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Additive Effect of Indomethacin and Methotrexate on Suppression of Growth in Rats

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ABSTRACT: The purpose of this study was to investigate the medium-term effects of methotrexate (MTX) and indomethacin on the growth of young rats. Four equal groups of Sprague–Dawley male rats (four animals in each group; mean $\pm$ S.D. body weight, 183 ± 13 g, in their rapid growth phase) were subjected to the following drug treatment: one group was given MTX (0.2 mg kg^{-1} body weight) subcutaneously on every fourth day, another received indomethacin (2.5 mg kg^{-1} body weight) subcutaneously daily and the third group was given both of these drugs (MTX on every fourth day and indomethacin daily). The fourth group was injected subcutaneously with physiological saline every day to serve as a control group. Total body weight, food and water consumption by animals in each group were monitored every second day for a period of 10 weeks. After this period, liver, spleen and kidneys were excised, weighed and analysed for MTX and dihydrofolate reductase activity. Compared with the groups, which received MTX alone, indomethacin alone, or physiological saline, mean increase (17 ± 11 g) in body weight of rats was minimal in the group receiving both MTX and indomethacin. The difference was statistically significant ($p = 0.001$) when the values of mean increase in body weight of rats in different treatment groups after a 10-week treatment were compared. The mean weights of liver and spleen in this group receiving both MTX and indomethacin were also found to be significantly less than the weights of these organs in the control group ($p < 0.01$). There also appears to be a decline in food consumption in this group ($p < 0.05$). This negative effect on growth of animals in this group appears to be not only due to decreased food consumption but also due to increased inhibition of *de novo* pathway of DNA synthesis. This is supported by increased accumulation of MTX and decreased dihydrofolate reductase activity in this group receiving both MTX and indomethacin, as compared with the group receiving MTX alone. The data indicate an additive effect of MTX and indomethacin on the suppression of growth in young rats, alluding to the notion that patients suffering from juvenile rheumatoid arthritis or acute lymphoblastic leukaemia receiving these two drugs concomitantly over a long period of time might be at a risk of experiencing short-term suppression of growth. Copyright © 1999 John Wiley & Sons, Ltd.

Key words: methotrexate; indomethacin; growth of rats; suppression of growth; folate antagonists; nonsteroidal anti-inflammatory drugs

Introduction

Methotrexate (MTX) is a synthetic analogue of folic acid. Being a potent inhibitor of dihydrofolate reductase (DHFR), the drug has been used in the treatment of leukaemia, choriocarcinoma and a number of other malignancies [1]. For the past few years, it is also being increasingly used in the treatment of juvenile rheumatoid arthritis (JRA) as a disease modifying drug [2–6]. It has turned out to be an effective therapy for most children with JRA. In addition, children with JRA are often concomitantly treated with nonsteroidal anti-inflammatory drugs (NSAIDs) which can alter MTX plasma clearance leading to toxicity [7–10]. Indomethacin is an inexpensive NSAID often used in JRA as analgesic and anti-inflammatory agent along with MTX. Both of these drugs may have an adverse effect on the growth of these children. Such an effect has not been reported so far. Therefore, in order to investigate the hypothesis that 'administration of MTX alone, and MTX and indomethacin in combination may result in suppression of growth', a rat model was used. Another objective of the study was to see whether increased accumulation of MTX in tissues following concomitant treatment with indomethacin was one of the possible causes of this effect.

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Materials and Methods

Chemicals

MTX and indomethacin with purity $>97\%$ were obtained from Sigma Chemical Company (St. Louis, MO, USA). Tritiated MTX (specific activity 11.2 Ci mmole^{-1}) was purchased from Amersham Corp. (Arlington Heights, IL, USA). All other chemicals were of reagent grade.

