ASPIRIN ALTERS METHOTREXATE DISPOSITION IN RHEUMATOID ARTHRITIS PATIENTS

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Intravenous methotrexate (MTX) (10 mg), either alone or with oral aspirin (ASA) (3,900 mg/day), was administered to 15 patients with rheumatoid arthritis. Systemic and renal clearance of MTX were lower, and the unbound fraction of MTX was higher when patients were also receiving ASA than when taking MTX alone. No acute hematologic, renal, or hepatic toxicity was observed with either treatment. The findings of this study therefore indicate that concomitant aspirin therapy acutely alters the clearance of low-dose MTX in patients with rheumatoid arthritis.

Methotrexate (MTX), a drug which has been widely used in the treatment of many disease states, has recently been approved by the FDA (Food and Drug Administration) for treatment of rheumatoid arthritis (RA) (1–7). The therapeutic effects of MTX do not occur until 0.5–3 months of treatment, but MTX often remains effective for several years with continued treatment (8,9). Because of the delay in onset of the drug's effects, nonsteroidal antiinflammatory drugs (NSAIDs), including aspirin (ASA), are commonly used with MTX to relieve acute symptoms (10,11). Interactions between aspirin and antirheumatic drugs have been recently reviewed (12,13), and in only 1 study of 4 patients receiving tritiated MTX and sodium salicylate was it suggested that an interaction between MTX and ASA had occurred (14). In that study, it was noted that the clearance of tritium was reduced by 26–42%, presumably due to inhibition of tubular secretion of MTX by salicylic acid, an organic acid. However, since those authors did not measure serum MTX concentrations, they could not conclude that the disposition of MTX was altered, only that the clearance of tritium was decreased (14). This interaction has not been evaluated in RA patients treated with short courses of intravenous infusions of MTX. In the current study, we sought to characterize the effects of salicylic acid on the disposition of low-dose (10 mg) MTX in patients with rheumatoid arthritis. Our findings, including the results of assessments of MTX systemic and renal clearance and plasma protein binding, are presented below.

PATIENTS AND METHODS

Patient population. Patients eligible for the study were between the ages of 25 and 65 years, had a diagnosis of RA, as defined by the American College of Rheumatology (formerly, the American Rheumatism Association) criteria (15), for at least 6 months, and had been receiving a stable dosage of MTX (maximum 20 mg/week) for at least 2 months. Patients who had a bilirubin or serum transaminase level greater than twice the upper limit of normal, a serum creatinine value >1.4 mg/dl, a creatinine clearance rate <60 ml/minute, radiologic evidence of pulmonary fibrosis, a

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platelet count <150,000/mm^3, or a white blood cell (WBC) count <4,000/mm^3, and those who were receiving concomitant therapy for any diseases other than RA were excluded from the study. Patients receiving treatment with gold salts, penicillamine, hydroxychloroquine, azathioprine, or other cytotoxic drugs within 2 weeks of the beginning of the study were also excluded.

Study schedule. The study protocol was approved by the Institutional Review Board, and written consent was obtained from each patient prior to entry into the study. Seven days prior to arm I of the study, a medical history was obtained, a physical examination was performed, and a laboratory assessment, including renal and liver function tests, urinalysis, and a complete blood cell count, was performed on each patient. All antirheumatic drugs were stopped 7 days prior to arm I. For both arms of the study, patients began fasting at 8:00 PM the night before, and were admitted to the Clinical Research Center (CRC) by 7:45 the next morning. All laboratory studies were performed prior to each arm of the study.

Arm I, intravenous MTX without aspirin. On day 0 at 8:00 AM, each patient was given 10 mg of MTX intravenously, over 5 to 10 minutes (exact time noted), and over the following 24 hours, serial samples of blood and urine were collected. All patients received a standard snack (i.e., coffee, ham, egg, and cheese biscuit) 1 hour after the MTX dose, and a standard lunch (i.e., meat, soup, vegetable, cake, fruit, and tea). After the final blood and urine samples were collected (at 24 hours), the aspirin was dispensed, and patients were instructed to take three 325-mg tablets 4 times daily for 1 week. A dosing schedule was maintained for each patient, indicating the date on which the ASA was administered and the bottle number. Compliance was documented by patient history and pill count.

