

Combination Therapy with Methotrexate and Hydroxychloroquine for Rheumatoid Arthritis Increases Exposure to Methotrexate

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ABSTRACT. Objective. To examine the bioavailability of methotrexate (MTX) in the presence of hydroxychloroquine (HCQ), and vice versa, to determine a possible pharmacokinetic explanation for the observation that combination treatment of rheumatoid arthritis with MTX and HCQ has been shown, clinically, to be more potent than MTX used alone.

Methods. In a randomized crossover study, 10 healthy subjects received, on each of 5 dosing occasions, MTX alone as tablets or intravenous solution, HCQ alone as a tablet or oral solution, or a coadministered dose of MTX tablets with an HCQ tablet. The area under the concentration-time curve (AUC) was determined for each subject, on each dosing occasion, for each compound.

Results. The mean AUC for MTX was increased ($p = 0.005$) and the maximum MTX concentration (C_{\max}) decreased ($p = 0.025$) when MTX was coadministered with HCQ, compared to MTX administered alone. The time to reach C_{\max} for MTX administration, t_{\max} , was also increased during the coadministration with HCQ ($p = 0.072$). The AUC of HCQ showed no significant difference ($p = 0.957$) between any of the dosing occasions.

Conclusion. These results may explain the increased potency of the MTX-HCQ combination over MTX as a single agent and also the sustained effects of MTX when administered with HCQ. In addition, the reduced C_{\max} of MTX observed during the coadministration may explain diminution of acute liver adverse effects. Extra vigilance for MTX adverse effects during combination therapy with HCQ is recommended, especially if renal function is known to be decreased. (J Rheumatol 2002;29:2077-83)

Key Indexing Terms:
METHOTREXATE
BIOAVAILABILITY

HYDROXYCHLOROQUINE
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Current practice in the treatment of rheumatoid arthritis (RA) advocates an aggressive therapeutic approach with use of disease modifying antirheumatic drugs (DMARD) early in the course of the disease¹⁻⁴.

Low dose methotrexate (MTX) is the most commonly used DMARD, either alone or in combination^{5,6}. Low dose MTX has been used in the treatment of psoriasis where liver toxicity was the main side effect⁷. However, concerns about hepatotoxicity have lessened and several studies have shown that serial monitoring of liver enzymes, along with abstinence from alcohol consumption, allowed prediction of

liver damage and subsequent reductions in MTX dose in at-risk patients^{8,9}. Risk factors for MTX induced pulmonary toxicity have similarly been identified and allow close monitoring of at-risk patients¹⁰⁻¹⁴. Other minor toxicities, e.g., stomatitis, can be avoided with concomitant use of folic acid¹⁵⁻¹⁷.

When MTX is used as a single agent, improvements in disease measures are seen relatively rapidly, but tend to plateau after about 6 months with no further improvement noted, even when patients are followed for long periods^{5,18,19}.

The interindividual variability in the bioavailability of MTX is high, ranging from 28 to 94%, and this may account for some treatment failures with single agent MTX^{20,21}. However, variability in absorption is not the complete picture and comparisons of monotherapy with combination therapy have shown a significant improvement in clinical signs when more than one DMARD was administered²²⁻²⁶.

In Canada and the US, MTX and hydroxychloroquine (HCQ) is a frequently used DMARD combination²⁷⁻²⁹. HCQ has antimalarial activity, but is also used as a second-line treatment for RA. Clinically, the use of HCQ is characterized by a long delay in the onset of action and, as a result, frequent withdrawal of treatment is due to inefficacy rather

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than from toxicity, as it has few serious adverse effects^{30,31}. Although the manufacturer's data sheet recommends ophthalmological monitoring twice a year, there is debate as to whether routine monitoring for retinopathy is actually required³²⁻³⁴.

The slow onset of action can be attributed to the pharmacokinetics of HCQ, and wide interpatient variability in steady-state concentrations achieved from the same dose has been observed³⁵. HCQ has a terminal half-life longer than 40 days, thus steady state is not reached until 6 months of treatment have been administered^{36,37} and correlations have been made between steady-state concentration and therapeutic effect^{35,38}. Significant variability in the bioavailability (ranging from 30 to 100%) of an oral dose has been described^{39,41}, in addition to a 2-fold range in clearance³⁶.

Combination therapy of MTX with HCQ has been shown to reduce the risk of acute liver damage seen with MTX alone. The exact mechanism of this interaction is not clear, but it has been postulated that HCQ stabilizes hepatic lysosomes, hence possibly allowing responding patients at risk of hepatic injury to continue with MTX treatment⁴². A second possibility is that the bioavailability of MTX is in some way reduced by HCQ, as noted for another anti-malarial drug, chloroquine⁴³. A concern is that reduced adverse effects may also be associated with reduced efficacy if, in combination therapy, bioavailability of MTX is being reduced.