Animal Studies

Sixteen Sprague–Dawley young growing male rats aged 2.0–2.5 months (a period of rapid growth) with mean $\pm$ S.D. weight, 183 ± 13 g, were divided into four groups, each comprising of four animals. They were kept untreated for 1 week for complete acclimation to the laboratory conditions. Then, one group was injected subcutaneously with MTX (0.2 mg kg^{-1} body weight) on every fourth day. Another group was given indomethacin (2.5 mg kg^{-1} body weight) subcutaneously every day. The third group received both MTX (0.2 mg kg^{-1} body weight) on every fourth day and indomethacin (2.5 mg kg^{-1} body weight) every day. MTX was administered in physiological saline in a solution form (0.2 mL), while indomethacin was injected in the form of a suspension in physiological saline (0.4 mL). The fourth group was injected daily with physiological saline (0.4 mL) and served as a control group. This treatment was continued for a period of 10 weeks and the injection sites were varied day by day. It is important to mention that the low dose of MTX was worked out after a series of trials with doses of the drug so that the effect of MTX could be studied over a period of 10 weeks. The trial doses of MTX for this pilot study were selected on the basis of the rat model used by Freeman-Narrod *et al*. [11] who used MTX in a dose, which was one-half of the LD_{10}. With an MTX dose of 0.2 mg kg^{-1} body weight on every fourth day, all the animals survived during the period of this study. There was no evidence of diarrhoea or gastrointestinal bleeding, which are the common complications of these two drugs when given over a long period of time. In the final 2 weeks of study, animals on MTX were found to be weak and lethargic.

Rats were bred at our animal house facility and maintained under standard conditions in a room with a daily photo-period of 16-h light at 23°C. Rats had free access to food and water. Food and water consumption and weight of animals in each group were determined every other day for a period of 10 weeks. Then the animals in each group were sacrificed and their liver, kidney and spleen were excised, cleaned with physiological saline, gently dried between folds of a filter paper and weighed. Homogenates of liver and kidney were prepared as described previously [12]. Briefly, liver and kidney were suspended in cold 0.05 M citrate buffer, pH 7.4 containing phenyl-methyl-sulphonyl-fluoride (3.5 mg L^{-1}) and 0.02% sodium azide using 3 mL of buffer for 1 g of tissue, and homogenized using a Potter–Elvehjem type tissue grinder. Insoluble debris was then pelleted by centrifugation at $30000 \times g$ for 30 min at 4°C and the supernates were analysed for MTX (total as well as free) and additional binding of [^{3}H]MTX at pH 4.8.

Measurement of Total MTX in Liver and Kidney Extracts

To measure the total MTX in liver and kidney extracts of rats, the homogenates were boiled for 5 min to denature the enzyme DHFR and release the bound drug. MTX in these extracts was measured using a modified ligand-binding radioassay by the principle of stoichiometric dilution analysis as reported previously [13].

A brief account of this assay is as follows: A total reaction volume of 0.5 mL in 0.06 M citrate buffer, pH 4.8, contained 48 μM NADPH, 2.2 nM [^{3}H]MTX, varying amounts of unlabelled MTX (0–4 ng) or the unknown sample, and 50 μl of DHFR that has been titrated to bind approximately 50% of [^{3}H]MTX. The reactions are incubated for 10 min at room temperature and then stopped by adding 0.4 mL of 1% Norit A neutral charcoal (Sigma Chemical Co., St. Louis, MO, USA) in 0.5% Dextran T-10 (Pharmacia Fine Chemicals, Piscataway, NJ, USA). After centrifugation, the radioactivity in 0.5 mL of supernatant solution, which contains the bound [^{3}H]MTX, is counted in an LS-3801 Spectrometer (Beckman Instruments Inc., Palo Alto, CA, USA) using 5 mL of 3a70 Scintillation fluor (Research Product International, USA). A dose–response curve is constructed by plotting the percent bound [^{3}H]MTX against the amount of unlabelled drug in the reaction. The amount of the drug in the unknown sample is determined using this dose–response curve.

The protein concentration of the unboiled extracts was determined by the Lowry method [14].

Measurement of Free MTX in Liver and Kidney Extracts

For measurement of free MTX in liver and kidney extracts, 1-mL samples of homogenates of liver and kidney of rats treated with MTX alone and MTX with indomethacin were dialysed extensively overnight against 0.15 M NaCl. The dialysed homogenates were then boiled for 5 min to denature DHFR and other MTX binding proteins to release the bound MTX. This bound MTX was determined by the MTX binding assay as described above. Free MTX in the extracts was calculated by subtracting the protein-bound MTX from the total MTX.

Measurement of Residual DHFR Activity

Residual DHFR activity in the dialysed extracts of liver and kidney of the rats treated with MTX alone and MTX with indomethacin was titrated by binding of [^{3}H]MTX [13] on the principle that the MTX stoichiometrically inhibits the enzyme by binding to its active site [15].

Statistical Analysis

The mean values have been presented as means $\pm$ standard deviation (S.D.). Statistical analysis of the data pertaining to effect of various drugs on body weight across the study period was carried out using repeated measures analysis of variance (ANOVA) followed by Tukey's multiple pair-wise comparisons.