Arm II, intravenous MTX with aspirin. On day 8, patients returned to the CRC, having fasted since 8:00 PM the night before. At 8:00 AM on day 8, intravenous MTX (10 mg) was administered as on day 0. The morning dose of aspirin was given immediately after the time 0 (predose) blood sample was drawn. Serial samples of blood and urine were collected for the next 24 hours. Following the last sample collection, patients were discharged from the CRC, and instructed to continue taking their usual NSAID.

Collection and handling of serum and urine samples. In each of the 2 treatment arms, 10 ml of blood was drawn before the MTX dose, and at 5 minutes, 30 minutes, 1 hour, 2, 4, 6, 8, 12, and 24 hours following the MTX dose. Blood samples were centrifuged at 3,000 revolutions per minute for 15 minutes, and the serum was removed and stored at −20°C. Urine was collected over 3 intervals: 0–6 hours, 6–12 hours, and 12–24 hours after MTX administration. The total volume for each interval was measured, the 3 samples were pooled, and after thorough mixing, a 15-ml aliquot was taken and stored at −20°C.

Drug assay procedures. Serum and urine samples were assessed for MTX with a radioenzymatic assay using dihydrofolate reductase as the binding protein, as previously utilized in our laboratory (16). The assay is extremely sensitive and specific, with <0.001% cross-reactivity with leucovorin and other major circulating serum folates, and <4% for 7-hydroxymethotrexate, the major extracellular metabolite (16). The limit of sensitivity for the MTX assay is 5 ng/ml (0.01 μM). The interday coefficient of variation was <10%. Salicylate concentrations were determined using a fluorescence polarization immunoassay (TDx; Abbott, North Chicago, IL), which has an interday coefficient of variation of <5%.

MTX protein-binding assay. MTX plasma protein binding was assessed by determining the unbound fraction (f_u) present in samples taken predose and at 1, 4, 8, and 12 hours. The extent of MTX binding to serum proteins was determined by equilibrium dialysis using tracer amounts of ^{3}H-labeled MTX. Initial studies indicated the time to equilibrium was between 3 and 4 hours; thus, 4 hours was selected as the duration of dialysis. Radiochemical purity was determined by thin-layer chromatography and was >95%. The unbound fraction of MTX was calculated from the ratio of the disintegrations per minute of ^{3}H-MTX in the buffer to the dpm in an aliquot of plasma, using an external standardization method for quench correction. The correction for volume shift was made by the method of Huang (17). Correction for radiochemical purity was made by the method of Bjornsson et al (18).

Pharmacokinetic evaluations. The area under the serum concentration–time curve (AUC) from time 0 to the time of the last measurable serum concentration ($AUC_{0\to t}$) was determined by the log-trapezoidal method (19). The $AUC_{0\to\infty}$ was calculated from the sum of the $AUC_{0\to t}$ and C_t divided by the terminal elimination rate constant (where C_t is the last measurable serum concentration). The terminal elimination rate constant was determined by log-linear least-squares regression of the plasma concentration time points in the terminal phase of the plasma disposition curve. Systemic clearance and renal clearance were calculated using standard pharmacokinetic equations (20). The clearance of unbound MTX was calculated by dividing either the systemic clearance or the renal clearance value by the MTX f_u value.

Statistical analysis. The mean and standard deviation were utilized to express the central tendency of the data. Comparison of laboratory values between the two treatment arms was done using the Mann-Whitney test. MTX pharmacokinetic parameters (e.g., AUC and clearance) during the control period (when patients were not receiving aspirin) were compared with pharmacokinetic parameters determined during the experimental period (when patients were receiving aspirin) using the nonparametric Wilcoxon paired-sample test (21). Simple linear and stepwise regression analyses were used to correlate measures of renal function (serum creatinine and creatinine clearance), MTX f_u, and salicylic acid concentration with MTX systemic and renal clearance. The preselected level of significance for all statistical tests was $P < 0.05$.

