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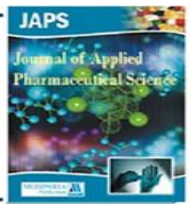

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Effect of Ketoprofen and Indomethacin on Methotrexate Pharmacokinetics in Mice Plasma and Tumor Tissues

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ABSTRACT

Methotrexate (MTX) has been used in combination with nonsteroidal anti-inflammatory drugs (NSAIDs) in the treatment of inflammatory diseases and malignancies. Severe adverse effects with this combination may occur, usually resulting from inhibition of renal transporters. Solid Ehrlich Carcinoma was induced by implantation of Ehrlich Ascites Carcinoma (EAC) cells subcutaneously into the thigh of mice and after 30 days, mice were divided into 3 groups , Group I served as control group received MTX (50 mg/kg, i.p.), Group II received Ketoprofen (100 mg/kg, i.p.) then after half an hour received MTX (50 mg/kg, i.p.), Group III received Indomethacin (10 mg/kg, i.p.) then after half an hour received MTX (50 mg/kg, i.p.). Plasma and tissue samples were collected at different times then MTX concentrations were determined by HPLC. The injection of Ketoprofen or Indomethacin before MTX injection caused significant increase in the AUC and C_{Pmax} of MTX ($p < 0.05$) and significant decrease in CL/F and Vd/F of MTX ($p < 0.05$) in mice plasma. The study showed that administration of ketoprofen or indomethacin prior to MTX caused significant decrease in MTX elimination and significant increase in MTX extent of absorption which may lead to severe adverse effects if coadministered in human.

Keywords: Methotrexate, Solid Ehrlich Carcinoma, Ketoprofen, Indomethacin, NSAIDS, Renal transporters.

INTRODUCTION

Breast cancer is malignant breast neoplasm which originates from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk . Cancers originating from ducts are known as ductal carcinomas; those originating from lobules are known as lobular carcinomas (Sariego, 2010). Despite significant advances in surgery, radiation therapy, and anticancer treatment in the past 30 years, chemotherapy resistance remains a major obstacle to improving a cancer patient's outcome. Because there are presently no proven predictors of a patient's response to chemotherapy, all cancer patients selected for chemotherapy receive the same treatment (Lee and Macgregor, 2004).

Based on currently available preclinical and clinical data, it can be expected that modulation of MRP members may represent a useful approach in the management of anticancer and antimicrobial drug resistance and possibly of inflammatory diseases and other diseases. Many agents of diverse structure and function that modulate MRP have been identified, including calcium channel blockers (e.g., verapamil), immunosuppressive drugs (e.g., cyclosporine A), antibiotics (e.g., erythromycin), probencid ,and NSAIDs (Löscher and Potschka , 2005).

Inhibition of ABC-transporters, by using certain inhibitors , was shown to increase the intracellular retention of several drugs like the anti-HIV drugs, and anticancers (Zhou *et al.*, 2008).

Methotrexate is the prototype folate antagonist cytotoxic drug, employed in the therapy of solid tumors and leukaemias, and recently also as an immunosuppressive agent in organ transplantation, in the treatment of some non-malignant diseases such as psoriasis, ankylosing spondylitis and rheumatoid arthritis (Majumdar & Aggarwal, 2001), and in the therapy of severe asthma (Rubino, 2001). Methotrexate and NSAIDs are often used concomitantly in clinical practice such as rheumatoid arthritis and cancer, the combination is reported to increase methotrexate-related adverse effects. The mechanisms responsible for NSAIDs-induced increase in methotrexate concentrations includes decrease in glomerular filtration of methotrexate by NSAIDs via reduction of renal blood flow with inhibition of prostaglandin synthesis (Brouwers and de Smet, 1994), inhibition of methotrexate tubular secretion (El-Sheikh *et al.*, 2007), and competition for protein-binding sites (Brouwers and de Smet, 1994). In general, main interaction mechanism has been known to be the inhibition of prostaglandin synthesis (Brouwers and de Smet, 1994). However, recently, in vitro studies have revealed many renal transporters for methotrexate and NSAIDs (Van Aubel *et al.*, 2000; Takeuchi *et al.*, 2001; Shibayama *et al.*, 2006). Organic anion transporters (OAT1, OAT3, OAT4, OAT-K1) (Masada *et al.*, 1997; Takeuchi *et al.*, 2000; Takeda *et al.*, 2002; Uwai *et al.*, 2004), multidrug-resistance proteins (MRP2, MRP4) (EI-Sheikh *et al.*, 2007 and Nozaki *et al.*, 2007b), and reduced folate carrier 1 (RFC-1) (Nozaki *et al.*, 2004) are competitive sites between methotrexate and NSAIDs. Considering that the methotrexate main elimination route is the tubular secretion (Rubin *et al.*, 1967 and Nierenberg, 1983), it is speculated that the competition for renal transporters is important mechanism of the interaction in humans.