One way ANOVA and Student's t-test were used for comparison of mean increase in body weight over a period of 10 weeks, and for comparison of mean food consumption, mean water consumption and the mean organs' weight in various treatment groups versus the control group. The data pertaining to total MTX, free MTX and residual DHFR activity in rats treated with indomethacin and MTX and MTX alone were also compared by Student's t-test. A $p < 0.05$ was considered significant, however, in order to highlight a negative (but not significant) effect of drug on growth, the $p < 0.1$ was also incorporated in certain tables.

Results

Effect of MTX and Indomethacin on Body Weight

Figure 1 shows the effect of MTX, indomethacin, and MTX plus indomethacin on body weight of growing rats from week 1 to week 10 of the treatment. MTX appears to have a growth retarding effect while indomethacin given along with MTX potentiates this growth retardation. When the mean increases in body weight of animals in these groups were calculated, it was noticed that the minimal mean $\pm$ S.D. increase (17 ± 11 g) was observed in the group receiving both MTX and indomethacin compared with the mean increase of 41 ± 10 g in the group receiving MTX alone, 58 ± 11 g increase in the control group and 56 ± 9 g increase in the group receiving indomethacin alone (Figure 2). In other words, compared with control group, there was 71% less weight increase in the group receiving both MTX and indomethacin, while there was 30% less weight increase in the group receiving MTX alone. When these mean increase values were compared by using one way ANOVA followed by Student's t-test, the group receiving both MTX and indomethacin was found to be significantly different from all other groups including the group receiving MTX alone (one way ANOVA $p = 0.0012$; F-statistic $= 10.9453$).

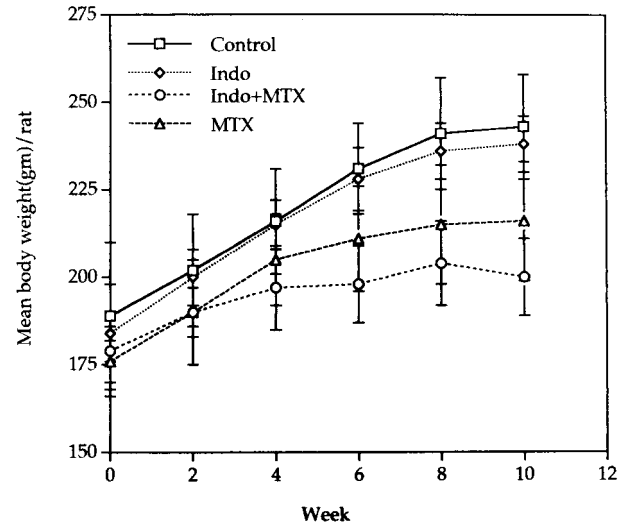

Figure 1. Effect of MTX and indomethacin on the body weight of rats over a period of 10 weeks. Indomethacin (2.5 mg kg^{-1} body weight) was given daily subcutaneously, while MTX (0.2 mg kg^{-1} body weight) was administered subcutaneously on every fourth day. There were four animals in each group. S.D. at each mean value is shown by error bars. Repeated measures ANOVA followed by Tukey's multiple pairwise comparisons showed that the interaction between time (in weeks) and drugs was significant (F-statistic $= 3.52$; $p = 0.0051$)

In order to see the effect of various drugs on weight gain across the whole study period, the data in Figure 1 were subjected to repeated measures ANOVA. Tukey's multiple pair-wise comparisons were used to compare the effect of drugs at various time intervals when the F-statistic was found to be significant.

Analysis showed that the interaction between time (in weeks) and drugs was significant

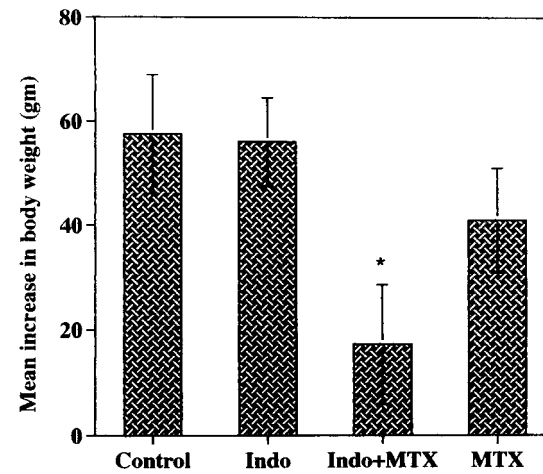

Figure 2. Mean increase in body weight of rats in various treatment groups from week 1 to week 10 of treatment. Indo refers to indomethacin. Each treatment group contained four animals. S.D. at each mean value is shown by error bars. Asterisk (*) indicates the treatment group significantly different from other groups. One-way ANOVA $p = 0.0012$