RESULTS

Thirteen women and 2 men with rheumatoid arthritis (age range 35–63 years) were entered into the study. Two were black and 13 were white. All patients completed the study with no adverse effects.

Renal clearance in 1 patient was considered inevaluable due to an incomplete urine collection.

There were no significant differences ($P > 0.05$, by Mann-Whitney test) between MTX alone and MTX + ASA for mean ± SD levels of serum creatinine (0.80 ± 0.19 versus 0.80 ± 0.16 mg/dl), creatinine clearance rates (87.4 ± 24.1 versus 92.1 ± 38.7 ml/minute), hemoglobin values (13.2 ± 1.3 versus 12.4 ± 1.2%), hematocrit levels (39.0 ± 4.0 versus 36.8 ± 3.6%), or WBC counts (7.3 ± 1.4 versus 7.1 ± 1.6 $\times 10^3$).

Figure 1 depicts the mean ± SD values of the MTX serum concentration over the 24-hour time period for patients receiving MTX with and without ASA. The MTX $AUC_{0\rightarrow\infty}$ was greater when ASA was coadministered than when MTX was given alone (3.6 ± 0.9 versus 2.8 ± 0.5 μM-hours/liter, $P = 0.03$, by Wilcoxon paired-sample test).

As depicted in Figure 2, systemic clearance of MTX was significantly lower during arm II (with ASA) than when MTX was taken alone (59.2 ± 11.3 versus 70.6 ± 18.5 ml/minute/m^2, $P = 0.03$, by Wilcoxon paired-sample test). There was a trend toward lower renal clearance of total MTX in patients receiving MTX alone than in those receiving MTX with ASA

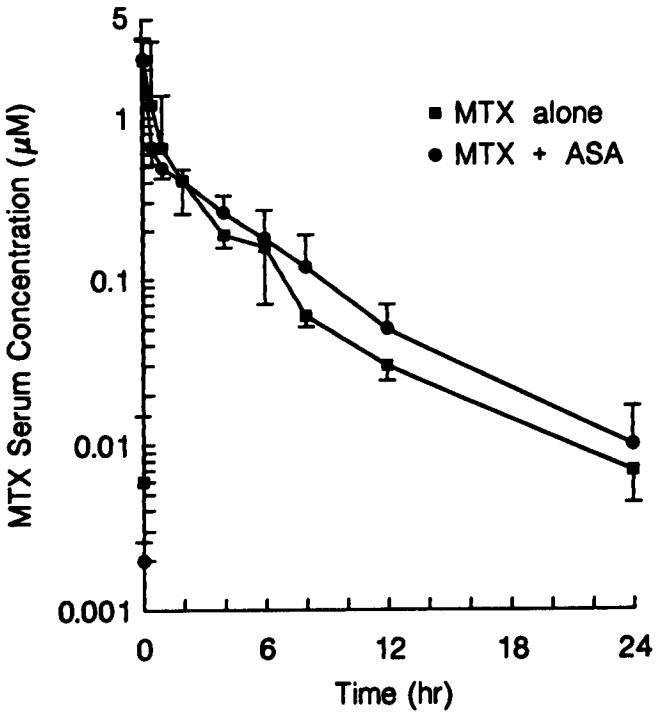

Figure 1. Serum concentration of methotrexate (MTX), with or without concomitant administration of aspirin (ASA), over 24 hours. Values are the mean and SD.