However, combination therapy of MTX with the anti-malarial HCQ has also been shown to be more potent than MTX used alone⁴⁴. HCQ has also been noted to sustain responses observed during combination therapy, even after stopping the MTX part of treatment⁴⁵. An explanation for this may be that HCQ in fact increases bioavailability of MTX or reduces the clearance of the drug.

We investigated the bioavailability of MTX in the presence of HCQ, and vice versa, to determine the pharmacokinetic interactions between antirheumatic agents used in combination therapy in an effort to improve treatment of RA.

MATERIALS AND METHODS

Healthy subjects. Informed written consent was obtained from 10 healthy volunteers following approval from the Research Ethics Committee at St. Vincent's Hospital. Subjects included 6 men and 4 women, ranging in age from 20 to 48 years and ranging in weight from 55 to 94 kg. Each volunteer had a full examination including determination of creatinine clearance, liver function tests, and hematological studies. All biochemical values and examinations were within normal ranges for inclusion into the study.

Subjects were required to fast from 10:00 PM in the evening prior to each dosing day, and a light breakfast (2 slices of toast, tea or coffee) was provided on the morning of drug administration. A predose sample was collected followed by drug administration according to a randomization protocol: (1) 15 mg MTX as tablets (Ledertrexate®); (2) 15 mg MTX intravenous solution; (3) 200 mg HCQ sulfate (HCQ-SO₄) tablet (Plaquenil®); (4) 200 mg HCQ-SO₄ as oral solution (this was used as the reference dose for HCQ-SO₄ in the absence of an intravenous formulation); (5) 200 mg HCQ-SO₄ tablet together with 15 mg MTX as tablets. All oral doses were taken with 200 ml water.

Blood was collected from an indwelling venous cannula at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 hours and via venepuncture at 24, 32, 48, 72, and 96 hours after the dose into tubes containing EDTA. Samples to be analyzed for MTX were centrifuged and the plasma portion stored at -20°C until analysis. Samples to be assayed for HCQ were stored at -20°C as whole blood for analysis.

Sample analysis. An enzyme multiplied immunoassay technique (EMIT®; Dade Behring, San Jose, CA, USA) was used to determine the higher MTX concentrations in plasma on a MIRA® machine (Roche Diagnostics, Basle, Switzerland). The calibration standards for EMIT range from 0.2 to 2.0 mol/l (roughly 100–900 ng/ml). As this study involved single doses of MTX, it was necessary to then use a highly sensitive high performance liquid chromatographic (HPLC) method to determine MTX plasma concentrations that were below the limit of quantitation (100 ng/ml) of EMIT⁴⁶. This enabled the collection of data points from later time samples (8 to 96 h) to form a complete concentration-time profile.

MTX quality controls were prepared in plasma at high concentrations (100, 400, and 800 ng/ml) for the EMIT, and at low concentrations (5, 15, and 25 ng/ml) to be analyzed with the low concentration samples by HPLC, and then stored with the healthy subject samples at -20°C.

HCQ concentrations were determined in whole blood using a well established HPLC method⁴⁷. HCQ quality controls were prepared in whole blood at low, mid, and high concentrations (160, 500, and 1000 ng/ml) and stored at -20°C prior to analysis by HPLC with the healthy subject samples.

Statistical analysis. The area under the concentration-time curve was determined by the trapezoidal rule for each dosing day (MTX tablets, MTX IV, HCQ-SO₄ tablet, HCQ-SO₄ solution, and MTX tablets with HCQ-SO₄ tablet) for each of the 10 subjects. The MTX AUC were extrapolated to infinity using the log-linear trapezoidal rule

$$AUC_{\text{last}}^{\infty} = \frac{C_{\text{last}}}{\lambda_z}$$

where $AUC_{\text{last}}^{\infty}$ was the AUC from the last sampling time (t_{last}) to infinity, C_{last} was the last measured concentration, and λ_z the terminal slope on a log_e scale. Due to the long half-life of HCQ the AUC were truncated at 32 h post-dose⁴⁸⁻⁵¹.

A one-way analysis of variance (ANOVA) was carried out on the AUC data for the MTX tablets, IV solution, and in the presence of HCQ. Similarly ANOVA was carried out for the AUC data for HCQ-SO₄ tablets, oral solution, and in the presence of MTX tablets. As ANOVA shows only the possibility of differences between the groups of data, multiple comparisons were also carried out using Fisher's LSD procedure (Minitab®, Release 12). This allowed calculation of the 95% confidence intervals between the 3 sets of data for each drug and determination of which treatment had AUC values that were truly different from the others. The statistical significance was determined as $p \leq 0.05$.

