

Navajo Neuropathy: Relation to MDR3 mRNA Deficiency

To the Editor:

Vu et al. (HEPATOLOGY 2001;34:116-120) recently showed depletion of mitochondrial DNA in liver from 2 patients with Navajo neuropathy (NN) and postulated that the primary defect is in nuclear regulation of mitochondrial DNA copy number.

In 1997, we proposed that liver disease in children with NN may result from MDR3 deficiency (HEPATOLOGY 26(suppl):369A). Northern analysis of RNA from the liver of an affected child revealed severe reduction in MDR3 messenger RNA (mRNA) abundance (Fig. 1A), as did reverse-transcription polymerase chain reaction (RT-PCR) analysis of liver biopsy samples from 3 other affected children. mRNAs for other canalicular transporters, including MDR1 and MRP2, showed no difference from normal controls. The relation between reduced MDR3 expression and the neurologic symptoms of NN was unclear. However, Northern analysis indicated that MDR3 is expressed in multiple areas of human brain (Fig. 1B). Mdr2, which is the murine form of MDR3, is expressed in the developing brain of normal mice, but is not detected in the brain or liver of *mdr2*^{-/-} mice. These findings suggested that MDR3 dysfunction may also contribute to neurologic aspects of NN.

To further assess whether MDR3 deficiency is a causative factor, we attempted to identify a mutation in the MDR3 gene that could account for reduced expression in NN. Although MDR3 message was severely depleted in the liver samples, a weak signal was evident by Northern and RT-PCR analysis, indicating the presence of MDR3 mRNA. Full-length complementary DNA clones were assembled from RT-PCR products generated from a patient's liver RNA. Sequence analysis of these clones failed to identify a mutation in the MDR3 mRNA, however, we could not exclude mutations in nontranscribed sequences. To determine whether mutations in MDR3 transcriptional regulatory sequences are associated with decreased mRNA levels, we obtained genomic DNA from 1 NN patient. Sequence was determined from 2.5 kb upstream of the transcriptional start site through exon 1, from the 28 MDR3 exons, and from ~100 to 300 nucleotides flanking each exon. No mutations were identified in these regions when the sequence of the patient was compared with that of 64 unaffected controls.

These data do not exclude MDR3 as a possible causative factor in NN. Reduced levels of MDR3 expression may result from mutations in the interior of introns, more distal regions of the promoter, or a cis-acting regulatory gene located elsewhere in the genome. Alternatively, selective reduc-

