Comparison of Methods for Identifying Early Methotrexate-Induced Hepatotoxicity in Patients with Rheumatoid Arthritis

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Hepatotoxicity may complicate therapy with methotrexate in patients with rheumatoid arthritis. Prevention of cirrhosis may depend upon early identification of liver damage, usually accomplished by serial biopsy. To determine the adequacy of noninvasive methods for identifying hepatotoxicity, 22 sets of data were obtained in patients undergoing therapy with methotrexate for rheumatoid arthritis. Comparisons were made between liver biopsy, hepatocellular enzymes and two noninvasive radioisotopic methods that have been shown to be abnormal in hepatocellular disease: the rate constant of excretion of the ^{14}C-aminopyrine and the time from injection to peak hepatic activity of ^{99m}Tc-diisopropylimidodiacetic acid. The hepatocellular enzymes and the time-to-peak-activity of diisopropyliminodiacetic acid were not useful predictors of methotrexate-induced hepatotoxicity. The aminopyrine breath test was abnormal in approximately half the patients with hepatotoxicity but showed poor specificity. Noninvasive methods remain inferior to biopsy for the detection of mild to moderate methotrexate-induced hepatotoxicity in patients with rheumatoid arthritis.

J Nucl Med 1993; 34:1905–1909

Methotrexate (MTX) has been shown to be an efficacious drug in the management of many patients with rheumatoid arthritis (RA). However, MTX has been implicated as causal in a spectrum of hepatic injury, up to and including cirrhosis. Although rare with current low-dose regimens, the risk of severe liver damage and cirrhosis dictates that most RA patients treated with MTX be monitored closely for early signs of hepatotoxicity.

Liver biopsy has traditionally been the best method for the detection of MTX-induced liver injury. Because of the invasive nature of a biopsy and the poor predictive value of hepatocellular enzymes, other noninvasive tests have been considered for identifying early hepatotoxicity in RA patients on MTX. In this study, we compared the value of liver biopsy to three different noninvasive modalities for the detection of mild hepatotoxicity in RA patients treated with MTX. The noninvasive tests included serum levels of the hepatic aminotransferases AST and ALT, the extraction efficiency of ^{99m}Tc-diisopropylimidodiacetic acid (DISIDA) and the rate of demethylation of ^{14}C-aminopyrine. Both of the latter methods have previously been shown to be reliably abnormal in patients with hepatocellular disease (*1–5*).

Received April 10, 1992; revision accepted July 18, 1993.

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MATERIALS AND METHODS

Twenty-two sets of comparisons were performed in 16 patients with RA over a 4-yr interval. The patients were receiving weekly low-dose MTX therapy and none of the patients were suspected of having significant hepatotoxicity. Each comparison included liver histology from a percutaneous biopsy, aminopyrine breath test, an index of the hepatic transit of DISIDA, and determination of the serum levels of hepatocellular enzymes. All studies in a given comparison were obtained within 48 hr of each other. Bayesian analysis of the data was performed on completion of the study.

Biopsy

All liver biopsies were stained with hemotoxylin and eosin, trichrome, reticulin, iron, periodic acid Schiff and diastase and were interpreted by a pathologist. Liver biopsies were graded from normal to Grade V according to a modification of the Roenigk classification criteria (*6,7*). By this modification, a normal biopsy has its own classification distinct from Grade 1. The Roenigk classification system evaluates liver histology based on fatty infiltration, nuclear variability, portal inflammation, portal tract expansion, fibrosis and cirrhosis. Characteristics of the classification system used in this study are listed in Table 1.

Aminopyrine Breath Test

With informed consent and approval by the Institutional Review Board, the aminopyrine breath test (ABT) was performed within 48 hr of liver biopsy. The content of $^{14}C\text{-}CO_2$ in a standard aliquot of exhaled breath was measured at 1, 2, 3, 4, 6, 8 and 10 hr after the oral administration of ^{14}C-aminopyrine, as previously described (*1,8*). By applying linear regression to the data, the rate constant of excretion (Kb) for ^{14}C-aminopyrine was determined

TABLE 1
Modified Roenigk Classification for Hepatic Injury

Grade	Histologic findings
Normal	Normal
Grade I	Mild fatty infiltration
	Mild nuclear variability
	Mild portal inflammation
Grade II	Moderate to severe fatty infiltration
	Moderate to severe nuclear variability
	Moderate to severe portal inflammation
	Necrosis
	Portal tract expansion
Grade IIIA	Mild fibrosis
	Fibrotic septae extending to the lobules
Grade IIIB	Moderate to severe fibrosis
Grade IV	Frank cirrhosis

for each patient. For the ABT, the normal reference range for the Kb is 16.8%–30.9% per hour (*2*). Prior to evaluating patients, our technique was validated by performing ABTs in five normal volunteers. The Kb for each of the five subjects was within this published range (data not shown).

