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Metadoxine accelerates fatty liver recovery in alcoholic patients: results of a randomized double-blind, placebo-control trial

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Background/Aims: **Our aim was to investigate the effectiveness of metadoxine (pyridoxol L, 2 pyrrolidone-5-carboxylate) in the treatment of alcoholic fatty liver.**
Methods: **A double-blind randomized multicenter trial involving 136 chronic active alcoholic patients diagnosed with fatty liver by clinical, biochemical and ultrasonographic criteria was performed. Patients were treated with 1500 mg/day of metadoxine ($n=69$) or placebo ($n=67$) for 3 months. Patients were clinically and biochemically evaluated every month. Ultrasonography was performed before and after treatment.**
Results: **At the end of the study there was a significant improvement in the liver function tests in both groups. However, the changes were more rapid and greater in patients treated with metadoxine, in whom significant changes in serum levels of bilirubin, aminotransferases and gammaglutamyl transpeptidase were already observed after 1 month of treatment, and normalization of these parameters was observed at the end. After treatment, the percentage of patients with ultrasonographic signs of steatosis was significantly lower in the metadoxine group (28% vs 70%, $p<0.01$) and the degree of steatosis was also lower in this group. Sixteen patients treated with metadoxine and 15 with placebo continued drinking. Alcohol intake was lower than initially, and similar in both groups. In the metadoxine group, the biochemical changes were similar in both the abstinent and the nonabstinent patients. In contrast, in the placebo group the improvement in the liver function tests was significantly higher in abstinents. Among patients who continued drinking, the prevalence (45% vs 92%, $p<0.05$) and the degree of steatosis were also significantly lower in patients treated with metadoxine.**
Conclusions: **In patients with alcoholic fatty liver, metadoxine accelerates the normalization of liver function tests and the ultrasonographic changes, even in those who do not completely abstain from alcohol intake. Thus, metadoxine could be useful in the treatment of the early stages of alcoholic liver disease.**

Key words: **Alcoholic liver diesease; Metadoxine; Steatosis; Ultrasonography.**

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FATTY LIVER occurs very frequently after the ingestion of moderate to large amounts of ethanol; the prognosis is usually good when fatty liver is not associated with other liver lesions. In fact, it is considered that with alcohol abstinence and an adequate diet the

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accumulation of fat may disappear from the liver within a few weeks (1), although several studies have suggested that alcoholic fatty liver may progress to cirrhosis (2–4). However, these latter studies included patients with some degree of liver fibrosis, which is recognized as a precursor of cirrhosis; Worner & Lieber (4) showed that the patients with steatosis and perivenular fibrosis were those who progressed to cirrhosis. A recent long-term prospective study including a large number of patients with "pure" alcoholic fatty liver demonstrated that nine out of 88 patients developed cirrhosis and seven others fibrosis, the features that predicted the development of fibrosis and/or cirrhosis being: the persistence of alcohol intake, female sex, the macro/microvesicular pattern of steatosis, the severity of steatosis and the presence of giant mitochondria (5). These findings show the need for studies to investigate specific treatments for this condition.

Increased free radical production and lipid peroxidation have been proposed as major cellular mechanisms involved in alcohol-induced liver injury (6). Ethanol modifies the redox state of hepatocytes, and it has been demonstrated that alcohol decreases the intracellular concentration of reduced glutathione, increases levels of reduced pyridine nucleotide levels and enhances the formation of reduced components of several oxidoreduction couples (7). The increased levels of reduced pyridine nucleotides inhibit the activity of tryptophan pyrrolase (TPO), and it has been shown that pyridoxine, which is low in alcoholics, is able to reverse TPO inhibition (8). Pyrrolidone carboxylate (PCA) is a cyclic lactam of glutamic acid, first reported as an intermediate in the γ-glutamyl cycle (9), a metabolic pathway that accounts for the synthesis and degradation of glutathione (10). Metadoxine (pyridoxol L,2 pyrrolidone-5-carboxylate) is a combination of pyridoxine and PCA. Experimental studies have demonstrated that this drug induces an increase in hepatic adenosine triphosphatase (ATP) concentration, protects against inhibition of TPO, and restores the hepatic levels of reduced glutathione (GSH) (8,11). Furthermore, metadoxine accelerates the plasma clearance of ethanol and acetaldehyde, and reduces the time exposure of the liver and other tissues to the toxic effect of ethanol and its metabolites (12). Although metadoxine has no effect either in human or in rat alcohol and aldehyde dehydrogenases *in vitro*, this compound prevents the loss of alcohol dehydrogenase activity in chronically alcohol-fed rats (13). On the basis of these experimental data, metadoxine has been tested in alcoholic patients, and studies including a small number of patients have found that it could be useful in the prevention and treatment of alcoholic fatty liver (14,15). This drug also improved liver function tests in alcoholic patients with more advanced liver disease when they abstained from alcohol (14).