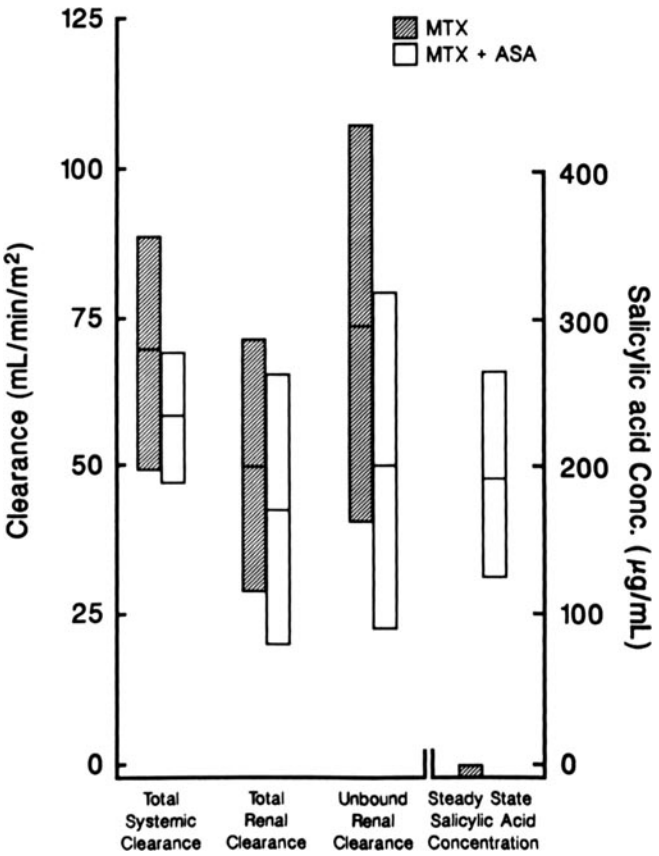

Figure 2. Methotrexate (MTX) total systemic clearance, MTX renal clearance (total and unbound), and salicylic acid plasma concentrations for arm I (MTX alone) and arm II (MTX plus aspirin [ASA]). The reduction in total systemic clearance ($P = 0.03$) and unbound renal clearance ($P = 0.01$) in the presence versus the absence of ASA was statistically significant. Bars show the mean ± SD.

(43.8 ± 23.4 versus 51.4 ± 21.1 ml/minute/m^2, $P = 0.11$, by Wilcoxon paired-sample test) (see Figure 2). Eleven of 14 patients had a decrease in MTX renal clearance in the presence of ASA. The mean renal clearance of unbound MTX was significantly lower when ASA was coadministered (51.5 ± 28.4 versus 74.4 ± 33.3 ml/minute/m^2, $P = 0.01$, by Wilcoxon paired-sample test).

Steady-state concentrations of salicylate attained during the interval following intravenous MTX administration showed little within-patient variation (average peak-to-trough ratio 16.4%); however, as much as a 500% difference in steady-state salicylate concentrations were observed between patients. The mean salicylate concentration during the 4-hour dosing interval was 199.6 ± 68.8 μg/ml (1,449 ± 498 μM). A trend was noted between renal clearance of unbound

MTX and the salicylic acid concentration ($r^2 = 0.16$, $P = 0.14$).

In arm I (MTX alone), a significant yet not highly predictive relationship was observed between serum creatinine levels and MTX systemic clearance ($r^2 = 31\%$, $P = 0.03$), systemic clearance of unbound MTX ($r^2 = 32\%$, $P = 0.28$), MTX renal clearance ($r^2 = 29\%$, $P = 0.04$), and renal clearance of unbound MTX ($r^2 = 29\%$, $P = 0.04$). In arm I (MTX alone), a significant yet not highly predictive correlation was observed between creatinine clearance and MTX renal clearance ($r^2 = 29\%$, $P = 0.04$) and renal clearance of unbound MTX ($r^2 = 28\%$, $P = 0.05$). Systemic and renal clearance of total and unbound MTX following 7 days of aspirin administration (arm II) was not significantly correlated with measures of renal function. Multiple stepwise regression analysis did not yield a model that explained additional variability in MTX pharmacokinetic parameters.

The ratio of the renal clearance of total or unbound MTX to the creatinine clearance rate was calculated when MTX was coadministered with ASA and when MTX was administered alone. For renal clearance of total MTX, there was a trend toward a decrease (average 15%) in the ratio during MTX + ASA therapy (ratio decreasing in 11 of 13 patients) ($P = 0.07$, by Wilcoxon paired-sample test). The decrease in the ratio (average 47%) for unbound MTX renal clearance was significant between the 2 treatment groups ($P = 0.02$, by Wilcoxon paired-sample test).