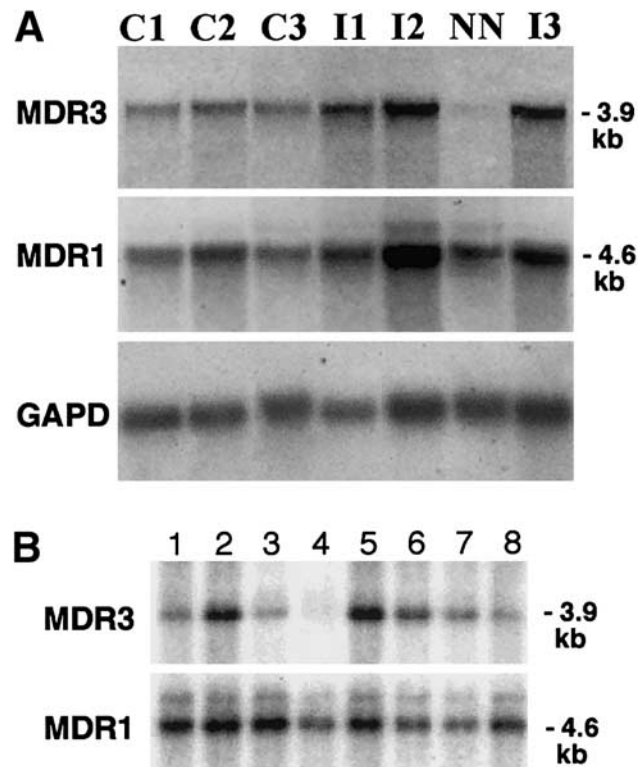


Fig. 1. Expression of MDR3 mRNA in NN liver and normal central nervous system. (A) Autoradiographs of a Northern blot containing polyA⁺ RNA isolated from livers of normal transplant donors (C1-C3) and children suffering from intrahepatic cholestasis (impaired bile secretion) (I1-I3) or NN (NN). The blot was hybridized with an MDR3-specific linker domain probe, stripped, and subsequently hybridized with MDR1 linker, or glyceraldehyde phosphate dehydrogenase probes. (B) Autoradiographs of a Northern blot (Clontech, Palo Alto, CA) containing RNA from the following adult human central nervous system regions: (1) cerebellum, (2) cortex, (3) medulla, (4) spinal cord, (5) occipital pole, (6) frontal lobe, (7) temporal lobe, (8) putamen. The blot was sequentially hybridized with MDR3- and MDR1-specific probes at high stringency. The different expression patterns and sizes of the transcripts indicate that the MDR3 signal is not caused by cross-hybridization of the MDR3 probe with MDR1 mRNA.

tion in MDR3 expression may be secondary to liver damage caused by the primary defect. The search goes on.

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The Effects of Portal Shunts on Intestinal Cytochrome P450 3A Activity

To the Editor:

The selective effects of liver disease on cytochromes P450 involved in human drug metabolism in both the liver and the intestine are poorly defined, and Chalasani et al.¹ are to be commended for clarifying many of the issues with respect to cytochrome P450 3A (CYP3A). We would like to add some further observations based on their data.

Chalasani et al.¹ reported a greater intestinal availability of midazolam (F_G) in cirrhotic patients with transjugular intrahepatic portosystemic shunts (TIPS) compared with cirrhotic patients without such shunts and healthy controls. They indicated that this might be a result of decreased intestinal CYP3A activity or a faster absorption of midazolam, thereby minimizing its contact time with the intestinal enzyme. Reference to reports of an increased cardiac output and splanchnic blood flow in patients with TIPS would support the latter explanation. However, Chalasani et al. considered that a faster drug absorption rate was unlikely for 2 reasons. First, 2 patients in the TIPS group did not require revision of their shunt, but their F_G values were comparable with those of the rest of the group. Second, there was no difference in the time to maximum plasma drug concentration (t_{max}) between the cirrhotic patients with and without TIPS.

We suggest that the data of Chalasani et al.¹ might, in fact, support a mechanism involving decreased contact with intestinal CYP3A secondary to an increase in splanchnic blood flow. Large interpatient variability in intestinal CYP3A content is well documented,²⁻⁴ and similar F_G values in TIPS patients with and without a requirement for revision may not necessarily reflect the difference in splanchnic blood flow. The t_{max} value has a discrete nature. It depends on sampling time, and the metric is considered to provide a poor measure of drug absorption rate.⁵⁻⁷ More robust measures of the latter include the partial area under the plasma drug concentration – time curve (AUC) up to t_{max} ^{5,6} and the initial slope of the plasma drug concentration.⁷ An assessment of the data presented in Fig. 3 of Chalasani et al.¹ indicates a 2- to 4-fold faster drug absorption rate in TIPS patients compared with the control cirrhotic patients and healthy subjects, based on estimates of AUC (0-15 minutes) and initial slope (ratio of concentrations at 5 minutes). These differences cannot reflect differences in drug elimination rate as net clearance, and elimination half-life was similar in the 2 groups of cirrhotic patients after intravenous administration of midazolam.

A number of proposals have been made with regard to the mathematical modeling of intestinal drug metabolism (and

associated drug – drug interactions).⁸⁻¹¹ In the absence of applicable model parameters in humans for this complex process, we have recently developed an operational model of the gut “first-pass” effect analogous to that of the “well-stirred” liver.¹²⁻¹³ Thus, the fraction of drug escaping first-pass metabolism in the intestinal wall (F_G) is given by

