The Beneficial Effect of Metadoxine (Pyridoxine-pyrrolidone-carboxylate) in the Treatment of Fatty Liver Diseases

JÁNOS FEHÉR, LÁSZLÓ VÁLI, ANNA BLÁZOVICS, GABRIELLA LENGYEL

2nd Department of Medicine, Faculty of Medicine,
Semmelweis University, Budapest, Hungary

Hepatic steatosis involves an imbalance between the processes of the hepatocytes' lipid uptake and lipid elimination, an overproduction results in the accumulation of excess triglycerides in the cells of the liver. Normally about 5% of the cells contain triglyceride; in steatosis this may exceed 50%. Under 50% the condition is called fatty infiltration, and over 50% it is called fatty liver. In mild forms this does not necessarily lead to disorders in cell functions, but in more severe forms it does; it often precedes the death of the cell. Fatty liver can be considered a pathologic condition which makes the liver more susceptible to other toxic influences. It is not a genuine disease; in most cases it is associated with a noxious state or other pathologic process. Alcohol-induced fatty liver is a current epidemie. The abnormal accumulation of fat in parenchymal organs, including the liver, is called fatty transformation or steatosis. Alone and limited to a certain degree of severity (the appearance of fibrosis), it represents a reversible damage; upon cessation of the underlying cause the liver clears its excess triglyceride content. The treatment is to be aimed at the underlying process; up to now there is no known specific medicine that could clearly reduce the fat accumulated in the hepatocytes. Although the etiologic factors of these diseases differ from each other, the pathological changes in the liver are very similar, thus certain drugs could be equally effective for treating them both. Metadoxine is one of those drugs, mainly due to its liver-protective effect against damage from free radicals. As an effective antioxidant, metadoxine regulates glutathione levels in the liver and throughout the body, thus positively influencing the maintenance of systemic redox homeostasis. The authors discuss in detail the effect of metadoxine in the treatment of fatty liver diseases (alcoholic and non-alcoholic), they also review the effect of metadoxine in both *in vitro and in vivo* experimental conditions.

Keywords: fatty liver, steatohepatitis, alcoholic, non-alcoholic, NASH, oxidative stress, metadoxine, antioxidant

Corresponding address: János Fehér MD, 2nd Dept. of Medicine, Semmelweis University, Szentkirályi u. 46., H-1088 Budapest, Hungary. E-mail: feher@bel2.sote.hu

Abbreviations

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AMA = anti-mitochondrial antibody; AMPK = AMP-activated protein kinase-alpha; ANA = antinuclear antibody; AST = aspartate aminotransferase; ATP = adenosine triphosphate, BMI = body mass index; CDT = carbohydrate deficient transferrin; CT = computer tomography; DPPH = 2,2- diphenyl-1-picrylhydrazyl radical; FFA = free fatty acid; FAEE = fatty acid-ethyl-ester; GGT = gamma-glutamyl transpeptidase; GLUT-4 = glucose transporter 4; IL = interleukin; IR = insulin resistance; IRS = insulin-receptor-substrate; MALT = Munich Alcoholism Test; MCV = mean corpuscular volume; MRC = mitochondrial respiratory chain; NAD = nicotinamide-adenine-dinucleotide; NAFLD = non-alcoholic fatty liver disease; NASH = non-alcoholic steatohepatitis; NF-κB = nuclear transcription factor-κB; NOSA = non-organ specific autoantibodies; OGTT = oral glucose tolerance test; PGC1 = PPAR-gamma co-activator (1 alpha and 1 beta); PPAR = peroxisome-proliferator-activated receptor; ROS = reactive oxygen species; SIRPH = serum immunoreactive prolyl hydroxylase; TGF = tissue growth factor; TNF = tumor necrosis factor.

Hepatic steatosis involves an imbalance between the processes of the hepatocytes' lipid uptake and lipid elimination, thus overproduction results in the accumulation of excess triglycerides in the cells of the liver. Normally about 5% of the hepatic cells contain triglyceride. In steatosis this may exceed 50%. Under 50% the condition is called fatty infiltration, and over 50% it is called fatty liver. In mild forms this does not necessarily lead to disorders in cell functions, but in more severe forms it does; it often precedes the death of the cell [1–5]. By itself, to a certain grade of severity (the appearance of fibrosis), it represents a reversible damage, upon cessation of the underlying cause the liver clears its excess triglyceride content. The treatment is to be aimed at the underlying process, up to now there is no known specific medicine that could clearly reduce the fat accumulated in the hepatocytes. Metadoxine is one of those drugs, mainly due to its liver-protective effect against damage from free radicals. As an effective antioxidant, metadoxine regulates glutathione levels in the liver and throughout the body, thus positively influences the maintenance of systemic redox homeostasis. In this review we would like to summarize the most important literary data about metadoxine in order to accordingly use this drug in the treatment of fatty liver diseases.