This study was carried out to examine the effect of two known MRP inhibitors (ketoprofen & indomethacin) on the distribution of methotrexate in experimentally induced mice tumor and also the effect of co-administration of these inhibitors on the overall pharmacokinetics of the drug .

MATERIALS AND METHODS

This study was carried out on adult female Swiss albino mice with an average body weight of 18 - 20 grams. Ehrlich Ascites Carcinoma (EAC) tumor cells obtained from the Pharmacology and Experimental Oncology Unit of the National Cancer Institute (NCI), Cairo University, Egypt were used where 2.5×10^6 EAC cells were implanted subcutaneously (S.C) into the right thigh of the lower limb of mice. A palpable solid tumor mass developed within 12 days.

On the 30^{th} day postinoculation, mice were randomly divided into the following three groups , each included 60 mice . mice in group I were given MTX (50 mg/ kg i.p.), mice in group II received Ketoprofen (100 mg/kg, i.p.) then after half an hour received MTX (50 mg/kg, i.p.), and mice in group III received Indomethacin (10 mg/kg, i.p.) then after half an hour received MTX (50 mg/kg, i.p.).

Blood and tumor tissue samples were collected at the following time intervals; 0.25, 0.5, 1, 2, 3, 4, 6, and 24 hours after MTX injection. Plasma was carefully separated and the excised tumors were sliced into cubes representing various areas of the tumor and all samples were kept frozen till analysis.

HPLC assay of MTX in mice plasma and tumor tissues

A high performance liquid chromatographic (HPLC) method described by Najjar, (1996) was used to measure MTX concentrations in plasma and tumor tissue samples. The system used consisted of Waters instrument equipped with a 600 E model pump 600 controller u6k injector with 20 µl loop, 486 uv/vis detector and photodiode array detector and the Millinium™ program (version 2.1) for data analysis. Initially, plasma samples were deproteinized with trichloroacetic acid. An aliquote of 40µl of the supernatant was the injected onto a reversed-phase 15 cm× 3.9 mm (i.d) C-18, 4µm Nova-pak™ column. The mobile phase was composed of phosphate buffer, methanol and Acetonitile (84:11:5), which was pumped at a flow-rate of 2.3 ml/min. The effluent was monitored at 313 nm.

Tumor samples were carefully weighed (500mg) , cut into small pieces , and 5 ml of 0.9% NaCl was added (1:5, w/v), then tissues were homogenized with a Polytron homogenizer. This produces 10% homogenate which was diluted in 1/15 phosphate buffe (pH 7.4 ± 0.1) and before the samples were injected into the chromatograph, protein denaturing and precipitation procedures were carried out. By adding 200 µl of 50:50 (v/v) mixture of methanol and 40% $ZnSO_4$ to 150 µl of sample. After one minute of vigorous vortex mixing the fluid was centrifuged at 1500 g for 10 min. The supernatent obtained was injected directly onto the HPLC system with a 200 µl fixed volume.

Pharmacokinetic Analysis

A two compartment model was utilized to describe the plasma concentration time profile of methotrexate. The values of maximum plasma concentration, CP_{max}; time of maximum concentration T_{max}; area under the plasma concentration time curve, AUC ; elimination rate constant , K_{10}; elimination half life , $t½\beta$; Vd/F and CL/F) were determined for each group of mice using the softwareWinNonlin standard (Version 2.0.0.0, Pharsight corporation, California, USA).

Statistical Analysis

Comparisons between groups were carried out by one way analysis of variance (ANOVA) followed by LSD post hoc test. The level of significance was set at P= 0.05.