The maximum concentration (C_{max}) and time of C_{max} (t_{max}) from each dose given to the 10 subjects were collated directly from the concentration-time data, and the effect of HCQ on the C_{max} and t_{max} of MTX tablets was also determined using a paired t test. The difference was considered to be significant where $p \leq 0.05$. The C_{max} and t_{max} of HCQ from each of the 3 treatments were compared using ANOVA.

RESULTS

The distributions and mean values of the AUC for each MTX and HCQ treatment are shown in Figures 1 and 2, respectively. Figure 1 shows that one subject, subject 7, had a MTX AUC following oral administration of 15 mg of 6331 ng ml⁻¹ h. As this was greater than the mean + (1.96 SD) for the group values (5157 ng ml⁻¹ h), subject 7 was considered to be an outlier and was excluded from further

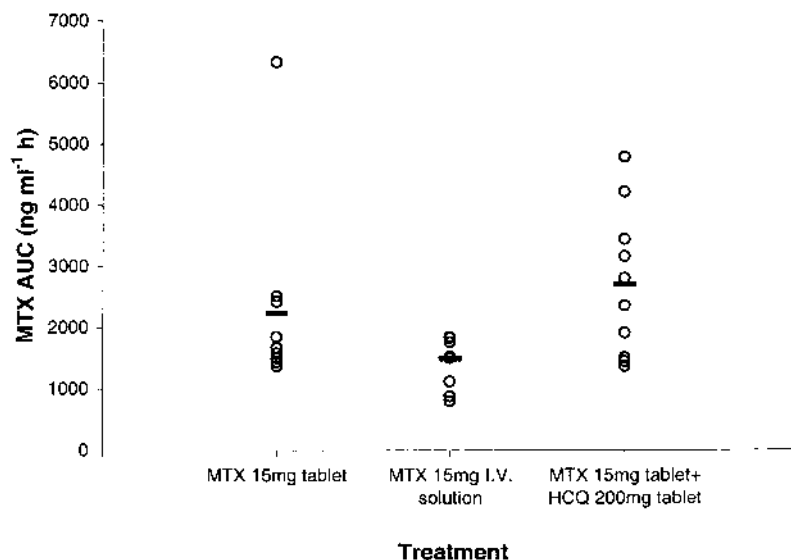


Figure 1. Distribution of AUC measurements for each MTX treatment. The mean is represented by the horizontal line.

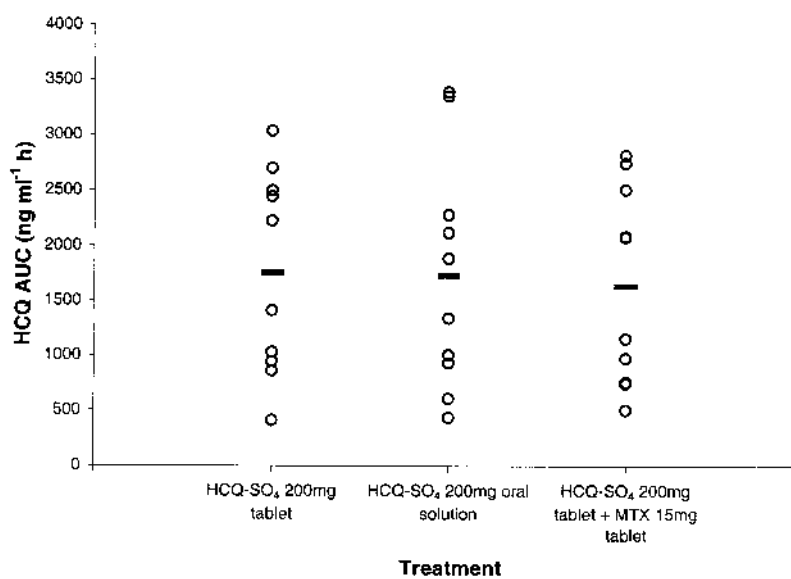


Figure 2. Distribution of AUC measurements for each HCQ treatment. The mean is represented by the horizontal line.

analysis in this group of AUC values. No outliers were noted for any of the HCQ treatments and the data from all 10 subjects were included.

The data for MTX and HCQ AUC, C_{max} , and t_{max} from each dosing day are presented in Table 1. ANOVA showed that a difference existed in the MTX AUC values between the 3 treatment days ($p = 0.005$). Table 2 details the 95% CI obtained for the differences between the 3 MTX treatments obtained by carrying out multiple comparison analysis, using Fisher's LSD method. The 95% CI for the comparison of AUC value for MTX tablets and MTX IV includes zero, showing that no difference existed between these treat-

ments. However, the differences between the single agent administrations of MTX and administration in combination with HCQ were both negative and the 95% CI did not include zero. The differences were calculated by subtracting the AUC values of the combination from the corresponding single agent administration AUC value. Hence, these negative values revealed that the AUC values measured when MTX was coadministered with HCQ were higher than when MTX was administered alone either as tablets or as IV solution.