(F-statistic = 3.52; $p = 0.0051$). This implies that the effect of drugs is different across different time intervals. When the control group was compared with the group treated with indomethacin plus MTX, the weight changes in the two groups at weeks 4, 6, 8 and 10, were found to be statistically significant, while at weeks 0 and 2, the weight changes in the two groups were not statistically different.

Similarly, when the control group was compared with the group treated with MTX alone, then at weeks 6, 8 and 10 the weight changes in the two groups were found to be statistically different, while up to week 4 the weight changes in the two groups were not statistically significant.

When the indomethacin-treated group was compared with the group treated with both indomethacin and MTX, then at weeks 4, 6, 8 and 10, the weight changes in the two groups were statistically significant, while at weeks 0 and 2, the weight changes in the two groups were not significantly different.

When the indomethacin-treated group was compared with the group receiving MTX alone, then at weeks 8 and 10, the weight changes in the two groups were statistically significant, while up to week 6, the weight changes were not statistically different.

When the group receiving indomethacin plus MTX was compared with the group receiving MTX alone, then at week 10, the weight change in the two groups was statistically significant, while at weeks 0, 2, 4, 6 and 8, the weight changes were not significantly different.

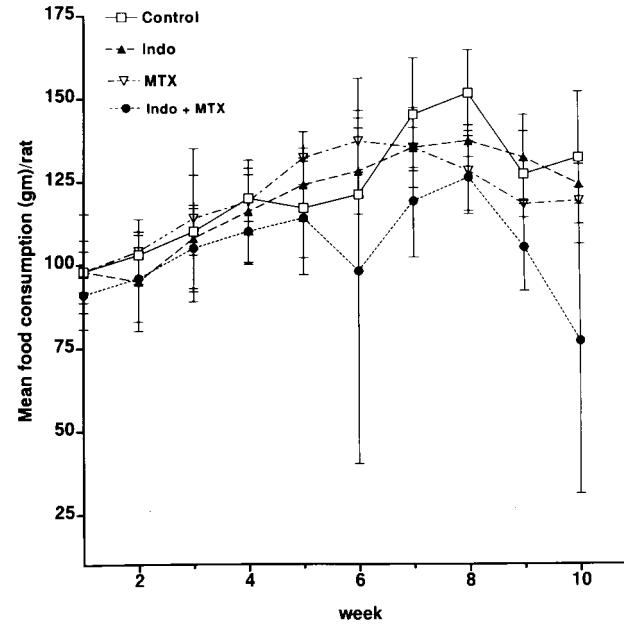

Figure 3. Effect of MTX and indomethacin on weekly food consumption of rats over a period of 10 weeks. Indomethacin (2.5 mg kg^{-1} body weight) was given daily subcutaneously, while MTX (0.2 mg kg^{-1} body weight) was administered subcutaneously on every fourth day. There were four animals in each group. S.D. at each mean value is shown by error bars. One-way ANOVA p values (comparing mean changes in food consumption between the group receiving both MTX and indomethacin versus the control group receiving physiological saline) at different weeks of treatment (week 1 = 0.757, week 2 = 0.566, week 3 = 0.858, week 4 = 0.577, week 5 = 0.313, week 6 = 0.668, week 7 = 0.157, week 8 = 0.031, week 9 = 0.088, week 10 = 0.05)

Effect of MTX and Indomethacin on Food and Water Consumption

Figure 3 shows the effect of MTX, indomethacin, and MTX plus indomethacin on weekly consumption of food by rats from week 1 to week 10 of the treatment. After week 7, MTX alone appears to have a somewhat negative effect (though not significant) on food consumption by rats when compared with the control group receiving no drug. However, MTX plus indomethacin has a considerably more negative effect on food consumption by rats after week 8 of treatment ($p = 0.031$). This suggests that decreased food consumption may also be a factor contributing to slower growth of rats in the group receiving both MTX and indomethacin. Water consumption throughout the study period, however, remained nearly the same among all the groups (data not shown).