RESULTS

Assay Validation

The calibration curves were linear over the concentration range of 2-100 µg/ml. The equation that describes the linear relationship between peak area ratio and the concentration in the standard curves of MTX was:

Peak area = 8781.262 (± 359.5394) concentration – 3376.816 (± 11364.39)

The correlation coefficient was always greater than 0.99 during the course of the validation.

The coefficient of variation for the within-day precision ranged from 2.08 % to 19.81 % and the coefficient of variation for the between-day precision ranged from 0.47 % to 12.7 %. The measured plasma MTX concentration relative to the nominal MTX concentration, which is a measure of the accuracy of the assay, ranged from 99.86 % to 119.83 % for the within-day accuracy and from 98.31% to 116.5% for the between-day accuracy. These results indicate good precision and accuracy of the method.

Methotrexate Pharmacokinetic Parameters in Plasma

The mean plasma concentration time curves of MTX (50 mg/kg i.p.), are shown in Figure (1). The mean plasma MTX concentrations observed after injection of MTX (50 mg/kg, i.p) to eight different mice in the three treatment groups are shown in Table (1). The individual MTX pharmacokinetic parameters estimated after injection of MTX (50 mg/kg i.p.) in the three treatment groups are presented in Tables (2), (3), and (4) respectively. The mean pharmacokinetic parameters for MTX after injection of MTX (50 mg/kg i.p.) in the three treatment groups are presented in Table (5).

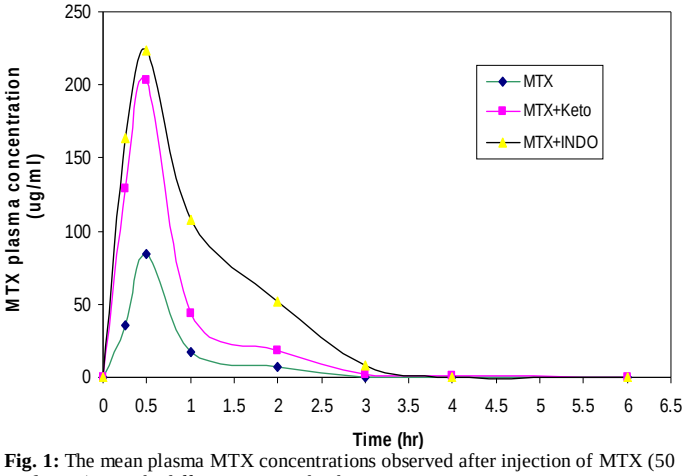

Fig. 1: The mean plasma MTX concentrations observed after injection of MTX (50 mg/kg, i.p) to eight different mice in the three treatment groups.

Table. 1: The mean plasma MTX concentrations observed after injection of MTX (50 mg/kg, i.p) to eight different mice in the three treatment groups.

Time (hr)	Mean Methotrexate Concentration (µg/ml)		
	Group I (MTX alone) (Control group) (n=8)	Group II (MTX+Ketoprofen) (n=8)	Group III (MTX+Indomethacin) (n=8)
0	0	0	0
0.25	31.54 ± 1.23	121.05 ± 2.29	155.71 ± 5.42
0.5	79.37 ± 1.59	208.11 ± 1.96	222.88 ± 17.40
1	16.01 ± 0.53	31.67 ± 3.00	107.95 ± 9.28
2	7.18 ± 0.09	14.26 ± 0.29	51.41 ± 10.36
3	0.21 ± 0.01	1.58 ± 0.04	8.01 ± 2.21
4	0.15 ± 0.01	0.84 ± 0.04	0.28 ± 0.09
6	0.09 ± 0.01	0.36 ± 0.02	0.11 ± 0.01

Table . 2: The individual MTX pharmacokinetic parameters observed after injection of mice with MTX (50 mg/kg i.p.).