Metadoxine is a new type of synthetic drug which is being used to treat alcoholic fatty liver. It is a pyridoxine-pyrrolidone-carboxylate compound *(Fig. 1)*. Its primary effect is to increase elimination of alcohol via the kidneys, and to help clear the by-products of alcohol decomposition, such as acetaldehyde, from the blood and tissues. The process of oxidizing ethanol into acetaldehyde and acetone consumes reduced glutathione levels *(Table 1)*. Following an insult from alcohol, metadoxine helps restore nicotinamide-adenine-dinucleotide (NAD), glutathione, and adenosine triphosphate (ATP) concentration in the liver and the brain, as well as normalizes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) levels, all of which are characteristic signs of liver regeneration [6–7].

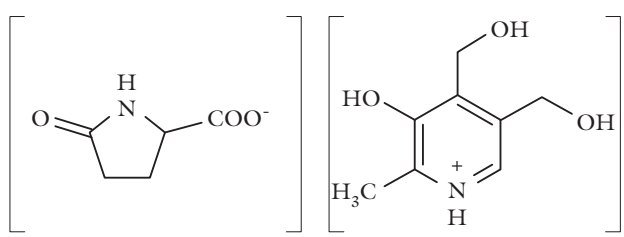


Fig. 1 Chemical structure of metadoxine

Table 1 Effects of metadoxine in the organism

– Antioxidant properties and radical-binding ability
– Increases the level of adenosine triphosphate
– Increases the level of reduced glutathione
– Reduces the rate at which damaging agents cause fibrosis
– Prevents depletion of glycogen stores
– Positively influences fatty acid metabolism
– Accelerates elimination of alcohol

Mechanism of Action

Metadoxine (pyridoxine-L-2-pyrrolidone-5-carboxylate) is a combination of the pyridoxine and pyrrolidone-carboxylate, glutamic acid cyclical-lactam, and the gamma-glutamyl cycle that is responsible for synthesizing and breaking down glutathione. The two sub-units of the molecule occur naturally. The pyridoxine component of the drug (a B6 vitamin) fulfills an important function in the system, such as during the metabolisation of amino acids, carbohydrates, sphingolipids and hemoglobin. It also plays a role in neutralizing and detoxifying biliary acid by synthesizing taurine [6–9].

In the tissues, the ion pair molecules could detach, forming N-oxide type molecules which structurally function as spin traps, thus are able to capture singlet oxygen, hydroxyl, and superoxide free radicals. Depending on the concentration, metadoxine acts as a proton donor in the presence of DPPH stable radicals, and the same is true for its reduction capacity [10– 11].

Alcoholic Liver Disease

Fatty liver is the first stage in the progression of alcoholic liver disease pathogenesis. The development of alcoholic hepatitis and cirrhosis confers a serious prognosis and a different course of treatment. The therapy has four main elements: abstinence, assuring adequate diet/artificial nourishment, medication, and as the last resort: liver transplantation. Early diagnosis and treatment depending on the severity and development of the disease within the liver are crucial to any attempt to retard progression and improve prognosis [12]. The metabolic effects of alcohol are due both to its direct action and to that of its first metabolite, and can also be connected with the changes in the redox state. Differences in ethanol distribution, bioavailability and hepatic metabolism can provide insight into the protective and predisposing factors in alcoholism, as well as gender differences of alcohol toxicity. Oxidative stress occurs following various conditions of ethanol consumption [13].

Metadoxine is suitable for increasing reduced glutathione level, which is key to the redox homeostasis of the liver and the whole body. The effectiveness of the drug is established both in acute and in chronic alcoholism. Moreover, it helps the patient to be abstinent [14–17].

In Russia, a case-control study was conducted in which 20 patients with liver disease of alcoholic origin received intravenously glucose and vitamins, lipotropic drugs and 1,500–2,000 mg/day of metadoxine, for thirty days. Metadoxine stimulated liver regeneration and the degeneration of liver cells normalized. It was well tolerated, did not cause side effects, and probably delayed the development of cirrhosis. Metadoxine does not activate alcohol-dehydrogenase or aldehyde-dehy-

drogenase in any of their forms [18], except at concentrations above 0.1 mM, and so has virtually no physiological impact. In alcoholic cases, metadoxine plays a protective role against the accumulation of triglycerides, in a manner similar to ubiquinone [19–20]. Metadoxine blocked the lowering of ATP concentrations in the brain and the liver [21].