$$F_G = 1 - E_G = 1 - \frac{CL_{int}}{Q' + CL_{int}}$$

in which E_G is the fraction of the amount of drug entering the enterocyte that is metabolized; CL_{int} is the intrinsic clearance by metabolism in the enterocyte (a direct measure of enzyme activity and capacity, V_{max}/K_m); and Q' is a hybrid parameter reflecting drug absorption rate from the gut lumen, drug transfer to splanchnic blood, volume of enterocytes, etc. Thus, in contrast to the “well-stirred” liver model, the associated flow term (Q') is nominal and does not refer to actual enterocytic blood flow. A value of Q' can be obtained by rearranging the equation when the other parameters are known. Accordingly, it has been estimated at 20 L/h, knowing the abundance of intestinal CYP3A and the K_m and V_{max} values of midazolam metabolism by the enzyme ($CL_{int} = 30$ L/h).^{12,13} A 2- to 4-fold increase in midazolam absorption rate, as indicated by the data of Chalasani et al.,¹ would increase the value of Q' by the same extent, thereby decreasing E_G from 0.6 to 0.3 to 0.4 and increasing F_G to 0.6 to 0.7. The average value of F_G observed in TIPS patients by Chalasani et al.¹ was 0.8. Thus, it seems reasonable to assume that most of the change in apparent intestinal drug metabolism occurs as a result of a faster removal of drug from the vicinity of the enzyme by an increased splanchnic blood flow.

These findings have implications for the use of *in vitro* cell systems (e.g., Caco-2 cells) to assess quantitative aspects of intestinal drug metabolism and transport. Addition of albumin to the basolateral aqueous reservoir has been shown to decrease the extent of midazolam metabolism, presumably by acting as a sink to decrease drug contact with the enzymes.¹⁴ Blood flow would have the same effect, suggesting that such *in vitro* systems might be more predictive of *in vivo* events if they utilized dynamic rather than static (nonsink) conditions.

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Reply:

We recently reported in *HEPATOLOGY*¹ that cirrhotic patients with TIPS displayed an almost complete loss of

effective gut wall CYP3A metabolism. Rostami-Hodjegan and Tucker have analyzed our mean concentration versus time curves for midazolam following oral administration and suggest that increased splanchnic blood flow is responsible for the increase in gut wall availability (F_G) that we observed. This conclusion was reached by considering a simple model for F_G , as described by Tozer² and Wachter et al.³ and modified by Yang et al.⁴:

$$F_G = Q'/(Q' + CL_{INT})$$

where CL_{INT} is the intrinsic clearance associated with gut wall metabolism. In this model, Q' does not refer to the actual physiologic blood flow. Rather, it represents a hybrid function combining the absorption rate from the intestinal lumen to enterocytes, the transfer rate to blood, the splanchnic blood flow, volume of enterocytes, etc.⁴ Moreover, Q' can be defined in terms of actual blood flow at the site of absorption

$$Q' = \frac{Q \cdot A}{Q + A}$$

where Q is the true splanchnic blood flow and A is a permeability constant that is determined by the aforementioned physicochemical factors (*i.e.*, absorption rate into enterocytes, transfer rate to blood, etc.). It follows, therefore, that true blood flow cannot be equated with Q' , as asserted by Rostami-Hodjegan and Tucker, unless absorption is blood flow limited ($A \gg Q$).

We would like to address two issues that will help clarify the conclusions presented by Rostami-Hodjegan and Tucker. First, F_G of midazolam averaged 0.42 in healthy volunteers and 0.32 in cirrhotic patients without TIPS in our study.¹ This parameter was increased to 0.83 in cirrhotic patients with portosystemic shunts. This roughly 2-fold increase of F_G , from 0.4 to 0.8, requires Q' to increase from 20 L/h to 120 L/h, assuming $CL_{INT} = 30$.⁴ Thus, the assertion that the observed effect primarily reflects "an increased splanchnic blood flow" would require a 6-fold or greater increase in blood flow in patients with TIPS relative to healthy volunteers. This is plainly an unlikely physiologic response to TIPS. Cardiac output and portal vein blood flow increase about 50% and 127%, respectively, in patients after TIPS placement,⁵ but these flow changes would have a modest effect on F_G .