Time-to-Peak-Activity of DISIDA

The time from injection to peak hepatic activity of ^{99m}Tc-DISIDA (TPD), the most commonly used biliary agent in nuclear medicine, was determined in each patient at the same time as the ABT. Informed consent and approval by the Institutional Review Board were obtained for the TPD. By a simple published method, each patient was injected intravenously with 2–3 mCi of ^{99m}Tc-DISIDA, and the TPD was determined by computer-assisted region-of-interest (ROI) analysis applied to serial scintigraphic images of the liver (*4,5*). By this method, the upper limit of normal (2 s.d.) is 16.3 min (*4*).

Hepatocellular Enzymes

Serum levels of hepatocellular aminotransferases were determined for each patient prior to liver biopsy. Measurements of both AST and ALT (formerly SGOT and SGPT, respectively) were made. The normal serum values for these enzymes at our institution are 9–32 U/liter (AST) and 7–55 U/liter (ALT).

RESULTS

A total of 22 sets of data were obtained from 16 patients over a 4-yr interval. The average and median duration of therapy was 3.35 yr (range 6 mo–8 yr). The average age of the patients was 54 yr (range 32–72 yr). Results of liver biopsy, ABT, TPD and hepatocellular enzymes are shown in Table 2. An analysis of this data is shown in Table 3.

The average duration of MTX therapy was 4.2 yr for patients with normal biopsy, 3.6 yr for those with Grade I biopsy, 2.9 yr for Grade II biopsy and 2 yr for Grade III liver biopsy. Therefore, the risk of an abnormal biopsy in this group did not increase with a longer duration of therapy. Those patients with the shortest duration of therapy were actually those with the most severe liver abnormalities on biopsy, although this inverse relationship was not statistically significant.

Hepatocellular enzymes were normal in 20 of the 22 cases. The two abnormal cases were in patients with Grade II and Grade III biopsies. Therefore, the sensitivity for the hepatocellular enzymes was only 10.5%, but the specificity was 100%. The ABT correctly identified nearly half of the patients with abnormal biopsies with a sensitivity of 47.4%. The abnormal values were distributed among patients with all grades of abnormal biopsies. However, the specificity for the ABT was poor, at 33%. The positive predictive values for an abnormal result were 100% and 81.85%, respectively, for the hepatocellular enzymes and the ABT. However, the negative predictive values for both the hepatocellular enzymes and the ABT in this series were very low (≤15%).

The TPD was within normal published limits for 21 of the subjects examined and was therefore the least discriminating of the tests. The test was only abnormal in a single patient (one with a Grade II biopsy). Therefore, the sensitivity for the TPD was only 5.3% for the detection of an abnormal biopsy.

As shown above, each of the noninvasive tests of liver function (hepatocellular enzymes, ABT, TPD) was evalu-

TABLE 2
Results of Methodologies

Liver biopsy	Patients (no.)	Duration of therapy (yr)	HE* (nl:abn)§	ABT Kb† (nl:abn)	TPD‡ (nl:abn)
Normal	3	4.2	3:0	1:2	3:0
Grade I	10	3.6	10:0	5:5	10:0
Grade II	8	2.9	7:1	4:4	7:1
Grade III	1	2	0:1	0:1	1:0

*Hepatocellular enzymes (ALT, AST)—elevation of one or both constitutes an abnormal result. Upper limits of normal: ALT = 7–55 U/liter, AST = 9–32 U/liter.

†Carbon-14-aminopyrine breath test (normal range 17%–32%/hr).

‡Time from injection to peak hepatic activity of ^{99m}Tc-DISIDA (upper limit of normal = 16.3 min).

§Normal to abnormal (nl:abn).

TABLE 3
Analysis of Data

	HE*	ABT†	TPD‡	TPD or HE§
True-positive	2	9	1	4
False-positive	0	2	0	0
True-negative	3	1	3	3
False-negative	17	10	18	15
Sensitivity	11%	47%	5%	21%
Specificity	100%	33%	100%	100%
Accuracy	23%	45%	18%	32%
Positive predictive value	100%	82%	100%	100%
Negative predictive value	15%	9%	14%	17%
1 – Negative predictive value¶	85%	91%	86%	83%

*Hepatocellular enzymes (ALT, AST)—elevation of one or both constitutes an abnormal result.
†Carbon-14 aminopyrine breath test.
‡Time from injection to peak hepatic activity of ^{99m}Tc-DISIDA.
§Results of other combinations of tests: Either abnormal ABT or HE: same as for HE alone; either abnormal ABT or TPD: same as for TPD alone; either abnormal ABT, HE or TPD: same as for ABT alone; both abnormal ABT and HE: same as for ABT alone; and both abnormal ABT and TPD: same as for ABT alone.
¶Post-test likelihood of abnormal biopsy with a negative (normal) result.
Total number of patients = 22; number of patients with abnormal biopsy = 19; and number of patients with normal biopsy = 3.

ated individually. In addition, various combinations of the tests were also evaluated to determine whether some combination might optimize the identification of patients with hepatic injury. As shown in Table 3, no combination of the three noninvasive tests resulted in an improvement over those considered individually.