The aim of the present study was to evaluate the effectiveness of metadoxine in the treatment of alcoholic fatty liver and also the safety and tolerability of this drug.

Patients and Methods

The study was carried out in 136 patients with long-standing alcoholism and clinical features of fatty liver consecutively admitted to one of the 14 participating hospitals for alcohol detoxification over a period of 2 years. The criteria for admitting patients to the study were: (1) age between 20 and 70 years; (2) a well-documented history of an average daily alcohol consumption exceeding 80 g/day and active drinking at the time of the study, (3) mild clinical and laboratory abnormalities suggestive of early-stage alcoholic liver disease; and (4) ultrasonographic evidence of fatty liver. Patients were mostly asymptomatic, tender hepatomegaly was present in 75%, and they had mild or moderate elevation of serum bilirubin, AST, ALT, GGT and alkaline phosphatase. Patients with more advanced alcoholic liver disease or with nonalcoholic liver disease were excluded. Other exclusion criteria were: allergy to pyridoxine or derivatives, regular treatments during the month prior to the study, renal failure, and other severe associated disease. The study was approved by the Ethics Committee of each hospital involved, and all the patients gave written informed consent to participate in the study.

The study was conducted in a randomized, double-blind fashion. A random code was prepared by computer for each participating center. The number of patients included in each center ranged from 6 to 40. Metadoxine in 500-mg tablets and identical placebo tablets were provided by the Laboratorio Zambon (Barcelona, Spain). A dose of 1500 mg of metadoxine or an identical placebo was divided into 3 equal doses and was given daily for 3 months.

At the time of randomization the clinical data were recorded and a number of liver, biochemical and hematologic tests performed. The tests were repeated monthly during the treatment period. Liver ultrasonography was also carried out before and after the treatment. Steatosis was graded from 0+ to 3+ (absent, mild, moderate and severe). The degree of steatosis was assessed by the difference of echo amplitude between liver and kidney and the loss of echoes from the walls of the portal veins and/or from the gallbladder wall (16,17). In order to exclude more advanced liver disease or cirrhosis, other ultrasonographic signs, such as

TABLE 1

Characteristics of the patients at inclusion in the study

	Metadoxine (n=69)	Placebo (n=67)	p
Age (years)	42.7±11.8	45.6±9.2	n.s.
Sex (male/female)	53/16	60/7	n.s.
Alcohol intake (g/day)	131.1±46.6	140.3±44.3	n.s.
Bilirubin (mg/dl)	1.33±1.1	1.20±0.8	n.s.
AST (U/l)	136.6±75.7	110.6±60.9	<0.05
ALT (U/l)	116.7±60	92.6±43.8	<0.05
GGT (U/l)	387.2±313.5	272.6±277.3	<0.05
Alkaline phosphatase (U/l)	302.2±296.4	229.7±103.8	n.s.
Prothrombin index (%)	88.7±16.3	91.1±12.6	n.s.
MCV (fl)	101.8±9.1	100.9±8.5	n.s.
Total protein (g/l)	73.6±9	71.1±8	n.s.
Cholesterol (mg/dl)	225±90.8	218.7±71.4	n.s.
Triglycerides (mg/dl)	169.6±115.6	198.1±126.9	n.s.
U.S. fatty liver +/++/+++	1/16/52	6/17/44	n.s.

AST=aspartate aminotransferase; ALT=alanine aminotransferase; GGT=gammaglutamyl transpeptidase; MCV=mean corpuscular volume of erythrocytes.

TABLE 2

Laboratory data of the patients treated with metadoxine at inclusion, at day 30 and at day 90 of the study

	Initial (n=69)	Day 30 (n=69)	Day 90 (n=57)
Bilirubin (mg/dl)	1.33±1.1[1]	0.79±0.44	0.58±0.5
AST (U/l)	136.6±75.7[1]	55.3±34.4[5]	28.5±12.5
ALT (U/l)	116.7±60[1]	57.8±37.9[6]	35.6±21.5
GGT (U/l)	387.2±313.5[1]	113.6±87.1	43.8±28.8
Alkaline phosphatase (U/l)	302.2±296.4[1]	183.5±91.2	171.1±116.4
Prothrombin index (%)	88.7±16.3[2]	92.2±10.1	96.8±5.7
Total protein (g/l)	73.6±9	71.7±1	72.1±5.8
Cholesterol (mg/dl)	225±90.8[3]	199.8±41.3	193.2±39.2
Triglycerides (mg/dl)	169.6±115[4]	129.5±45.3	96.8±5.7