Effect of MTX and Indomethacin on Organ Weight of Rats

In order to see whether these drugs affect the growth of different organs of the rats especially the ones with a relatively high cell turnover rate, mean $\pm$ S.D. weights of liver, spleen and kidney of rats in various treatment groups were determined, including the control, and the values were compared by Student's t-test. As shown in Table 1, the mean weights of liver and spleen in the group receiving indomethacin plus MTX were found to be significantly less ($p < 0.01$ in liver and spleen) than those of control rats. MTX as a single drug had a significant effect only on the mean weight of liver ($p = 0.021$). Indomethacin as a single drug had no significant effect on the growth of these organs.

Effect of Indomethacin on the Accumulation of MTX

The concentration levels of total MTX and free MTX in the extracts of liver and kidney of the rats treated with MTX alone and MTX plus indomethacin for a period of 10 weeks are shown in Table 2. It is noteworthy that, compared with the rats treated with MTX alone, there is significantly more concentration of both total and free MTX in liver and kidney of the rats treated concomitantly with indomethacin and MTX ($p < 0.025$).

Table 1. Weight of spleen, kidney and liver of rats following 10 weeks of drug treatment (means ± S.D.)

Treatment group	No.	Spleen weight (g)	p^a	Kidney weight (g)	p^a	Liver weight (g)	p^a
Control	4	0.521 ± 0.07	—	1.265 ± 0.08	—	6.7 ± 0.45	—
Indomethacin	4	0.5118 ± 0.06	NS	1.288 ± 0.07	NS	6.4 ± 0.46	NS
MTX	4	0.4919 ± 0.07	NS	1.145 ± 0.10	0.055 (NS)	5.9 ± 0.43	0.021
Indomethacin + MTX	4	0.3577 ± 0.06	0.006	1.065 ± 0.20	0.056 (NS)	5.56 ± 0.30	0.001

[a] The p values compare treatment groups with the untreated (control) group. NS, not significant.

Effect of Indomethacin on the Residual DHFR Activity

Additional binding of [^{3}H]MTX at pH 4.8 by the extracts of liver and kidney is indicative of residual DHFR activity in these organs. Table 3 shows the additional binding of [^{3}H]MTX by the extracts of liver and kidney of the rats treated with indomethacin plus MTX, compared with the extracts of these organs of the rats treated with MTX alone. There is significantly less residual DHFR activity in liver and kidney of rats treated with both MTX and indomethacin ($p < 0.025$).

Discussion

Growth by definition is an increase in size of an animal and includes both the linear increase due to bone growth and the body weight increase due to tissue growth in the rapid phase of development. Administration of MTX has been shown to adversely affect the growth of cells [16]. Recently, it has been have reported that high-dose MTX causes short-term suppression of linear growth in rabbits [17]. These authors, however, studied the effect of the drug for 5 days only. Similarly, it has also been reported that short-term low dose MTX has little effect on the weight gain by rats [18]. These authors administered MTX intraperitoneally on days 1, 8 and 15 at a dose of 0.3 or 3 mg kg^{-1} and studied the effect of the drug only for 18 days, a period too short to see any significant growth inhibition by MTX. This observation is also supported by the data (Figure 1), which show that MTX has little effect on body weight gain during the first 2 weeks of treatment. Apart from these studies, the medium-term or long-term effects of the drug have not been analysed before. This becomes important when children suffering from JRA or acute lymphocytic leukaemia (ALL) have to be treated with MTX for a prolonged time. The results in this study show that MTX in low dosage (0.2 mg kg^{-1} body weight) can adversely affect the development of body weight in growing rats especially when it is given concomitantly with indomethacin. The effect was observed on the growth of individual organs as well, most noticeably in liver and spleen (Table 1). There could be a number of reasons for the observed suppression of growth in rats. Decreased food intake by the animals and malabsorption could be one of them. MTX is known to cause weight loss, anorexia and prevents regeneration of gastrointestinal mucosa [19]. Indomethacin, too, has been reported to cause anorexia, nausea, abdominal pain, diarrhoea and gastrointestinal bleeding [20]. The data show that concurrent administration of these two drugs appears to significantly reduce ($p = 0.05$) food consumption by the animals (Figure 3), thereby contributing to slowing the increase in their body weights.