Mouse No.	K10 (hr^{-1})	CL/F (ml/hr)	Vd/F (ml)	AUC (µg.hr/ml)	T_{max} (hr)	Cp_{max} (µg/ml)	½β (hr)
1	2.26	5.3	2.35	188.7	0.41	165.19	3.04
2	2.34	5.37	2.29	186.22	0.4	168.01	2.63
3	2.46	5.55	2.25	180.2	0.39	166.15	0.95
4	2.28	5.76	2.53	173.61	0.38	163.86	2.91
5	2.42	5.55	2.29	180.25	0.39	167.46	2.01
6	2.47	5.72	2.32	174.69	0.39	165.21	1.10
7	2.41	6.18	2.57	161.77	0.38	154.77	0.32
8	2.43	5.72	2.35	174.67	0.41	157.69	0.57
Mean	2.38	5.64	2.37	177.51	0.39	163.55	1.69
± S.D.	0.08	0.27	0.12	8.41	0.01	4.77	1.09

Table. 3: The individual MTX pharmacokinetic parameters observed after injection of mice with MTX (50 mg/kg i.p) half an hour after injection of Ketoprofen (100 mg/kg i.p).

Mouse No.	K10 (hr^{-1})	CL/F (ml/hr)	Vd/F (ml)	AUC (µg.hr/ml)	T_{max} (hr)	Cp_{max} (µg/ml)	½β (hr)
1	2.26	5.3	2.35	188.7	0.41	165.19	3.04
2	2.34	5.37	2.29	186.22	0.4	168.01	2.63
3	2.46	5.55	2.25	180.2	0.39	166.15	0.95
4	2.28	5.76	2.53	173.61	0.38	163.86	2.91
5	2.42	5.55	2.29	180.25	0.39	167.46	2.01
6	2.47	5.72	2.32	174.69	0.39	165.21	1.10
7	2.41	6.18	2.57	161.77	0.38	154.77	0.32
8	2.43	5.72	2.35	174.67	0.41	157.69	0.57
Mean	2.38	5.64	2.37	177.51	0.39	163.55	1.69
± S.D.	0.08	0.27	0.12	8.41	0.01	4.77	1.09

Table. 4: The individual MTX pharmacokinetic parameters observed after injection of mice with MTX (50 mg/kg i.p) half an hour after injection of Indomethacin (10 mg/kg i.p) .

Mouse No.	K10 (hr^{-1})	CL/F (ml/hr)	Vd/F (ml)	AUC (µg.hr/ml)	T_{max} (hr)	Cp_{max} (µg/ml)	½β (hr)
1	1.68	3.69	2.2	270.72	0.47	204.76	2.84
2	2.07	4.09	1.98	244.08	0.43	205.35	0.34
3	1.42	3.6	2.54	277.39	0.42	203.39	5.62
4	1.84	3.81	2.07	262.16	0.47	204.5	0.38
5	1.79	3.92	2.19	254.88	0.41	193.14	2.06
6	1.45	3.78	2.61	264.22	0.44	193.55	5.18
7	1.03	3.56	3.43	280.72	0.43	185.59	0.44
8	1.05	3.67	3.48	272.3	0.45	179.3	0.54
Mean	1.54	3.77	2.57	265.81	0.44	196.2	2.18
± S.D.	0.37	0.18	0.59	12.13	0.02	9.95	2.19

Table. 5: The mean MTX pharmacokinetic parameters observed after injection of mice with MTX (50 mg/kg i.p) in the three treatment groups.

Treatment group	K10 (hr^{-1})	CL/F (ml/hr)	Vd/F (ml)	AUC (µg.hr/ml)	T_{max} (hr)	Cp_{max} (µg/ml)	½β (hr)
Group I (MTX alone) (Control gp.) (n=8)	2.25± 0.11	16.62± 0.41	7.4± 0.49	60.22± 1.47	0.41± 0.01	54.15± 1.56	1.49 ± 0.6
Group II (MTX + ketoprofen)(n=8)	2.38± 0.08	5.64± 0.27*	2.37± 0.12*	177.51± 8.41*	0.39± 0.01	163.55 ± 4.77*	1.69 ±1.09
Group III (MTX + Indomethacin)(n=8)	1.54± 0.37	3.77± 0.18*	2.57± 0.59*	265.81± 12.13*	0.44± 0.02	196.2± 9.95*	2.18 ±2.19

* Significantly different from group I ($p < 0.05$).