A comparison of the maximum concentrations from MTX tablet alone or when given with HCQ showed a signif-

Table 1. Comparison of pharmacokinetic parameter values for HCQ and MTX. Data are mean (SD) [range].

Parameter	15 mg Tablets	MTX		p
		15 mg IV Solution	15 mg Tablets + 200 mg HCQ Tablet	
AUC _{0-∞} , ng ml ⁻¹ h	1775* (415)* [1367-2506]*	1489 (415) [796-1844]	2695 (1196) [1368-4776]	0.005
C _{max} , ng ml ⁻¹	351 (120) [197-563]	—	292 (77) [225-459]	0.025 [†]
t _{max} , h	1.61 (0.52) [1.00-2.52]	—	1.93 (0.58) [1.02-3.05]	0.072 [†]

Parameter	200 mg Tablet	HCQ		p
		200 mg Oral Solution	200 mg Tablet + 15 mg MTX Tablets	
AUC ₀₋₃₂ , ng ml ⁻¹ h	1754 (924) [408-3036]	1732 (1060) [434-3389]	1634 (900) [502-2814]	0.957
C _{max} , ng ml ⁻¹	155 (94) [78-406]	160 (111) [72-421]	134 (100) [55-392]	0.833
t _{max} , h	3.31 (1.07) [1.48-4.55]	3.04 (1.69) [0.50-5.67]	3.72 (1.6) [2.00-7.42]	0.596

*Subject 7 not included; [†] Paired t test.

Table 2. 95% confidence intervals for differences between MTX AUC for the 3 treatments using Fisher's LSD method. The confidence intervals represent the difference between the column treatment minus the row treatment.

Treatment	MTX 15 mg Tablet	MTX 15 mg IV
MTX 15 mg IV	-450 to 1023	—
MTX 15 mg tablet + HCQ-SO ₄ 200 mg tablet	-1656 to -183	-1923 to -489

icant decrease in C_{max} (p = 0.025) when the 2 drugs were coadministered. The time to reach maximum concentration (t_{max}) also increased in the presence of HCQ, although this result did not reach significance (p = 0.072). The increase in AUC and t_{max}, coupled with a decrease in C_{max} when HCQ was coadministered with MTX is displayed in the concentration-time profile example shown in Figure 3.

There was no significant difference in AUC values when HCQ was administered alone as tablets or oral solution or when administered in combination with MTX (p = 0.957). Similarly, ANOVA did not detect any treatment difference in the C_{max} or t_{max} data obtained from the HCQ concentrations (p = 0.833 and p = 0.596, respectively). A concentration-time profile for HCQ is shown in Figure 4.

DISCUSSION

Coadministration of MTX and HCQ resulted in AUC_{0-∞} values for MTX that were on average 65% higher than those achieved when MTX was administered alone, either orally or intravenously. In addition, C_{max} was lower and t_{max} longer for MTX on the day of the combination of drugs being

administered. No significant differences were recorded for HCQ AUC, C_{max}, or t_{max} values on any of the dosing days.

Subject 7 was excluded from the analysis of MTX AUC following the single oral administration of 15 mg. This subject's AUC value was 6331 ng ml⁻¹ h, greater than the mean + (1.96 SD) for the group (5157 ng ml⁻¹ h). No errors were found in the assay or analysis methods for subject 7. However, on each of the days when MTX was administered orally, subject 7 had the highest C_{max} of the 10 subjects. This was 61% and 57% higher than the mean C_{max} for the group on the single MTX tablet day and MTX-HCQ combination day, respectively. The MTX AUC on the combination administration day was also highest for subject 7, but within the limit of mean + (1.96 SD) for the group values and hence was not excluded from the analysis. Interindividual variability in the bioavailability of MTX is high, ranging from 28 to 94%, and this may account for the high MTX AUC observed for subject 7^{20,21}.

Predictive models using the pharmacology of DMARD have been examined for potential beneficial therapeutic effects of combinations of drugs⁵². These models are limited by the lack of clear information regarding the exact mechanisms of actions of many of the DMARD and have occasionally predicted a successful combination that had previously been proved to be inadequate (D-penicillamine and HCQ)⁵³. However, the MTX-HCQ combination was predicted to have a reasonable chance of therapeutic benefit, with complementary pharmacological profiles. In particular, the slow onset of action of HCQ would be offset by the more rapid onset of MTX. This has been confirmed in clinical practice, where the combination has shown the clinical benefit of increased effectiveness over MTX used alone^{27,44}.