Italian researchers conducted double-blind studies on the effects of metadoxine, in which 60 chronic alcoholics participated. They tested the effects of metadoxine given twice daily in 500 mg doses to chronic alcoholics, who were divided in two groups: those treated with metadoxine and an untreated group. Metadoxine brought about much quicker improvement in liver enzyme values (AST, ALT, and GGT). This improvement was accompanied by significant reduction in MCV for 20 of the patients. Based on their medical histories, the MALT (Munich Alcoholism Test) score for this group of 60 chronic alcoholics was significantly lower than those of the control group, and their need for benzodiazepine and/or neurolepticum had decreased. Metadoxine appeared to have helped maintain abstinence, at least in the short-term period [22].

Alcohol abuse and alcoholism are responsible for a wide variety of medical problems. Chronic alcoholism is accompanied by fatty-acid metabolic disturbance. The levels of free fatty acid (FFA) and fatty acid-ethyl-ester (FAEE) have been measured in various organs of rats treated with alcohol. The level of fatty acid-ethyl-esters gradually increases under the influence of alcohol. Administering metadoxine prophylactically one hour prior to alcohol consumption significantly blocked the accumulation of both free fatty acid and fatty acid-ethyl-ester in all organs that were sampled. Metadoxine primarily blocked the formation of the saturated and mono unsaturated fatty acids with carbon chains. Metadoxine also changes the metabolism of fat in alcohol consuming patients [23–25].

The pharmaco-therapeutic aspect of alcoholism includes the use of drugs, with different actions and objectives. Among them, metadoxine seems to be of interest. Metadoxine is able to accelerate the elimination of alcohol from the blood and tissues, to help restore the functional structure of the liver and to relieve neuro-psychological disorders associated with alcohol intoxication. Metadoxine also seems to be safe; more than 15 years of post-marketing surveillance revealed only minor aspecific and reversible events in patients exposed to the treatment [26]. According to recent studies, 900 mg intravenous metadoxine once daily could be recommended for the patients with ethanol intoxication [27].

Chronic alcohol consumption may lead to primary and secondary malnutrition. In particular, protein energy malnutrition not only aggravates alcoholic liver disease but also correlates with impaired liver function and increased mortality. Therefore, in these patients, adequate nutritional support should be implemented in order to improve their prognosis. Clinical trials addressing this issue have shown that nutritional therapy either enterally or parenterally improves various aspects of malnutrition, and there is increasing evidence that it may also improve survival. Therefore, malnourished alcoholics should be administered a diet rich in carbohydrate- and protein-derived calories preferentially via the oral or enteral route. Micronutrient deficiencies typically encountered in alcoholics, such as thiamine and folate, require specific supplementation. Fatty liver represents the early stage of alcoholic liver disease, which is usually reversible with abstinence. Metadoxine appears to improve fatty liver but confirmatory studies are necessary [28].

A multicentric, randomized, double-blind study was conducted on the effects of metadoxine therapy on liver function, in which 136 chronic alcoholics participated. Those who received metadoxine experienced much quicker and more complete recovery than the control group. Metadoxine accelerates liver regeneration: it normalized liver function values, and the ultrasonic picture improved. This is another study where metadoxine proved to be an effective treatment for early stage alcoholic fatty liver [29].

In a recent three months follow-up study the authors examined the efficacy of metadoxine in a cohort of alcoholics admitted to the hospital at the University of Pisa (Italy). They analyzed the clinical data, psychometric tests and blood tests of 160 alcoholics on admission and after 3 months of treatment. They compared 58 patients treated with metadoxine with 102 patients who did not receive any drug as an adjunct to the psycho-educational interventions. At follow-up, the patients in treatment with metadoxine showed a significant improvement in the rate of complete abstinence. Furthermore, the number of drop-outs at three months of treatment was also significantly lower in the metadoxine group than in the control group. The authors conclude the use of metadoxine in the management of alcohol dependence in order to help patients to adhere to treatment programs and to prevent the development of mental and physical complications due to chronic and heavy alcohol consumption [30].

Metadoxine in Acute Alcoholic Liver Toxicity

In order to study the role of metadoxine in acute alcoholic poisoning a double-blind, randomized, placebo-controlled study was done. Fifty-eight acute alcoholic intoxicated patients were included in the study, of whom 29 individuals received a one-time, intravenous 900 mg dose of metadoxine, and 29 persons received a placebo [25]. In patients with acute ethanol intoxication metadoxine accelerated the elimination of ethanol from blood, which led to faster recovery from intoxication, and improved the behavioral toxic symptomatology. On the basis of these data the authors suggested the use of metadoxine in the management of acute ethanol intoxication.