[1]p<0.001 vs day 30 and day 90; [2]p<0.001 vs day 90; [3]p<0.05 vs day 90; [4]p<0.05 vs day 30 and day 90; [5]p<0.001 vs day 90; [6]p<0.05 vs day 90.
Abbreviations as in Table 1.

hepatic marginal irregularities, enlarged portal vein, abdominal collaterals and splenomegaly, were also evaluated (18). All the ultrasounds were reassessed blindly at the end of the study.

At each visit, compliance with the treatment, possible adverse effects and concomitant medication were recorded. Compliance with treatment was assessed by counting the remaining tablets. Alcohol abstinence during the treatment period was carefully controlled by questioning the patient and relatives, and by serial urine alcohol determination (19).

According to an unpublished pilot study at one of the participating centers, the minimal difference observed with respect to the liver function tests after 90 days of treatment was 20%. Based on this value, we calculated that 82 patients were needed in each group to show differences with an alpha error of 5% and a beta error of 10%. We therefore decided to include 196 patients (98 per group) because of an estimated loss of 20% of the patients. When the estimated time for inclusion was reached, only 136 patients had been included. Nonetheless, an intermediate analysis was done at that time and, on observation of the results achieved, we decided to discontinue the study. Comparison between patients from both groups was performed by using Student's t-test or the Mann-Whitney U-test for quantitative variables and the chi-square test with Yates' correction for qualitative variables. All these calculations were made with the SAS statistical package for Windows (SAS Institute Inc., Cary, NC, USA). Results are expressed as mean±SD.

Results

Of the 136 patients included in the study, 69 received metadoxine and 67 placebo. Twelve patients in the metadoxine group and 13 in the placebo group were excluded prematurely from the trial, mainly because of patient refusal to continue and loss to follow-up. Tolerance to treatment was excellent or good in all but one patient from each group, who did not tolerate the treatment. The only adverse effect reported was an episode of transient diarrhea in a patient treated with metadoxine. After a few days, metadoxine was given again and the patient completed the treatment with no further problems.

The demographic data, clinical features, and initial laboratory studies of the patients are shown in Table 1. The patients from the two groups did not differ with respect to sex, age, duration and amount of alcohol consumption, clinical data and degree of steatosis. No signs suggesting the presence of portal hypertension or liver cirrhosis were found in the basal ultrasonography. Patients treated with metadoxine had aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gammaglutamyl transpeptidase (GGT) values slightly, but significantly higher than patients treated with placebo, whereas there was no statistically significant difference between the groups in any of the other biochemical values.

The values of the different biochemical parameters at the beginning, after 1 month and after 3 months of treatment with metadoxine or placebo are shown in Tables 2 and 3, respectively. After 1 month, most of the values improved significantly in patients treated with metadoxine, whereas in the placebo group there were no significant differences compared to the initial values. At the end of the study, all the values improved significantly in both groups, although normalization

TABLE 3

Laboratory data of the patients treated with placebo at inclusion, at day 30 and at day 90 of the study

	Initial (n=67)	Day 30 (n=64)	Day 90 (n=54)
Bilirubin (mg/dl)	1.20±0.8[1]	0.99±0.6	0.76±0.38
AST (U/l)	110.6±60.9[2]	92.7±67.7	74.5±60.1
ALT (U/l)	92.6±43.8[2]	84.5±48.8	65.1±49.5
GGT (U/l)	272.6±277.3[2]	207.7±181.5	153.1±154.7
Alkaline phosphatase (U/l)	229.7±103.8	204.1±80.8	194.7±75.2
Prothrombin index (%)	91.1±12.6[3]	93.9±9.9	96.3±7.1
Total protein (g/l)	71.1±8	72.4±6.3	72.3±5.1
Cholesterol (mg/dl)	218.7±71.4	212.1±41.9	214.1±37.9
Triglycerides (mg/dl)	198.1±126.9[2]	157.2±86.6	142.1±83.5

[1]$p<0.001$ vs day 90; [2]$p<0.01$ vs day 90; [3]$p<0.05$ vs day 90.
Abbreviations as in Table 1.