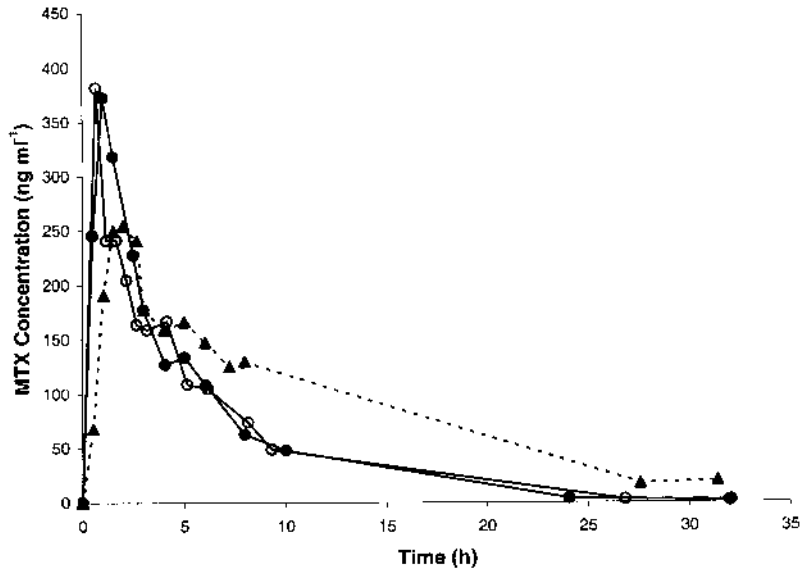


Figure 3. MTX plasma concentration-time profile for a healthy subject following a single dose of MTX 15 mg tablets (●), MTX 15 mg IV (○), or coadministration of 15 mg MTX tablet and 200 mg HCQ-SO₄ tablet (▲).

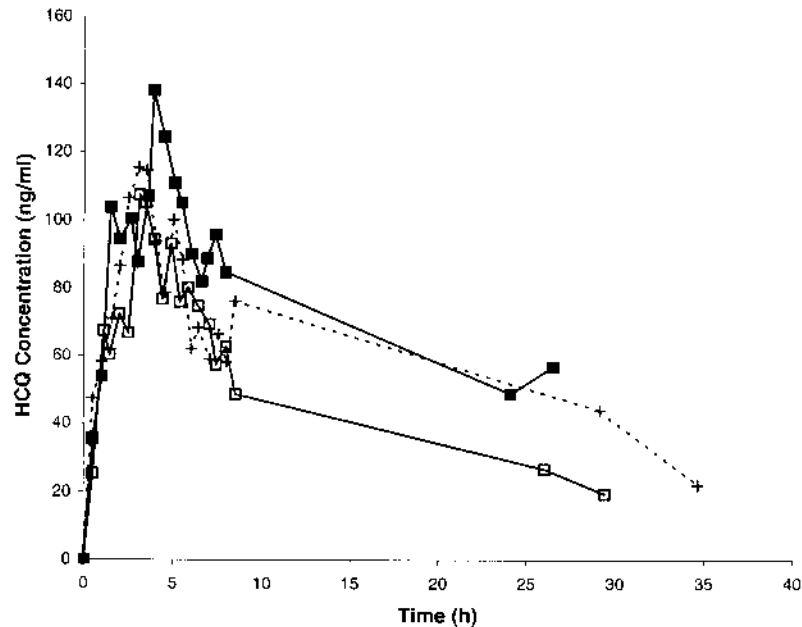


Figure 4. HCQ plasma concentration-time profile for a healthy subject following a single dose of 200 mg HCQ-SO₄ tablet (■), 200 mg HCQ-SO₄ solution (□), or coadministration of 200 mg HCQ tablet and 15 mg MTX tablet (+).

Although the success of the MTX-HCQ combination can be accounted for to some degree by the pharmacology, to date no studies detailing the pharmacokinetic interactions have been published. Postulations of altered bioavailability have been made, as one study reported that chloroquine reduced the bioavailability of MTX⁴³. Within our study the area under the concentration-time curve was measured for MTX and HCQ after 5 different dosing events in healthy

volunteers, to establish subject exposure to either drug. Intersubject variability in AUC was high for both drugs on each of the dosing days, with coefficient of variation for the 10 subjects in excess of 20% and 50% for MTX and HCQ, respectively, regardless of coadministration of the 2 drugs. These values correlate with published values for variability in bioavailability of these drugs^{20,21,39-41}. The observed values of AUC, C_{max}, and t_{max} for the single administrations

of MTX were also similar to values available in the literature^{19,54}. Altered bioavailability is unlikely to be the explanation for the increased AUC observed in this study when MTX was coadministered with HCQ, as there were no significant differences between the oral and intravenous AUC measurements when MTX was administered alone (Table 2).