The MTX f_u following ASA administration was significantly greater than that following MTX alone (0.85 ± 0.06 versus 0.69 ± 0.05, $P < 0.05$, by Wilcoxon paired-sample test). MTX f_u was directly correlated with salicylate concentration ($r^2 = 0.69$, $P < 0.001$) (Figure 3). The $AUC_{0\rightarrow\infty}$ for unbound MTX when ASA was coadministered was significantly higher than the $AUC_{0\rightarrow\infty}$ for unbound MTX when MTX was administered alone (3.05 ± 0.89 versus 2.21 ± 1.04 μM-hour/liter, $P = 0.013$, by Wilcoxon paired-sample test).

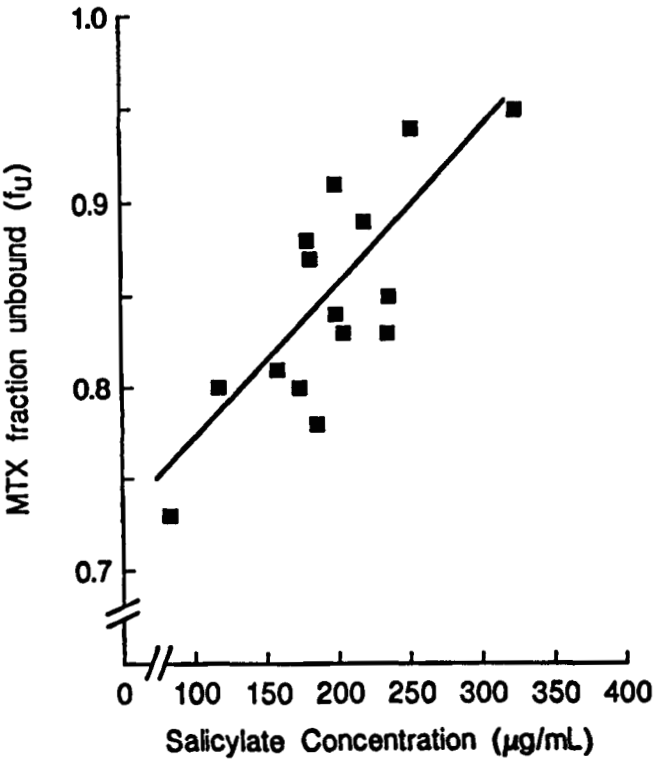

Figure 3. Correlation between methotrexate (MTX) f_u and the mean salicylate concentration (SA) ($f_u = 0.000869 * [SA] + 0.735$; $r^2 = 0.69$, $P < 0.001$).

DISCUSSION

Several studies have confirmed the efficacy of low-dose methotrexate in treating patients who have rheumatoid arthritis (6–8). Several case reports describing toxic and sometimes fatal interactions between NSAIDs and methotrexate have been published (22–26). Zuik and Mandel reported a retrospective evaluation of 176 patients who had received intraarterial infusions of methotrexate (27). Seven patients developed a rapid and severe pancytopenia; 6 of these 7 were receiving salicylates (dosage not specified). In this same study, Zuik and Mandel evaluated the interaction between MTX and ASA in mice, and observed severe leukopenia and decreased survival time when MTX and ASA were coadministered. Because of the results of these studies, clinicians may be uncertain of the safety of these drugs when administered in combination to RA patients. We have previously reported that naproxen (1 gm/day) does not significantly alter the clearance of low-dose MTX (28), but studies of a potential MTX–ASA interaction are limited.

Liegler and associates evaluated the effects of various organic acids on the renal clearance of low-dose tritiated methotrexate (5–15 mg) administered as a continuous infusion to 4 patients with leukemia or disseminated malignancies (14). When sodium salicylate was coadministered, renal clearance of tritium was reduced by 26–42%; this presumably resulted from an inhibition of the tubular secretion of MTX by

the salicylic acid. In the presence of salicylate, a 30% decrease in the MTX plasma protein binding was observed. Those authors concluded that salicylate inhibits the renal tubular secretion of MTX and displaces it from its plasma protein binding sites.