There were no significant differences between Tmax and β½ in group II which was injected with MTX (50 mg/kg i.p) half an hour after injection of ketoprofen (100 mg/kg i.p) and group I which was injected with MTX (50 mg/kg i.p) alone. The observed Tmax was 0.41 ± 0.01 hr versus 0.39 ± 0.01 hr, and β½ was 1.49 ± 0.6 hr versus 1.69 ± 1.09 hr, for group I and group II, respectively. However, there were significant differences between the AUC, CPmax, CL/F, and Vd/F in the two treatment groups. The observed AUC was 60.22 ± 1.47 µg.hr/ml versus 177.51 ± 8.41 µg.hr/ml, CPmax was 54.15 ± 1.56 µg/ml versus 163.55 ± 4.77 µg/ml, CL/F was 16.62 ± 0.41 ml/hr versus 5.64 ± 0.27 ml/hr, and Vd/F was 7.4 ± 0.49 ml versus 2.37 ± 0.12 ml for group I and group II, respectively. Therefore it can be concluded that ketorprofen decreased the elimination of MTX and increased its extent of absorption represented by the increase in CPmax and AUC .

By comparing group III which was injected with MTX (50 mg/kg i.p) half an hour after injection of indomethacin (10 mg/kg i.p) with group I which was injected with MTX (50 mg/kg i.p) alone, we can determine the effect of indomethacin on MTX pharmacokinetics. There were no significant differences between Tmax and β½ in the two treatment groups. The observed Tmax was 0.41 ± 0.01 hr versus 0.44 ± 0.02 hr, and β½ was 1.49 ± 0.6 hr versus 2.18 ± 2.19 hr, for group I and group III, respectively. However, there were significant differences between the AUC, CPmax, CL/F, and Vd/F in the two treatment groups. The observed AUC was 60.22 ± 1.47 µg.hr/ml versus 265.81 ± 12.13 µg.hr/ml, CPmax was 54.15 ± 1.56 µg/ml versus 196.2 ± 9.95 µg/ml, CL/F was 16.62 ± 0.41 ml/hr versus 3.77± 0.18 ml/hr, and Vd/F was 7.4 ± 0.49 ml versus 2.57 ± 0.59 ml for group I and group III, respectively. Therefore it can be concluded that indomethacin decreased the elimination of MTX and increased its extent of absorption represented by the increase in CPmax and AUC.

By comparing group III which was injected with MTX (50 mg/kg i.p) half an hour after injection of indomethacin (10 mg/kg i.p) with group II which was injected with MTX (50 mg/kg i.p) half an hour after injection of ketoprofen (100 mg/kg i.p), we can determine the comparative effects of indomethacin and ketoprofen on MTX pharmacokinetics. There were no significant differences between Tmax and β½ and Vd/F in the two treatment groups. The observed Tmax was 0.39 ± 0.01 hr versus 0.44 ± 0.02 hr, β½ was 1.69 ± 1.09 hr versus 2.18 ± 2.19 hr, and Vd/F was 2.37 ± 0.12 ml versus 2.57 ± 0.59 ml for group II and group III, respectively. However, there were significant differences between the AUC, CPmax, CL/F, in the two treatment groups. The observed AUC was 177.51 ± 8.41 µg.hr/ml versus 265.81 ± 12.13 µg.hr/ml, CPmax was 163.55 ± 4.77 µg/ml versus 196.2 ± 9.95 µg/ml, CL/F was 5.64 ± 0.27 ml/hr versus 3.77± 0.18 ml/hr, for group II and group III, respectively. Therefore it can be concluded that indomethacin has a more powerful inhibitory effects on MTX elimination than ketoprofen does represented by the decresed CL/F and increased the extent of MTX absorption more than ketoprofen represented by the increased CPmax and AUC .

Methotrexate Pharmacokinetic Parameters in Tumor Tissues

By the analysis of tumor tissue samples collected at the previously specified time intervals by HPLC, Methotrexate could not be detected by this method in any of the samples in the three treatment groups.