DISCUSSION

The folate vitamins are a class of essential co-factors with one-carbon groups. These groups are required for the synthesis of both purines and thymidilic acid, both of which are essential for DNA synthesis and for cell division. Thus, in the design of agents to inhibit DNA synthesis, folic acid and its derivatives are logical targets. MTX is a 4-NH_2, N-10 methyl analog of folic acid and is the most widely used anti-folate metabolite. In addition to being a folate analog, MTX may also inhibit the enzyme dihydrofolate reductase, a critical enzyme in the conversion of inactive to active forms of folic acid.

MTX is a widely used anti-metabolite with a role in the treatment of such diverse diseases as cancer and rheumatologic abnormalities and as an immunosuppressive agent in bone marrow transplants. The various applications of MTX are outlined in a review by Jolivet (*9*). In high doses, MTX plays a role in the therapy of leukemias, non-Hodgkins lymphoma, osteosarcoma, choriocarcinoma, head and neck carcinomas, breast carcinoma and ovarian cancer. MTX is used in bone marrow transplants for the suppression of graft versus host reaction. It is a therapeutic alternative in the treatment of severe psoriasis and is also used in the treatment of various rheumatic diseases.

Hepatic injury has previously been a limiting factor in patients receiving MTX. Hepatotoxicity from MTX was first described by Colsky in 1955, although it was not until the mid 1970s that the recognition of MTX-induced hepatotoxicity became firmly established (*10–12*). The histologic pattern of MTX-induced hepatotoxicity includes macrovesicular steatosis and hepatocellular necrosis with predominantly portal and periportal inflammation. The development of periportal, portal-central and portal-portal bridging fibrosis may herald the development of cirrhosis (*13*).

Initial studies suggested that fibrosis and cirrhosis could develop in up to 25% of individuals treated more than 5 yr or after cumulative doses of greater than 2 g (*13*). However, subsequent studies showed that histologic liver injury may be related to the type of underlying disease as well as the frequency of MTX dosing (*14*). Patients with psoriasis are at greater risk for MTX-induced hepatic injury than those with RA (*11,14*). Recently, long-term treatment of rheumatoid arthritis with weekly low doses of MTX has become popular in the United States and Europe. The low dose regimen may greatly reduce the risk of hepatotoxicity in patients with RA. Prospective trials using small (7.5–25 mg) MTX doses at weekly intervals were significant for a decrease in the development of cirrhosis in both psoriatic patients as well as those with RA (*15,16*). In fact, no study has conclusively demonstrated a significant risk of cirrhosis in patients with RA with weekly pulse doses of MTX below 15 mg.

Despite the decreased risk of cirrhosis in RA patients on low dose MTX, these patients can have histologic findings of liver injury on biopsy. These changes are usually mild, and include fibrosis, fatty change, nuclear variability and other morphologic abnormalities. To some degree, these abnormalities may be due to the underlying disease (*16,17*). However, there is some evidence that these underlying abnormalities may be worsened by MTX (*18*). Therefore, most patients receiving even low-dose MTX are monitored closely for signs of hepatic injury. When these

signs are present, many physicians will discontinue therapy rather than risk a small chance of cirrhosis.

Hepatocellular enzymes may be elevated in up to 48% of patients with RA receiving MTX therapy (*7*). Elevations in hepatocellular enzymes, however, have not been shown to correlate with histologic evidence of liver injury (*13*). Normal transaminase levels are often present with abnormal liver biopsies and, conversely, aminotransferase levels can be elevated without histologic evidence of hepatic injury (*13*). The best serologic predictor for the development of MTX-induced hepatic fibrosis is the development of hypoalbuminemia. However, this is a late finding of hepatic injury (*13*). In our series, an elevation in hepatocellular enzymes was only seen in 2 of 22 patients with RA who had either Grade II or III biopsies. The determination of serum aminotransferase levels was therefore of insufficient sensitivity to be a useful predictor of mild hepatocellular disease in RA patients treated with MTX.