Folate deficiency due to repeated use of MTX could be another possible cause for the observed suppression of growth in rats [21]. At the cellular level, inhibition of DHFR activity due to increased accumulation of MTX in tissues could be another possible mechanism for suppression of growth. Indomethacin has been shown to increase the cellular uptake of MTX [22]. In a clinical study by Dupuis *et al*. indomethacin appears to prolong the elimination half-life of MTX in children with chronic arthritis [23]. These investigators actually attempted to study the pharmacokinetics of low dose oral MTX alone and in the presence of NSAIDs (indomethacin, tolmetin, naproxen and aspirin) in seven children (age 3.5–14 years) with chronic arthritis. Six of the

Table 2. Concentration of total and free MTX in extracts of liver and kidney of rats treated with MTX alone and with MTX and indomethacin (means ± S.D.)

Treatment administered	No.	Concentration in extracts (ng mg^{-1} protein)							
		Liver				Kidney			
		Total MTX	p^a	Free MTX	p^a	Total MTX	p^a	Free MTX	p^a
MTX	4	10.84 ± 1.98	—	4.08 ± 1.01	—	6.89 ± 1.044	—	0.45 ± 0.57	—
Indomethacin + MTX	4	15.9 ± 1.04	0.001	7.86 ± 2.68	0.019	8.71 ± 0.77	0.015	1.9 ± 0.36	0.001

[a] The p values compare the group treated with indomethacin plus MTX with the group treated with MTX only.

children were receiving multiple NSAIDs. Among the three patients who were receiving indomethacin (50–75 mg day^{-1}) two were also receiving naproxen (500 mg) and the third one was on aspirin (2600 mg day^{-1}). After maintaining the patient on his/her normally prescribed NSAIDs, MTX dose (3.75–12.5 mg) was given the next day along with NSAIDs dose and then the blood sampling was carried out for a period of 6 h. Their results showed that the mean MTX elimination half-life was significantly prolonged when NSAIDs were coadministered. In six of the seven patients, the area under the serum MTX concentration–time curve (AUC) increased from 19 to 140% in the presence of NSAIDs suggesting that these patients may be at a greater risk of developing MTX toxicity. However, neither the mean apparent MTX clearance nor mean volume of distribution was significantly altered by the administration of NSAIDs.

A severe interaction between MTX and indomethacin has also been reported in a patient of non-Hodgkin's lymphoma [8]. All these reports allude to the possibility that potentiation of the negative effect on growth by indomethacin in this study might be due to increased accumulation of MTX in the animals. Keeping that in mind, the concentration of total and free MTX in liver and kidney of the rats treated with MTX alone and MTX plus indomethacin was also monitored. It is important to note that compared with the concentration of MTX in liver and kidney of rats treated with MTX alone, there is a significantly higher concentration of MTX in these organs of rats treated with indomethacin plus MTX. These results also conform well to those reported by Nierenberg [24] who has shown that indomethacin and other NSAIDs competitively inhibit tubular secretion of MTX, thereby increasing the concentration of this drug in serum and tissues.

In a similar study pertaining to interaction between indomethacin (2.5 μg mL^{-1}) and MTX (25 μg mL^{-1}), Statkevich *et al*. [25] have also demonstrated a highly significant effect of indomethacin on secretion of MTX in urine. They showed a significant decline ($p < 0.001$) in both total clearance and tubular clearance of MTX in the presence of indomethacin suggesting that concomitant administration of indomethacin with MTX *in vivo* would lead to increased accumulation of MTX in the body. This increased accumulation of MTX in tissues may have a significant effect on the growth of the tissues with high cell turnover rate by inhibiting the pathway of *de novo* DNA synthesis.

Since DHFR is the target enzyme of MTX in the pathway of *de novo* DNA synthesis [26], the residual activity of this enzyme in liver and kidney of animals treated with MTX alone and MTX plus indomethacin was also focused on. The results pertaining to decreased residual DHFR activity as determined by the additional [^{3}H]MTX binding at pH 4.8 (Table 3) and increased MTX concentration (Table 2) in liver and kidney of the rats treated with indomethacin plus MTX further suggest the possibility of greater inhibition of the pathway of *de novo* DNA synthesis in this group of rats compared with those which were treated with MTX alone, thereby resulting into reduced weights of these organs in this group of rats (Table 1). Similar findings have been reported by Tsukamoto and Kojo [27] who have also shown that reduced weight of liver in MTX-treated rats was possibly through the inhibition of the pathway of *de novo* DNA synthesis by MTX.