DISCUSSION

The comparison of the pharmacokinetic parameters estimated when ketoprofen was injected half an hour before MTX indicates that some pharmacokinetic parameters like AUC, CPmax, CL/F, and Vd/F were significantly changed. The AUC increased to about 2.9 folds while CPmax increased to about 3 folds when ketoprofen was injected before MTX. On the other hand, there was about 2.9 and 3 folds decrease in CL/F, and Vd/F respectively after injection of ketoprofen. There were no significant difference in Tmax and β½ between the two groups. These results indicates that ketorprofen decreased the elimination of MTX and increased its extent of absorption represented by the increase in CPmax and AUC . The comparison of the pharmacokinetic parameters estimated when Indomethacin was injected half an hour before MTX indicates that some pharmacokinetic parameters like AUC, CPmax, CL/F, and Vd/F were significantly changed. The AUC increased to about 2.9 folds while CPmax increased to about 3 folds when indomethacin was injected before MTX. On the other hand, there was about 2.9 and 3 folds decrease in CL/F, and Vd/F respectively after injection of indomethacin. There were no significant difference in Tmax and β½ between the two groups. These results indicates that indomethacin decreased the elimination of MTX and increased its extent of absorption represented by the increase in CPmax and AUC.

The results obtained in the current study are in agreement with previous experiments involving enhancement of MTX plasma concentrations after the use of different NSAIDs. Thyss *et al.* (1986) reported that simultaneous administration of ketoprofen and MTX was associated with prolonged and striking enhancement of serum MTX levels.

The increase in MTX plasma concentrations in the present study is attributed to the interaction between MTX and NSAIDs that involves inhibition of transporters in proximal renal tubules, and this is supported by several studies. Reid G al. (2003) reported that Indomethacin and ketoprofen inhibited both MRP4 and MRP1 transport but MRP4 proven to be more senitive than MRP1.

MTX is primarily excreted into urine in the unchanged form, and renal handling involves tubular secretion and reabsorption in addition to glomerular filtration. Renal tubular secretion of MTX has been thought to be a major site of interaction with other drugs (Takeda *et al.*, 2002). El-Sheikh *et al.*, (2006) studied the effect of various NSAIDs on MTX transport in membrane vesicles isolated from cells overexpressing the proximal tubular apical efflux transporters human multidrug resistance protein (MRP2 and MRP4) and found that a wide variety of NSAIDs like indomethacin and ketoprofen inhibited MRP2 and MRP4 mediated methotrexate transport at concentrations to which the transporters may be exposed under therapeutic conditions.

Maeda *et al.*, (2008) determined pharmacokinetic interaction between methotrexate and NSAIDs in rats and found that serum methotrexate concentrations were increased in proportion to NSAID concentration and suggested that this interaction involves inhibition of the transporters in proximal renal tubules. Nierenberg (1983) reported that methotrexate uptake in rabbit kidney slices was inhibited by various NSAIDs. Statkevich *et al.* (1993) demonstrated that tubular clearance of methotrexate was depressed by the concomitant administration of indomethacin or flurbiprofen in the isolated perfused rat kidney. Uwai *et al.* (2000) showed that uptake of methotrexate in the Xenopus laevis oocytes was inhibited by Indomethacin and Ketoprofen. Uwai *et al.*, (2011) represented that the NSAIDs significantly increased the area under the blood concentration-time curve of methotrexate and concluded that NSAIDs increase blood levels of methotrexate by influencing renal excretion of the antifolate. On the other hand, several studies suggested that NSAIDs do not significantly affect the disposition of methotrexate, on the contrary to some of the earlier reports. Iqbal *et al.*, (1998) by their studies on the pharmacokinetics of methotrexate in patients with rheumatoid arthritis concurrently taking the most commonly used non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, diclofenac, naproxen, indomethacin, and ibuprofen showed that the area under the curve, the total systemic clearance, the distribution volume, and the half-life of methotrexate in patients receiving concurrent NSAID therapy did not change significantly.

In the current study, no MTX was detected by the analysis of any of the tumor tissue samples taken at the specified time intervals in any of the three treatment groups by the described HPLC method. West *et al.*, (1980) suggested that methotrexate has a limited ability to penetrate into avascular tumor masses and concluded that the limited ability of methotrexate to penetrate solid tumor masses offers an explanation for the limited effectiveness of methotrexate when used for osteosarcoma. Cowan and Tannock (2001) by their studies of the penetration of radiolabelled methotrexate through multicellular layers (MCL) of murine EMT-6 and human MCF-7 cells grown on semiporous teflon membranes had provided an evidence that tissue penetration of methotrexate is through the extracellular space, that its distribution in solid tissue may be limited. Therefore it can be concluded that concurrent use of NSAIDs like ketoprofen or indomethacin with MTX can cause significant increase in MTX extent of absorption which may lead to severe adverse effects and care must be given in such situations.

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