Second, we have analyzed the individual patient data sets in the fashion recommended by Rostami-Hodjegan and Tucker (*e.g.*, AUC from 0 to T_{MAX} and the initial slope of the concentration vs. time curve). The correlations between these metrics and F_G were not good ($r^2 < 0.3$). The mean of the AUC to T_{MAX} was $3.3 \pm 2.0 \text{ ng} \cdot \text{h} \cdot \text{mL}^{-1}$ in the cirrhotic patients with TIPS compared with 2.1 ± 1.5 in the cirrhotic controls

and 1.4 ± 1.3 in the healthy volunteers. The initial slope value (0-15 minutes) was higher ($P < .05$) in the cirrhotic patients with TIPS ($123 \pm 115 \text{ ng} \cdot \text{h}^{-1} \cdot \text{mL}^{-1}$) compared with the cirrhotic controls (64 ± 30) and the healthy volunteers (55 ± 30). Presumably, both of these surrogates for the rate of drug absorption should vary in proportion to the changes in Q' . However, the magnitude of these differences was small. Clearly, the 2- to 3-fold differences observed do not correspond to the 6-fold increase required for Q' in the Rostami-Hodjegan and Tucker analysis. As we previously concluded, the most likely cause for the increase in F_G in cirrhotic patients with TIPS was a decrease in intestinal CYP3A activity. Similar changes in F_G have been reported in studies of CYP3A inhibition by clarithromycin⁶ and by grapefruit juice⁷ in healthy volunteers.

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Long-Term Effect of Treatment of Acute Budd-Chiari Syndrome With a Transjugular Intrahepatic Portosystemic Shunt

To the Editor:

In the January 2002 issue of *HEPATOLOGY*, Perello et al. reported on 13 patients with Budd-Chiari syndrome (BCS) who were treated with a transjugular intrahepatic portosystemic shunt (TIPS). In only 3 patients TIPS was patent without any dysfunction after 3, 6, and 12 months. In addition, there were 7 patients with stenosis of their TIPS. Reestablishment of shunt patency was necessary in 2 patients and in 5 patients stenosis was not corrected. In one patient a surgical portocaval shunt was inserted because of early stenosis of the TIPS. Another patient received a liver transplant 2 years after TIPS. So TIPS patency was ultimately maintained in 5 patients with a maximum follow-up of 80 months.¹

BCS is a rare clinical disorder caused by hepatic venous outflow obstruction.² There are 4 different treatment options: medical therapy, surgical portal decompression, TIPS, and liver transplantation.³ However, medical ther-

apy is only an option for the milder cases. For the more severe cases one has to choose from surgical decompression, TIPS, and liver transplantation. After TIPS or surgical decompression, liver transplantation might still be necessary. Mortality rate after liver transplantation is 20% in the first year (www.eltr.org). In relation to liver transplantation in the acute phase of BCS, one has to realize that the underlying disease, for example polycythaemia vera, is not yet treated, thereby creating a risk for thrombotic complications like hepatic artery thrombosis or recurrence of BCS. So on theoretical grounds portal decompression is to be preferred. In addition when comparing TIPS with surgical shunts, TIPS will not hamper future liver transplantation.⁴ Although we also recognized the occurrence of TIPS dysfunction as a major disadvantage, in clinical practice this mostly means once in a year an angiography. Furthermore the incidence of TIPS dysfunction might be expected to reduce with the newer Gore TIPS.⁵

In the period of 1996 to 2002 we have seen 6 patients with BCS all secondary to polycythaemia vera. One received a mesocaval shunt because of thrombosis of the jugular vein and another was treated conservatively. Four patients received a TIPS. Two patients developed stenosis of the TIPS but patency was not corrected because both patients were asymptomatic due to intrahepatic recanalization. The other two still have a patent TIPS although both have had stenosis of the TIPS. They are in good clinical condition 72 months after TIPS insertion, resumed a normal life and are not on a liver transplant waiting list.

In the literature there are only a few reports about the use of TIPS in the treatment of BCS. In accordance with Perello et al., we want to emphasize the beneficial long-term effect of TIPS in BCS. We believe that in patients with BCS the use of TIPS should be considered first, because it is effective in the preservation of liver function and a good quality of life with a low risk of mortality. However, because of the rarity of the disease, clinical trials can probably never be performed. All one-center studies will in fact remain a collection of case reports. Therefore we would like to plead for a European or world-wide registry of the use of TIPS in BCS.