TABLE 4

Mean differences between the initial and final main laboratory data of patients treated with metadoxine and placebo

	Metadoxine (n=57)	Placebo (n=54)	p
Bilirubin (mg/dl)	−0.79±1.1	−0.41±0.7	n.s.
AST (U/l)	−103.9±60.8	−33.2±46.7	<0.001
ALT (U/l)	−85.8±60.5	−24.9±44.4	<0.001
GGT (U/l)	−335.4±323.1	−119.1±220.5	<0.001
Alkaline phosphatase (U/l)	−139.6±221.1	−27.8±86.8	<0.001
Prothrombin index (%)	+8.82±15.8	+4.42±10.6	<0.05

Abbreviations as in Table 1.

TABLE 5

Mean differences between the initial and final main biochemical parameters in patients treated with metadoxine and placebo according to the persistence of alcohol intake

	Metadoxine		Placebo	
	Abstinent n=53	Nonabstinent n=16	Abstinent n=52	Nonabstinent n=15
Bilirubin (mg/dl)	−0.8±0.7	−0.8±0.3	−0.4±0.7*	−0.4±0.6**
AST (U/l)	−131±61	−111±63	−42±43*	−5±44**
ALT (U/l)	−87±60	−80±61	−34±37*	−2±55**
GGT (U/l)	−344±346	−402±210	−143±235*	−32±145**
AP (U/l)	−149±244	−99±68	−27±96*	−29±52**

*$p<0.05$ vs abstinents treated with metadoxine. **$p<0.05$ vs nonabstinents treated with metadoxine.
Abbreviations as in Table 1.

of values was achieved only in patients treated with metadoxine (Table 2). Furthermore, the percentage change in the different parameters was significantly higher in the metadoxine group (Table 4).

Signs of fatty liver remained evident in the ultrasonography performed at the end of the study in 16 patients from the metadoxine group and in 38 from the placebo group ($p<0.01$). The degree of steatosis was also different in both groups. Fifteen of the 16 patients with steatosis in the metadoxine group had mild steatosis, whereas in the placebo group 18 patients had mild, 11 moderate and nine severe steatosis ($p<0.01$) (Fig. 1).

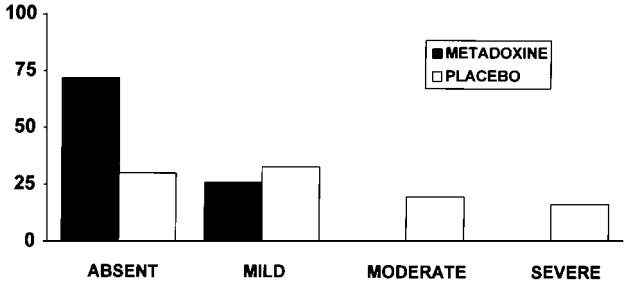

Fig. 1. Degree of fatty liver at the end of the study in patients treated with metadoxine and placebo.

Alcohol abstinence was strongly recommended to all patients at the beginning of the study and throughout follow-up. However, 16 patients in the metadoxine group and 15 in the placebo group continued drinking. The alcohol intake during the study was significantly lower than initially and was similar in both groups (47.7±39 g/day in the metadoxine group and 57.3±31 g/day in the placebo group, p: n.s.). In the metadoxine group the changes in AST, ALT, and GGT values were similar in abstinent and nonabstinent patients. In contrast, in the placebo group the improvement in these biochemical tests was significantly higher in abstinent than in non-abstinent patients (Table 5). In both groups of patients the prevalence of steatosis was higher in nonabstinent patients (45% vs 24% in patients treated with metadoxine and 92% vs 63% in patients treated with placebo). However, among the nonabstinents, the prevalence and the degree of steatosis were also significantly lower in patients treated with metadoxine ($p<0.05$).

Discussion

It is well established that alcohol withdrawal is essential in the treatment of alcoholic liver disease and, in the early stages of the disease, usually leads to disappearance of hepatic lesions. The trend towards improvement in the liver function tests and in the hepatic ultrasonography in the abstinent patients in the placebo group confirms this general agreement.

Our study has also shown that metadoxine-treated patients have a more rapid and greater improvement in liver function tests than patients from the placebo group. After 1 month of treatment significant changes in serum levels of bilirubin, AST, ALT, and GGT were

already observed in patients in the metadoxine group in whom a normalization of the biochemical parameters was observed at the end of the study. The percentage of changes in the biochemical values and the fact that their normalization was achieved only in the metadoxine group argue against a possible influence in the results of the slight but significant differences in transaminases and GGT observed at entry. Furthermore, the percentage of patients in whom the ultrasonographic changes of fatty liver disappeared was higher in those treated with metadoxine, and in this group the degree of steatosis at the end of the study was also lower. The results of the present study confirm the beneficial effects of metadoxine in the treatment of fatty liver observed in previous clinical studies involving a small number of patients. Metadoxine appears to be a well-tolerated drug and no serious side-effects were observed.

