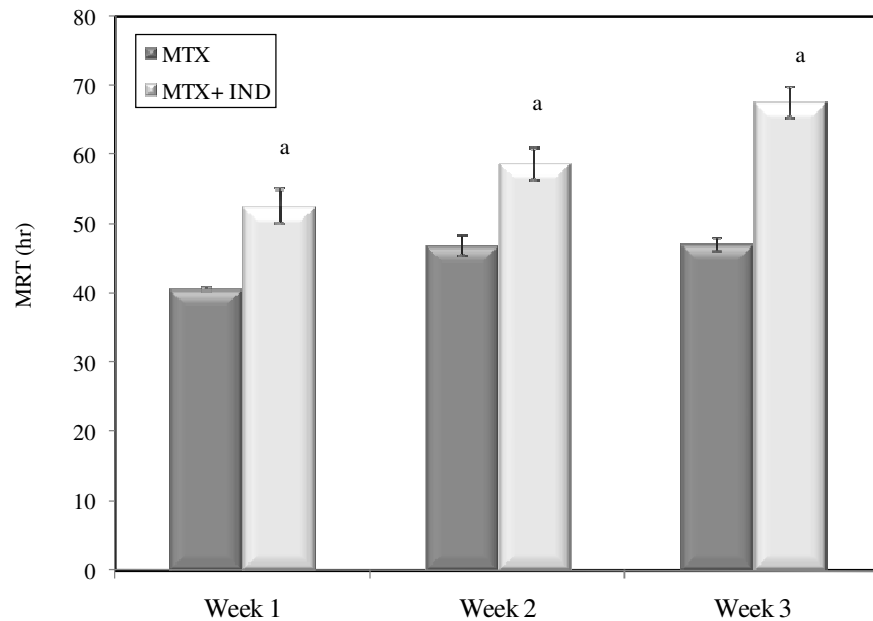
Figure 4. The mean residence time (MRT) of methotrexate (MTX) during first, second and third week of administration of MTX alone in IV weekly doses of 10 mg/kg and in combination with IV daily indomethacin (IND) doses of 4 mg/kg. Data presented as mean ± SD of 6 observations, [a]$P < 0.05$ vs. MTX alone.

Figure 5. The area under the plasma concentration time curve (AUC) of methotrexate (MTX) during first, second and third week of administration of MTX alone in IV weekly doses of 10 mg/kg and in combination with IV daily indomethacin (IND) doses of 4 mg/kg. Data presented as mean ± SD of 6 observations, [a]$P < 0.05$ vs. MTX alone.

prostaglandin E$_2$, which plays an important role in renal hemodynamics may be an added factor (Llinás et al., 2001). The assumption of reduced clearance is supported by the findings of Tracy et al. (1992) who demonstrated a significant reduction in the clearance of MTX in patients receiving concurrent NSAIDs. An expected result of higher plasma levels of MTX would be prolonged half-life and increased AUC values, which were again seen in our study. By inspecting the results of the two treatment regimens, in our study, it could be seen that co-administration of indomethacin increased the AUC after one week by 22.4%. Such difference widened to 54.8% by the end of the third week of treatment. It should also be noted that there was a notable stepwise weekly increase in AUC in the group receiving the combined treatment, where the difference between first and third week AUC values was 41.4%. Similar variations in AUC were reported by Dupuis et al. (2009). The underlying cause of these effects appears to by reduced clearance of MTX. However, a previous short-term study evaluating the effect of indomethacin on continuous MTX infusion, in rabbits, over a period of 240 min reported insignificant change in plasma MTX concentration and excluded the possibility of its delayed elimination as a cause of the observed toxicity caused by such combination (Najjar et al., 1992).

In conclusion, results of the present study clearly indicate that the plasma concentration of MTX should be monitored and the dosages of both MTX and indomethacin should be modified in order to minimize or prevent possible toxicity or side effects.

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