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Serum Versus Intrahepatic HCV RNA and Liver Histology

To the Editor:

The study by Barrett et al.¹ shows the absence of hepatitis C virus (HCV) RNA in frozen liver tissue of 30 women positive for anti-HCV antibodies confirmed by recombinant immunoblot assay (RIBA; 4 RIBA indeterminate) but negative for HCV RNA in serum. This seems to contradict our own results² and those of Haydon et al.³ We have analyzed several matching paraffin-embedded liver biopsy specimens of patients included in the study of Barrett et al. by the nested reverse-transcription polymerase chain reaction (RT-PCR) method for formalin-fixed tissue established by us. Except for a blinded control, all of them tested negative, confirming the results presented. After scrutinizing all clinical and histologic data given in the study we even estimate the negative tissue RT-PCR for HCV RNA in good accordance with our own study for the following reasons: Of the 33 serum PCR-negative women, 91% had no or minimal inflammation (histologic activity index [HAI] 0-3). Except for the presence of lymphofollicular portal infiltration, such low grades of inflammation cannot be considered suggestive of chronic hepatitis C, a fact that is also emphasized by the authors.

Only 2 patients had significant portal inflammation (HAI portal score 2), 1 with histopathologic diagnosis of steatohepatitis, the other with granuloma, excessively elevated body mass index, and indeterminate RIBA. Both patients belonged to the group of 3 patients with a mild grade of inflammatory activity (HAI 5 and 6), the third patient also having steatohepatitis and being obese. Only 5 of 33 patients had elevated alanine transaminase levels, 3 with histologically established steatohepatitis, 1 being obese, and 1 with HAI 0, no fibrosis, and indeterminate RIBA. Twenty-eight patients had no fibrosis at all, most of them more than 20 years after infection, whereas 4 of the remaining 5 patients with only minimal fibrosis were obese, had steatohepatitis, or grade 3 fatty liver. Taking these data into account, there is not a single patient left actually suspicious of having chronic hepatitis C. So the study confirms that complete and lasting elimination of HCV is possible, a fact that is important when considering such patients in the context of organ transplantation donors, and for immunosuppression or chemotherapy in case of later disease. In contrast, all serum HCV-RNA negative patients included in our study² had either elevated alanine

transaminase levels or histology suggestive of hepatitis C, and only 2 of 28 patients testing positive for HCV RNA in liver tissue had minimal HAI (score 2 and 3). Therefore, the data from our own study and those from the study of Barrett et al. are compatible.

In contrast we have serious objections regarding 2 main conclusions by the authors, stating that “negative serum PCR status appears to reflect cleared past-exposure in liver” (abstract) and “neither liver histology nor PCR testing of liver tissue is necessary to diagnose active HCV infection” (discussion).¹

First of all, only women infected by anti-D rhesus prophylaxis were investigated, a unique group of patients who have been shown in several studies to differ from the typically heterogeneous group of hepatitis C patients in many aspects. No general conclusions can be drawn on such a restricted basis. For this reason, the type of patient cohort investigated should have been mentioned in the abstract. Furthermore, the statement that negative serum PCR reflects cleared infection is doubtlessly contradicted by thousands of HCV-RNA negative end-of-treatment responders who relapse during the following 24 weeks and by alternative detection methods of superior sensitivity.⁴

Furthermore, we disagree with the statement that “neither liver histology nor PCR testing of liver tissue is necessary to diagnose active HCV infection.”¹ There is no doubt that the diagnosis of hepatitis C is made by serologic testing, but active infection can only be determined by liver biopsy procedure with the additional advantage that alternative liver disease can be evaluated or excluded (as is shown by the present study). We would like to stress that in 21 of 33 liver biopsy specimens evaluated by Barrett et al., relevant information in diagnostic, therapeutic, and prognostic respect was rendered (4 symptomatic patients had steatohepatitis, 1 patient had grade 3+ hemosiderosis, and 16 of 20 symptomatic patients could be proven

free of relevant chronic liver disease [no fibrosis]), which again clearly underlines the usefulness of liver biopsy.

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