The ABT has been efficacious in identifying the severity of disease in patients with cirrhosis and chronic hepatitis, and in predicting surgical risk in patients with known liver disease (*19–21*). The technique has also been used to monitor hepatotoxicity in patients treated with MTX. In psoriatics, the ABT has proved to be ineffective in identifying early MTX-induced liver disease (*22*). However, in a single study (Ytterberg et al.) evaluating the efficacy of ABT in RA patients treated with MTX, results were inconclusive, possibly because these patients were treated for less than 2 yr and had minimal histologic liver abnormalities by biopsy (*23*). Nonetheless, the ABT identified no patients in the Ytterberg group as being falsely abnormal.

There are several differences between our study and that of Ytterberg et al: the average length of treatment for patients in our study is longer, at 3.35 yr; a greater percentage of our subjects had abnormal biopsies; and 41% of our subjects had biopsy abnormalities of Grade II or higher. (However, within this group, there was no direct correlation between length of treatment and severity of biopsy finding.) We also used the Kb for excretion of aminopyrine, rather than the percent of excretion at 2 hr as used by Ytterberg et al. We have found the 2-hr value to be more prone to false-positives than the Kb, possibly as a result of differences in gastric emptying that might affect absorption and early excretion (unpublished results). Although the study of Ytterberg et al. found a low false-positive rate with the ABT, two of the three patients in our series with normal biopsies had abnormal ABTs. The ABT correctly identified only 9 of 19 patients with abnormal biopsies. Therefore, in our series, the ABT lacked both sensitivity and specificity for identifying patients with early hepatocellular abnormalities.

The TPD has not been previously tested for identifying hepatic injury in patients receiving MTX. However, it has been shown to be abnormal in patients with noncalculous partial obstruction of the cystic duct and from hepatocellular disease from a variety of etiologies (*4,5*). In our series, all patients except for one (a true-positive) had values within the published normal range. Therefore, the TPD does not appear to be useful as a predictor of hepatocellular disease in RA patients treated with MTX.

We conclude that serum levels of hepatic aminotransferases and the time to peak activity of DISIDA are not useful for predicting MTX-induced hepatotoxicity in patients with RA. The aminopyrine breath test may be marginally useful for predicting MTX-induced histologic changes, with a sensitivity of 47% in our series. However, a normal ABT was not useful for excluding MTX-induced liver changes. Liver biopsy may be invasive but it remains the most accurate method for detecting mild-moderate histologic liver abnormalities in RA patients on MTX. Although a less invasive or noninvasive method for detecting early hepatotoxicity would be desirable, our results suggest that the ABT, time-to-peak activity of DISIDA, and the serum levels of hepatic aminotransferases, either alone or in combination, do not represent this alternative.

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ERRATUM

Due to a production error, Figure 3 in the article "Nitrates Improve Detection of Ischemic but Viable Myocardium by Thallium-201 Reinjection SPECT" by He et al. (J Nucl Med 1993;34:1472–1477) was printed incorrectly. The corrected figure is shown below.

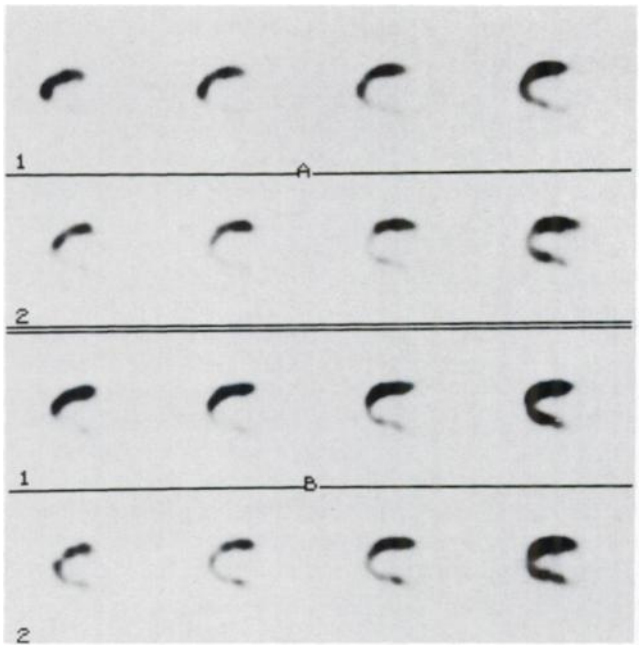

FIGURE 3. Example of a 73-yr-old male patient with coronary artery disease but without myocardial infarction (50% LAD, 50% LCx and 80% RCA stenoses). Protocol A: exercise SPECT images (A1) showed an inferior defect which was fixed on delayed imaging with reinjection alone (A2). Protocol B: exercise SPECT images (B1) also showed an inferior defect which completely normalized on delayed imaging with nitrates and reinjection (B2).