As bioavailability is not a viable explanation for the increased MTX AUC observed on coadministration with HCQ, other postulations are that either the clearance of MTX is reduced in some way by the presence of HCQ or HCQ may increase the active reabsorption of MTX.

MTX is eliminated almost entirely by the kidneys, and low dose treatment for RA has been shown to impair renal function over time^{55,56}. In light of the results of this study, caution should be advised before administration of the combination to patients with reduced renal function, as this may further increase the AUC of MTX. The elderly are a specific patient group who may benefit from extra vigilance during the use of this combination as they may have reduced kidney function before beginning therapy⁵⁷⁻⁵⁹. However, it must also be restated that the pharmacokinetics of MTX are variable and dose adjustments should be based on the individual patient, not merely on creatinine clearance⁶⁰.

Our results showing increased MTX AUC when coadministered with HCQ may explain those obtained by Clegg, *et al*⁴⁵, where the combination therapy was administered for 6 months before withdrawal of MTX. They showed that continued maintenance therapy with HCQ alone prolonged the time until disease flare, whereas withdrawal of MTX treatment usually results in a rapid disease flare. In addition, the reduced C_{max} observed in this study may also explain the observation of reduced liver toxicity with the combination therapy, as measured by elimination of elevations in hepatic enzymes⁴². Raised hepatic enzymes are generally a measure of acute liver injury and this may be linked to high C_{max} values.

This study has shown that patient exposure to MTX is increased when HCQ is administered simultaneously, which may explain some of the clinical benefits observed with combination and the lesser degree of disease flare upon MTX withdrawal. In addition our observation indicates a need for extra vigilance for patients receiving the combination, who may be at increased risk of MTX induced toxicities.

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REFERENCES

- Gordon DA, Hastings DE. Clinical features of early, progressive and late disease. In: Klippel JH, Dieppe PA, editors. *Rheumatology*. 2nd ed. London: Mosby; 1998:5.3.1-5.3.14.
- Pincus T, O'Dell JR, Kremer JM. Combination therapy with multiple disease-modifying antirheumatic drugs in rheumatoid arthritis: a preventive strategy. *Ann Intern Med* 1999;131:768-74.
- Mottonen TT, Hannonen PJ, Boers M. Combination DMARD therapy including corticosteroids in early rheumatoid arthritis. *Clin Exp Rheumatol* 1999;17 Suppl 18:S59-65.
- Fries JF. Current treatment paradigms in rheumatoid arthritis. *Rheumatology* 2000;39 Suppl 1:30-5.
- O'Dell JR. Methotrexate use in rheumatoid arthritis. *Rheum Dis Clin North Am* 1997;23:779-96.
- Kremer JM. Methotrexate and emerging therapies. *Clin Exp Rheumatol* 1999;17 Suppl 18:S43-6.
- Haustein UF, Rytter M. Methotrexate in psoriasis: 26 years' experience with low-dose long-term treatment. *J Eur Acad Dermatol Venereol* 2000;14:382-8.
- Kremer JM, Alarcon GS, Lightfoot RW Jr, et al. Methotrexate for rheumatoid arthritis. American College of Rheumatology suggested guidelines for monitoring liver toxicity. *Arthritis Rheum* 1994;37:316-28.
- Kremer JM, Furst DE, Weinblatt ME, Blotner SD. Significant changes in serum AST across hepatic histological biopsy grades: prospective analysis of 3 cohorts receiving methotrexate therapy for rheumatoid arthritis. *J Rheumatol* 1996;23:459-61.
- Kremer JM. Safety, efficacy, and mortality in a long-term cohort of patients with rheumatoid arthritis taking methotrexate: followup after a mean of 13.3 years. *Arthritis Rheum* 1997;40:984-5.
- Salaffi F, Manganelli P, Carotti M, Subiaco S, Lamanna G, Cervini C. Methotrexate-induced pneumonitis in patients with rheumatoid arthritis and psoriatic arthritis: report of five cases and review of the literature. *Clin Rheumatol* 1997;16:296-304.
- Green L, Schattner A, Berkenstadt H. Severe reversible interstitial pneumonitis induced by low dose methotrexate: report of a case and review of the literature. *J Rheumatol* 1988;15:110-2.
- Hargreaves MR, Mowat AG, Benson MK. Acute pneumonitis associated with low dose methotrexate treatment for rheumatoid arthritis: report of five cases and review of published reports. *Thorax* 1992;47:628-33.
- Alarcon GS, Kremer JM, Macaluso M, et al. Risk factors for methotrexate-induced lung injury in patients with rheumatoid arthritis. A multicenter, case-control study. *Ann Intern Med* 1997;127:356-64.
- Morgan SL, Baggott JE, Vaughn WH, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med* 1994;121:833-41.
- Morgan SL, Baggott JE, Vaughn WH, et al. The effect of folic acid supplementation on the toxicity of low-dose methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum* 1990;33:9-18.
- Ortiz Z, Shea B, Suarez-Almazor ME, Moher D, Wells GA, Tugwell P. The efficacy of folic acid and folinic acid in reducing methotrexate gastrointestinal toxicity in rheumatoid arthritis. A metaanalysis of randomized controlled trials. *J Rheumatol* 1998;25:36-43.
- Weinblatt ME, Maier AL, Fraser PA, Coblyn JS. Longterm prospective study of methotrexate in rheumatoid arthritis: conclusion after 132 months of therapy. *J Rheumatol* 1998; 25:238-42.
- Bannwarth B, Pehourcq F, Schaefferbeke T, Dehais J. Clinical pharmacokinetics of low-dose pulse methotrexate in rheumatoid arthritis. *Clin Pharmacokinet* 1996;30:194-210.
- Seideman P. Methotrexate — the relationship between dose and clinical effect. *Br J Rheumatol* 1993;32:751-3.
- Lebbe C, Beyeler C, Gerber NJ, Reichen J. Intraindividual variability of the bioavailability of low dose methotrexate after oral administration in rheumatoid arthritis. *Ann Rheum Dis* 1994;53:475-7.
- Tugwell P, Pincus T, Yocum D, et al. Combination therapy with