Although the bone growth of rats was not monitored in this study, the effect of MTX on bone metabolism cannot be discounted. Even in low doses it has been shown to cause osteopenia in normal animals [28] and a decrease of osteoprogenitor cell colony growth in normal rats [18]. In a more recent *in vitro* study, it has been shown not to affect mature osteoblast population, but instead suppresses the proliferating cell fraction [29]. All these reports lend further support to the hypothesis that increased accumulation of MTX in the body in the presence of indomethacin may cause suppression of growth by inhibiting the pathway of *de novo* DNA synthesis especially in organs with a high cell-turnover rate.

Low-dose MTX therapy has been frequently used in JRA patients, but no growth related long-term or short-term effects of this treatment have been studied in these patients. Growth retardation after chemotherapy in children suffering from acute lymphocytic leukaemia has been observed [30,31] but the regimen used was a combination of drugs including MTX, hence, making it difficult to

Table 3. Additional binding of [^{3}H]MTX by extracts of liver and kidney of rats treated with MTX alone and with MTX and indomethacin (means ± S.D.)

Treatment administered	No.	Concentration of additional [^{3}H]MTX bound (ng mg^{-1} protein)			
		Liver	p[a]	Kidney	p[a]
MTX	4	0.83 ± 0.09	—	1.3 ± 0.17	—
Indomethacin + MTX	4	0.7 ± 0.03	0.017	0.87 ± 0.13	0.002

[a] The p values compare the group treated with indomethacin plus MTX with the group treated with MTX alone.

conclude that the observed effect was exclusively due to MTX. Since MTX is often used as a single disease-modifying drug in JRA, it would be important to study its long-term effect on growth of such patients. It is noteworthy that the dosage of MTX (0.2 mg kg^{-1} every fourth day) used in this study is comparable with the dosage of the drug normally used in JRA. For example, it has recently been reported that, in at least ten studies, the mean weekly MTX dosage used in JRA was around or below 0.4 mg $week^{-1}$ [32]. Similarly, the indomethacin used in this study was in a dosage which corresponds to the highest dosage used in clinical practice. Therefore, the data regarding the additive effect of indomethacin and MTX on suppression of growth in rats allude to the possibility that JRA patients receiving these two drugs concomitantly over a long period of time might be at a risk of experiencing short-term suppression of growth.