Hepatic stellate cells play a key role in hepatic fibrogenesis (20) and their proliferation and activation are attributed to a response to necroimflammation mediated, at least in part, by Kupffer cell-derived factors (21). Activation of stellate cells and correlation between alcohol-induced hepatic stellate cell activation and the severity of steatosis have recently been observed in liver biopsies of patients with fatty liver without evidence of alcoholic hepatitis (22). Another recent study has shown that both acute and chronic steatosis were associated with lipid peroxidation in mice, suggesting that this might be a mechanism for the development of steatohepatitis lesions (23). The role of lipid peroxidation in the pathogenesis of liver fibrosis and cirrhosis has been well documented both *in vivo* and *in vitro* (24,25). The recent study by Reeves et al. (22) provides a clear link between oxidative stress, steatosis and hepatic stellate cell activation, supporting the potential progression of fatty liver to more advanced liver lesions.

Experimental studies in ethanol-fed rats have demonstrated that metadoxine restores hepatic glutathione content and prevents the decrease in hepatic ATP concentration caused by ethanol (11). Maintenance of normal intracellular redox homeostasis is important for preventing fatty liver and hepatic necrosis caused by ethanol or other toxic substances (26). On the other hand, metadoxine accelerates the hepatic oxidation of ethanol and acetaldehyde and, as a consequence, diminishes their tissular concentration after alcohol intake (12). This effect of metadoxine on ethanol catabolism could explain the beneficial role of this drug in alcoholic fatty liver, despite the persistence of alcohol intake. In fact, in patients treated with metadoxine the biochemical changes were similar in the abstinents and in those who did not remain total abstainers, whereas in patients receiving placebo significant changes were observed only in abstinents. Similarly, although the percentage of patients with ultrasonographic signs of fatty liver was higher among nonabstinents, it was significantly lower in the metadoxine group than in the placebo group.

Liver biopsy is required for definitive assessment of alcoholic liver disease (27). In the present study liver biopsy was not considered to be justified because most of the patients were asymptomatic and the biochemical abnormalities were moderate. The accuracy of ultrasound in diagnosing the presence and severity of diffuse parenchymal liver diseases, including those which are alcohol-related, remains uncertain (28–31). In the different series a few cases of liver cirrhosis were not diagnosed. However, in the present study cirrhosis could be reasonably excluded because of the lack of clinical stigmata, suggestive biochemical data, and ultrasonographic changes in hepatic parenchyma or signs of portal hypertension. In contrast, the presence of some degree of fibrosis or histological features of alcoholic hepatitis could not be completely excluded. The difficulty of performing liver biopsies in these patients has been pointed out by Schäfer et al. (32), who, in a recent study, classified alcoholic patients according to clinical, biochemical and ultrasonographic findings in mild, more advanced and severe alcoholic liver disease.

Despite some discrepancies, most of the studies show that ultrasound has a good sensitivity and specificity for the detection of steatosis (16,33,34), although it is very difficult to recognize by ultrasound whether steatosis is associated with mild fibrosis. In this context, Saverymuttu et al. (16) reported a sensitivity of 87% and a specificity of 89% in detecting fatty infiltration or fibrosis of the liver that had been proven by histology. When both alterations were considered separately, ultrasound was more sensitive in detecting steatosis (94%) than fibrosis (57%). There is a frequent association between steatosis and fibrosis, and the lower sensitivity in detecting fibrosis may be due to the fact that in cases of mild fibrosis the features of steatosis predominate, masking the fibrotic component. In the current study, in which liver biopsy was not performed, the presence and intensity of fibrosis and/or mild alcoholic hepatitis as well as its possible influence on the biochemical changes could not be evaluated. However, experimental data suggest that metadoxine could be useful in the early stages of alcoholic liver disease regardless of the presence of fibrosis. In this context, in a rat model of carbon tetrachloride-induced fibrosis, metadoxine slowed the development of liver

fibrosis. After 6 weeks of repeated injections of CCl_4, the simultaneous administration of metadoxine reduced the size of liver lesions, normalized the serum levels of immunoreactive prolyl hydroxylase, an enzyme involved in collagen synthesis, and reduced the increase of pro-alpha$_2$ (I) collagen mRNA content in the liver observed in these animals (35,36).

In conclusion, in alcoholic fatty liver metadoxine accelerates the normalization of liver function tests and the ultrasonographic changes, even in patients who do not completely abstain from alcohol intake. Thus, metadoxine could be useful in the treatment of the early stages of alcoholic liver disease.

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