- cyclosporine and methotrexate in severe rheumatoid arthritis. *N Engl J Med* 1995;333:137-41.
23. Calguneri M, Pay S, Caliskaner Z, et al. Combination therapy versus monotherapy for the treatment of patients with rheumatoid arthritis. *Clin Exp Rheumatol* 1999;17:699-704.
 24. O'Dell JR, Haire CE, Erikson N, et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. *N Engl J Med* 1996;334:1287-91.
 25. O'Dell JR. Triple therapy with methotrexate, sulfasalazine, and hydroxychloroquine in patients with rheumatoid arthritis. *Rheum Dis Clin North Am* 1998;24:465-77.
 26. Kremer JM. Rational use of new and existing disease-modifying agents in rheumatoid arthritis. *Ann Intern Med* 2001;134:695-706.
 27. Bensen W. Aim for remission or "personal best" using combination DMARD therapy with methotrexate and hydroxychloroquine. *Clin Exp Rheumatol* 1999;17 Suppl 18:S95-101.
 28. Khraishi MM, Singh G. The role of anti-malarials in rheumatoid arthritis — the American experience. *Lupus* 1996; Suppl 1:S41-4.
 29. Zvaifler NJ. Antimalarials in the treatment of rheumatoid arthritis. *Mod Treat* 1971;8:769-77.
 30. Richter JA, Runge LA, Pinals RS, Oates RP. Analysis of treatment terminations with gold and antimalarial compounds in rheumatoid arthritis. *J Rheumatol* 1980;7:153-9.
 31. Silman A, Shipley M. Ophthalmological monitoring for hydroxychloroquine toxicity: a scientific review of available data. *Br J Rheumatol* 1997;36:599-601.
 32. Shipley M, Silman A. Should patients on hydroxychloroquine have their eyes examined regularly? *Br J Rheumatol* 1997;36:514-5.
 33. Blyth C, Lane C. Hydroxychloroquine retinopathy: is screening necessary? *BMJ* 1998;316:716-7.
 34. May K, Metcalf T, Gough A. Screening for hydroxychloroquine retinopathy. Screening should be selective. *BMJ* 1998;317:1388-9.
 35. Tett S, McLachlan A, Day R, Cutler D. Insights from pharmacokinetic and pharmacodynamic studies of hydroxychloroquine. *Agents Actions* 1993;44 Suppl:145-90.
 36. Tett SE, Cutler DJ, Day RO, Brown KF. A dose-ranging study of the pharmacokinetics of hydroxy-chloroquine following intravenous administration to healthy volunteers. *Br J Clin Pharmacol* 1988;26:303-13.
 37. Tett S, Cutler D, Day R. Antimalarials in rheumatic diseases. *Baillieres Clin Rheumatol* 1990;4:467-89.
 38. Tett SE, Day RO, Cutler DJ. Concentration-effect relationship of hydroxychloroquine in rheumatoid arthritis — a cross sectional study. *J Rheumatol* 1993;20:1874-9.
 39. McLachlan AJ, Tett SE, Cutler DJ, Day RO. Bioavailability and in vivo dissolution of hydroxychloroquine in fed volunteers. *Br J Clin Pharmacol* 1993;36:405-11.
 40. McLachlan AJ, Tett SE, Cutler DJ, Day RO. Bioavailability of hydroxychloroquine tablets in patients with rheumatoid arthritis. *Br J Rheumatol* 1994;33:235-9.
 41. Tett SE, Cutler DJ, Day RO. Bioavailability of hydroxychloroquine tablets assessed with deconvolution techniques. *J Pharm Sci* 1992;81:155-9.
 42. Fries JF, Singh G, Lenert L, Furst DE. Aspirin, hydroxychloroquine, and hepatic enzyme abnormalities with methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1990;33:1611-9.
 43. Seideman P, Albertioni F, Beck O, Eksborg S, Peterson C. Chloroquine reduces the bioavailability of methotrexate in patients with rheumatoid arthritis. A possible mechanism of reduced hepatotoxicity. *Arthritis Rheum* 1994;37:830-3.