Furst et al recently reported that in 12 RA patients receiving MTX (10 mg/m^2 intravenously), ASA administration (3,400 mg/day) had no effect on the systemic clearance of MTX (29). However, they reported that if one outlier was excluded from the analysis, a statistically significant decrease in MTX systemic clearance was observed when patients received ASA. When the values for MTX systemic clearance from Furst's study and those from the present study are combined (27 patients), a statistically significant decrease in clearance is noted when patients receive ASA ($P = 0.02$, by Wilcoxon paired-sample test). Since Furst et al did not report MTX renal clearance and protein binding values or salicylate concentrations, further comparisons are not possible.

In contrast to the studies by Furst and associates, our study revealed a significant decrease in MTX systemic clearance and renal clearance when ASA (3,900 mg/day) was coadministered. Our results are thus consistent with those of the previous studies by Liegler et al (14), who observed a decrease in the clearance of low-dose intravenous MTX when sodium salicylate was coadministered to cancer patients.

In our study, we observed a trend for MTX renal clearance to be lower in patients receiving ASA, with renal clearance of total MTX decreasing in 12 of 15 patients. Moreover, renal clearance of *unbound* MTX was significantly lower when ASA was coadministered. It is plausible that renal clearance of *total* MTX was not significantly lower because a reduction in tubular secretion or glomerular filtration was offset by a concomitant increase in unbound MTX available for filtration or secretion due to displacement from plasma proteins by ASA.

MTX renal clearance consists of glomerular filtration, tubular secretion, and tubular reabsorption (30), and the relative effects that an increased concentration of free drug may have on these processes has been discussed by Levy (31) and by Cook and Smith (32). Several studies have demonstrated that the short-term administration of aspirin may result in decreased glomerular filtration both in medical patients and in normal subjects (33–36). Those studies did not describe a predictive concentration–effect relationship for the salicylate-induced decrease in renal function. However, in the present study, variability in salicylate concentrations was shown to be associated with variability in MTX renal clearance. Since the MTX f_u was significantly correlated with the salicylate concentration, an increase in unbound (free) MTX may have increased renal clearance of MTX via glomerular filtration, offsetting the reduction in MTX tubular secretion which salicylates are known to produce.

The lower unbound MTX renal clearance when ASA was coadministered is consistent with ASA decreasing MTX renal clearance via either tubular secretion or glomerular filtration of unbound MTX. However, since the creatinine clearance rate did not decrease when ASA was coadministered, it is probable that ASA had a greater effect on tubular secretion of MTX, rather than glomerular filtration. This is supported by the significant decrease (average 47%; $P = 0.02$) in the ratio of renal clearance of unbound MTX to creatinine clearance. Further, the ~20% increase in MTX f_u and ~30% decrease in unbound MTX renal clearance, when creatinine clearance did not change, is also consistent with the relatively small change (~16% decrease) we observed in renal clearance of *total* MTX. Moreover, renal clearance of unbound MTX was significantly related to serum creatinine and creatinine clearance (data not shown) only in the group of patients that received MTX alone. No correlation between creatinine clearance and MTX renal clearance (total or unbound) was noted when patients received both MTX and ASA. This feature is consistent with ASA altering MTX renal clearance without affecting creatinine clearance in a similar manner.

Rooney and Furst have published in abstract form a study which showed no difference in toxicity in MTX-treated RA patients who were also receiving aspirin or other NSAIDs (37). Interestingly, we recently published results of studies in our laboratory, in which no change in MTX clearance was found when naproxen (1 gm/day) was coadministered (28). Results of the present study demonstrate that short-term ASA therapy can acutely lower the systemic and renal clearance of low-dose MTX in patients with RA, which indicates that this combination is likely to yield greater pharmacologic effects (e.g., toxicity) at any dosage of MTX. Taken together, these studies indicate that it may be preferable to coadminister an NSAID other than ASA (e.g., naproxen) with MTX in patients with rheumatoid arthritis.

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