Metadoxine Versus Placebo for the Treatment of Non-alcoholic Steatohepatitis: A Randomized Controlled Trial

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Objective and design: **The study aimed at assessing the therapeutic efficacy and safety of metadoxine versus placebo on the ultrasonographic and histological features of non-alcoholic steatohepatitis (NASH).** ***Subjects:*** **134 subjects with biopsy-confirmed NASH were randomized to receive metadoxine 500 mg two times daily (*n* = 75) or placebo (*n* = 59) added to the standard of care, over 16 weeks.** ***Efficacy endpoints:*** **Originally, the primary efficacy endpoint was the composite of: reduction in the steatosis by ≥1 grade, reduction in hepatic necro-inflammation by ≥1 grade and ALT normalization. Since >50% of patients refused the second biopsy, it was decided to analyze only the individual parameters.** ***Results:*** **There was no significant difference between the treatment and the placebo groups in either liver histology or ALT or AST. Overall, as expected both groups showed reduction in serum ALT and AST compared to baseline. Compared to placebo (9 out 54), patients on metadoxine (34 out of 75) had significantly higher rates of improvement in 1-point in steatosis grade on ultrasound (*P*-value <0.001). Safety and tolerability did not differ between treatments.** ***Conclusion:*** **Metadoxine is not effective in improvement of liver histology or serum ALT or AST in patients with NASH. However, there was significant improvement of steatosis assessed by ultrasound. To properly estimate the effects on histology and transaminases, further studies of longer duration and at higher doses are needed.** **(J Clin Exp Hepatol 2014;4:94–100)**

NAFLD

Non-alcoholic fatty liver disease (NAFLD) is an adaptive response of the liver to insulin resistance. It is an increasingly common condition and has high prevalence among those with obesity and diabetes.[1] A meta-analysis of 23 randomized controlled trials in non-alcoholic steatohepatitis (NASH) or NAFLD[2] showed that most RCTs were small and did not exceed 1-year duration. Thiazolidinediones improved steatosis and inflammation but yielded significant weight gain. Randomized clinical trials (RCTs) with antioxidants had issues related to type and dose of drug, duration and implementation of lifestyle intervention. Pentoxifylline, telmisartan and L-carnitine improved liver histology in at least one RCT in NASH. Vitamin E was superior to placebo for the treatment of non-alcoholic steatohepatitis in adults without diabetes. In a recent meta-analysis, it has been shown that pioglitazone improves steatosis, ballooning degeneration, and lobular inflammation and there is a suggestion that it may also improve fibrosis when pioglitazone was analyzed alone.[3] In a recent systematic review of pentoxifylline in NAFLD/NASH, it was found that the drug reduced AST and ALT levels and may improve liver histology in patients with NAFLD/NASH, but did not appear to affect the cytokines.[4]

Metadoxine (pyridoxine-L-2-pyrrolidone-5-carboxylate) exhibits multifactorial pharmacological properties suited for its utilization in NAFLD, NASH, and alcoholic liver disease (ALD). These include restoration of nicotinamide adenine dinucleotide (NADH), glutathione (GSH) and adenosine triphosphate (ATP) levels, as well as the proportion between saturated and unsaturated fatty acids and esters in the liver, and reduces oxidative stress.[5–9] Metadoxine also decreased the synthesis of fibronectin and procollagen and the activity of proline hydroxylase in the liver after

Keywords: NASH, metadoxine, controlled clinical trial
Received: 8.1.2014; *Accepted:* 3.3.2014; *Available online:* 27.3.2014
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Abbreviations: ALD: alcoholic liver disease; ALT: alanine transaminase; ANCOVA: analysis of covariance; AST: aspartate transaminase; ATP: adenosine triphosphate; GGT: gamma-glutamyl transferase; GSH: glutathione; HOMA-IR: homeostasis model assessment for insulin resistance; ITT: intention to treat; MRI: magnetic resonance imaging; MRS: MR spectroscopy; NADH: nicotinamide adenine dinucleotide; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; PDFF: proton-density fat-fraction; RCTs: randomized clinical trials; RIQ: range interquartile; TNF: tumor necrosis factors
http://dx.doi.org/10.1016/j.jceh.2014.03.041

CCl_4 challenge.[10,11] In hepatic stellate cells, metadoxine prevented the increase in collagen and attenuated tumor necrosis factors (TNF)-alpha secretion caused by acetaldehyde.[12] Thus, metadoxine appears to be a potentially effective strategy to manage non-alcoholic steatohepatitis and non-alcoholic fatty liver disease (NAFLD). Based on experimental and early clinical studies,[13] it has been hypothesized that metadoxine may be useful in NASH and NAFLD. Since, however, clinical investigations were so far focused on alcoholic liver disease,[14] we designed this study to monitor whether similar results could be obtained in NASH.