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References

1. Jolivet J, Cowan KH, Curt GA, Clendeninn NJ, Chabner BA. The pharmacology and clinical use of methotrexate. *New Engl J Med* 1983; **309**: 1094–1104.
2. Rose CD, Padman RN, Wesdock KA, Singsen BH. Methotrexate safety in juvenile rheumatoid arthritis (JRA). *Arthritis Rheum* 1990; **33**: S95 (abstract).
3. Giannini EH, Brewer EJ, Kuzmina N, Shaikov A, Maximov A, Vorontsov I, Fink CW, Newmen AJ, Cassidy JT, Zemel LS. Methotrexate in resistant juvenile rheumatoid arthritis—results of the USA–USSR double-blind, placebo-controlled trial. *New Engl J Med* 1992; **326**: 1043–1049.
4. Rose CD, Singsen BH, Eichenfield AH, Goldsmith DP, Athreya BH. Safety and efficacy of methotrexate therapy for juvenile rheumatoid arthritis. *J Pediatr* 1990; **117**: 653–659.
5. White PH, Ansell BM. Methotrexate for juvenile rheumatoid arthritis. *New Engl J Med* 1992; **326**: 1077–1078.
6. Giannini EH, Brewer EJ, Shaikov A, Kuzmina N, Maximov A. Low dose methotrexate in recalcitrant JRA—results of the USA–USSR randomized controlled trial. *Arthritis Rheum* 1991; **34**: 53.
7. Wallace CA, Bleyer WA, Sherry DD, Salmonson KL, Wedgwood RJ. Toxicity and serum levels of methotrexate in children with juvenile rheumatoid arthritis. *Arthritis Rheum* 1989; **32**: 677–681.
8. Maiche AG. Acute renal failure due to concomitant action of methotrexate and indomethacin. *Lancet* 1986; **I**: 1390 (letter).
9. Singh RR, Malavia AN, Pandey JN, Guleria JS. Fatal interaction between methotrexate and naproxen. *Lancet* 1986; **I**: 1390 (letter).
10. Thyss A, Milano G, Kubar J, Namer M, Schneider M. Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. *Lancet* 1986; **I**: 256–258.
11. Freeman-Narrod M, Narrod SA, Yarbro JW. Choline antagonism of methotrexate liver toxicity in the rat. *Med Paed Oncol* 1977; **3**: 9–14.
12. Rothenberg SP, Iqbal MP, daCosta M. Effect of folate compounds on the accumulation of methotrexate and the activity of dihydrofolate reductase in liver, kidney and small intestine of the mouse. *J Pharmacol Exp Ther* 1982; **223**: 631–634.
13. Rothenberg SP, Iqbal MP, daCosta M. Ligand-binding radioassay for the antifolate compounds. Application in patients receiving methotrexate. *Cancer Treat Rep* 1977; **61**: 575–584.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
15. Werkheiser WC. Specific binding of 4-amino folic acid analogues by folic acid reductase. *J Biol Chem* 1961; **236**: 888–893.
16. McBurney MW, Whitmore GF. Mechanism of growth inhibition by methotrexate. *Cancer Res* 1975; **35**: 586–590.
17. Möell C, Garwicz S. High-dose methotrexate causes short term suppression of growth in rabbits. *Acta Paediatr* 1995; **84**: 1237–1240.
18. Suzuki Y, Nakagawa M, Masuda C, Ide M, Uehara R, Ichikawa Y, Mizushima Y. Short term low dose methotrexate ameliorates abnormal bone metabolism and bone loss in adjuvant induced arthritis. *J Rheumatol* 1997; **24**: 1890–1895.
19. Zaloga GP, Roberts P, Black KW, Prielipp R. Gut bacterial translocation + dissemination explains the increased mortality produced by parenteral nutrition following methotrexate. *Circulatory Shock* 1983; **39**: 263–288.
20. Insel PA. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In *Goodman and Gilman's The Pharmacological Basis of Therapeutic* (9th edn), Hardman JG, Limbird LE (eds). McGraw-Hill: New York, 1995; 633–634.
21. Morgan SL, Baggott JE, Altz-Smith M. Folate status of rheumatoid arthritis patients receiving long-term, low-dose methotrexate therapy. *Arthritis Rheum* 1987; **30**: 1348–1356.
22. Bennett A, Gaffen JD, Melhuish PB, Stamford IF. Studies on the mechanism by which indomethacin increases the anticancer effect of methotrexate. *Br J Pharmacol* 1987; **91**: 229–235.
23. Dupuis LL, Koren G, Shore A, Silverman ED, Laxer RM. Methotrexate—nonsteroidal anti-inflammatory drug interaction in children with arthritis. *J Rheumatol* 1990; **17**: 1469–1473.
24. Nierenberg DW. Competitive inhibition of methotrexate accumulation in rabbit kidney slices by nonsteroidal anti-inflammatory drugs. *J Pharmacol Exp Ther* 1983; **226**: 1–6.
25. Statkevich P, Fournier DJ, Sweeney KR. Characterization of methotrexate elimination and interaction with indomethacin and flurbiprofen in the isolated perfused rat kidney. *J Pharmacol Exp Ther* 1993; **265**: 1118–1124.
26. Blakley RL. *The Biochemistry of Folic Acid and Related Pteridines*. North-Holland Publishing Co.: Amsterdam, 1969; 477–483.
27. Tsukamoto I, Kojo S. Effects of methotrexate on rat liver regeneration after partial hepatectomy. *Clin Sci Colch* 1992; **82**: 181–184.
28. May KP, West SG, McDermott MT, Huffer WE. The effect of low dose of methotrexate on bone metabolism and histomorphometry in rats. *Arthritis Rheum* 1994; **37**: 201–206.
29. Scheven BA, van der Veen MJ, Damen CA, Lafeber FP, Van Rijn HJ, Bijlsma JW, Duursma SA. Effects of methotrexate on human osteoblasts *in vitro*: modulation by 1, 25-dihydroxyvitamin D_3. *J Bone Miner Res* 1995; **10**: 874–876.
30. Kirk J, Raghupathy AP, Stevens MM, Cowell CT, Menser MA, Bergin M, Tink A, Vines RH, Silink M. Growth failure and growth hormone deficiency after treatment for acute lymphoblastic leukaemia. *Lancet* 1987; **I**: 190–193.
31. Clayton PE, Shalet SM, Morris-Jones PH, Price DA. Growth in children treated for acute lymphoblastic leukaemia. *Lancet* 1988; **I**: 460–462.
32. Wallace CA. The use of methotrexate in childhood rheumatic diseases. *Arthritis Rheum* 1998; **41**: 381–391.