 44. Trnavsky K, Gatterova J, Linduskova M, Peliskova Z. Combination therapy with hydroxychloroquine and methotrexate in rheumatoid arthritis. *Z Rheumatol* 1993;52:292-6.
 45. Clegg DO, Dietz F, Duffy J, et al. Safety and efficacy of hydroxychloroquine as maintenance therapy for rheumatoid arthritis after combination therapy with methotrexate and hydroxychloroquine. *J Rheumatol* 1997;24:1896-902.
 46. McCrudden EA, Tett SE. Improved high-performance liquid chromatography determination of methotrexate and its major metabolite in plasma using a poly(styrene-divinylbenzene) column. *J Chromatogr B Biomed Sci Appl* 1999;721:87-92.
 47. Tett SE, Cutler DJ, Brown KF. High-performance liquid chromatographic assay for hydroxychloroquine and metabolites in blood and plasma, using a stationary phase of poly(styrene divinylbenzene) and a mobile phase at pH 11, with fluorimetric detection. *J Chromatogr* 1985;344:241-8.
 48. Chen ML, Lesko L, Williams RL. Measures of exposure versus measures of rate and extent of absorption. *Clin Pharmacokinet* 2001;40:565-72.
 49. Endrenyi L, Tothfalusi L. Truncated AUC evaluates effectively the bioequivalence of drugs with long half-lives. *Int J Clin Pharmacol Ther* 1997;35:142-50.
 50. Gaudreault J, Potvin D, Lavigne J, Lalonde RL. Truncated area under the curve as a measure of relative extent of bioavailability: evaluation using experimental data and Monte Carlo simulations. *Pharmacol Res* 1998;15:1621-9.
 51. Jackson AJ, Ouder Kirk LA. Truncated area under the curve as a measure of relative extent of bioavailability: evaluation using experimental data and Monte Carlo simulations. *Pharmacol Res* 1999;16:1144-6.
 52. Munster T, Furst DE. Pharmacotherapeutic strategies for disease-modifying antirheumatic drug combinations to treat rheumatoid arthritis. *Clin Exp Rheumatol* 1999;17 Suppl 18:S29-36.
 53. Bunch TW, O'Duffy JD, Tompkins RB, O'Fallon WM. Controlled trial of hydroxychloroquine and D-penicillamine singly and in combination in the treatment of rheumatoid arthritis. *Arthritis Rheum* 1984;27:267-76.
 54. Godfrey C, Sweeney K, Miller K, Hamilton R, Kremer J. The population pharmacokinetics of long-term methotrexate in rheumatoid arthritis. *Br J Clin Pharmacol* 1998;46:369-76.
 55. Seideman P, Muller-Suur R, Ekman E. Renal effects of low dose methotrexate in rheumatoid arthritis. *J Rheumatol* 1993;20:1126-8.
 56. Kremer JM, Petrillo GF, Hamilton RA. Pharmacokinetics and renal function in patients with rheumatoid arthritis receiving a standard dose of oral weekly methotrexate: association with significant decreases in creatinine clearance and renal clearance of the drug after 6 months of therapy. *J Rheumatol* 1995;22:38-40.
 57. Gardner G, Furst DE. Disease-modifying antirheumatic drugs. Potential effects in older patients. *Drugs Aging* 1995;7:420-37.
 58. Tett SE, Triggs EJ. Use of methotrexate in older patients. A risk-benefit assessment. *Drugs Aging* 1996;9:458-71.
 59. Bressolle F, Bologna C, Kinowski JM, Arcos B, Sany J, Combe B. Total and free methotrexate pharmacokinetics in elderly patients with rheumatoid arthritis. A comparison with young patients. *J Rheumatol* 1997;24:1903-9.
 60. Bressolle F, Bologna C, Kinowski JM, Sany J, Combe B. Effects of moderate renal insufficiency on pharmacokinetics of methotrexate in rheumatoid arthritis patients. *Ann Rheum Dis* 1998;57:110-3.