METHODS

The study was designed as a randomized, placebo-controlled, double-blind, multicentre trial in parallel groups of patients. We enrolled patients of either sex, aged between 18 and 60 years, drinking <20 g/day of ethanol, with obesity, upper abdominal symptoms, dyspepsia or elevated transaminases. Liver biopsy and ultrasound scan of the abdomen were performed to confirm the presence of NASH, defined as association of steatosis and hepatocyte ballooning with a pattern of centrilobular accentuation or association of steatosis, hepatocyte ballooning, and perisinusoidal fibrosis with a pattern of centrilobular accentuation. Patients with advanced liver disease, taking >20 g/day of ethanol, positive for hepatitis B or C, under established medications known to cause steatosis, with renal failure or who had recently used the test medication were not recruited. All patients received the standard of care each center normally applied to patients with NASH (individualized nutritional counseling for adequate caloric intake and appropriate lifestyle modifications, though there was no specific dietary prescription or exercise across centers). In addition, after signing the informed consent, the patients were randomly assigned to receive the test treatment (metadoxine 500 mg tablets) or matching placebo twice daily for 16 weeks, followed by 4-week untreated monitoring. The study statistician prepared a computer-generated randomization list, which was kept blinded until the end of the statistical analysis. No emergencies occurred that required breaking the blind.

Patients were monitored at 4-week intervals from randomization to week 20. Clinical history and physical examination were completed at each visit, as were hematology, hematochemistry, compliance and adverse events except at week 20. Laboratory tests included liver enzymes, complete blood count and prothrombin time. There was no centralized laboratory; however all the centers were using similar auto analyzers and standards for normal values. Fasting insulin and fasting glucose were used to calculate insulin resistance according to the homeostasis model assessment technique (HOMA-IR) at baseline. We scored the extent of steatosis at screening and end of the study (as 0 = absent, 1 = mild, 2 = moderate, 3 = severe) by the difference in echo amplitude between liver and kidney and the loss of echoes from the walls of the portal veins and/or gall bladder wall.[15,16]

The liver pathologists at each center determined the presence and severity of histological diagnosis of NASH at baseline and end of treatment. The study pathologists confirmed the adequacy of the liver biopsy specimens for evaluation. We graded and staged the histological conditions of the biopsies according to Brunt et al.[17] Necroinflammation was graded as 0 = absent; 1 = mild, occasional ballooned hepatocytes, scattered and mild lobular inflammation, no or mild portal inflammation; 2 = moderate, obvious hepatocyte ballooning, mild lobular inflammation, mild to moderate portal inflammation; 3 = severe, marked hepatocyte ballooning, scattered lobular inflammation and polymorphonuclear cells, mild to moderate portal inflammation. Fibrosis was staged as 0 = no fibrosis; 1 = zone 3 perisinusoidal fibrosis, focal or extensive; 2 = perisinusoidal fibrosis, focal or extensive, and periportal fibrosis, focal or extensive; 3 = bridging fibrosis, focal or extensive; grade 4 cirrhosis.

Using the criteria for steatosis by ultrasound scan, radiologists interpreted the findings at each center as this could not be centralized.

We originally set as primary endpoint the composite of: reduction in steatosis by ≥1 grade, reduction in necroinflammation by ≥1 grade and ALT normalization, however, after closing the study and in view of the small number of patients suitable for such analysis, we decided to analyze only the individual components. The rationale for the primary endpoint was based on the placebo effect observed in clinical trials. 15–33% of subjects receiving placebo showed 1-point improvement in steatosis, ballooning degeneration, lobular inflammation, NASH fibrosis and combined inflammation scores.[18]

Statistics

Based on the information from the studies in AFLD, we anticipated a difference of 30 percent points with the test medication over the placebo effect. Accounting for an expected loss of information (refused second biopsy) of approximately 50% and retaining a total alpha error ≤0.05, a sample of approximately 200 patients would have had 80% power to detect the anticipated difference with P ≤0.025 two-tailed for each correlated analysis, in the intention to treat (ITT) population (all randomized patients) and in the efficacy population (only patients with valid final measurement).

Normally distributed measures were summarized as mean and standard deviation (SD) and compared with the independent samples t-test or, where appropriate, with the analysis of covariance (ANCOVA) for repeated measures, using treatment and sex as fixed factors, center