A recent randomized, open-label study [31] was carried out to evaluate the efficacy of 300 mg metadoxine (given intravenously) to supplement the standard treatment managing the physical and psychological signs of acute alcohol intoxication. Two groups were used in the study. One that received metadoxine in addition to the control treatment and one that continued the standard treatment alone. Fifty-two acutely intoxicated patients were randomly assigned to one of two groups and followed during a two-hour period. Changes in clinical symptoms, degree of intoxication, and blood alcohol level were monitored. Patients receiving metadoxine in addition to standard therapy significantly improved by at least one degree of intoxication (one clinical category) compared with those receiving standard treatment alone (76.9% versus 42.3%, respectively). Metadoxine-treated patients also exhibited a significantly greater decrease in blood alcohol concentration compared with those receiving standard treatment alone (-105.4 ± 61.5 mg/dl versus -60.1 ± 38.6 mg/dl, respectively). Metadoxine improved the clinical signs of acute alcohol intoxication and accelerated alcohol clearance from the blood, thus supporting existing data. No adverse effects were observed with use of metadoxine therapy [31].

In acute alcohol intoxication the clinical manifestations are heterogeneous and involve different organs and apparatuses, with behavioral, cardiac, gastrointestinal, pulmonary, neurological, and metabolic effects. The management of an intoxicated patient occurs mainly in the emergency department and is aimed at stabilizing the clinical condition of the patient, depending on his/her clinical presentation. According to the most recent literary data one specific drug that is useful in the treatment of acute alcohol intoxication is metadoxine, which is able to accelerate ethanol excretion. In patients presenting with acute alcohol intoxication, alcohol-related disorders should be detected so that the patient can be directed to an alcohol treatment unit, where a personalized, specific treatment can be established [32].

Non-alcoholic Fatty Liver

Non-alcoholic fatty liver (NAFLD) is a multi-factorial liver disease *(Table 2)*. It includes a wide spectrum of liver damage characterised by histological changes of alcoholic origin (ranging from uncomplicated fatty liver to steatohepatitis, fibrosis and cirrhosis) in non-alcoholics (< 20 g/day ethanol consumption). It is developed mainly by insulin resistance and oxidative stress, but the exact metabolic and cellular mechanisms are not fully understood [33–35].

Table 2 Causes of fatty liver and steatohepatitis

Dietary causes	*Pregnancy*
Hyperalimentation, obesity	Hyperemesis gravidarum
Malnutrition (starvation, kwashiorkor etc.)	Acute gestational steatosis hepatis
Alcohol	*Infections*
Jejunoileal bypass surgery	Chronic hepatitis C
Gastric bypass, gastric banding	Fulminant viral hepatitis
Parenteral nutrition	HIV infection
Metabolic disorders	Tuberculosis etc.
Type 2 diabetes mellitus	*Endocrinopathies*
Hyperlipidaemia	Cushing's disease
Gout	Myxedema
Reye's syndrome etc.	Acromegaly
Chronic diseases	*Pharmacologic agents, toxins*
Ulcerative colitis, Crohn's disease	Amiodarone
Wilson's disease	Glucocorticoids
Chronic right heart insufficiency	Methotrexate
Cystic pancreatic fibrosis	Tetracycline
	Salicylates etc

Researches have identified the factors that can play a causative role: oxidative stress, abnormal cytokine production, fatty-acid metabolic disturbance and insulin resistance *(Figs 2–3)*. The pathophysiology of non-alcoholic fatty liver involves insulin resistance and production of reactive oxygen species, which stimulate the synthesis of several cytokines through the upregulation of their transcription by nuclear factor-κB (NF-κB). The combination of these events causes hepatocyte injury via direct oxidative injury, TNF-α induced apoptosis, or inflammation [34–39].

James and Day have distinguished three stages in the progression of NASH: 1. fat builds up without other visible damage, 2. formation of fatty liver, 3. formation of fibrosis [40]. Clinically the fatty liver of alcoholic origin can be hardly differentiated from the NAFLD. There is data that is diagnostically characteristic of one of these diseases *(Table 3)*. Besides anamnesis (knowing the alcohol consumption) there are no specific clinical symptoms [41]. In biochemical examinations the values of the mean corpuscular volume (MCV) and carbohydrate deficient transferrin (CDT) are higher in alcohol induced fatty liver. It also follows that the AST/ALT rate can help in the diagnostic procedure [42].

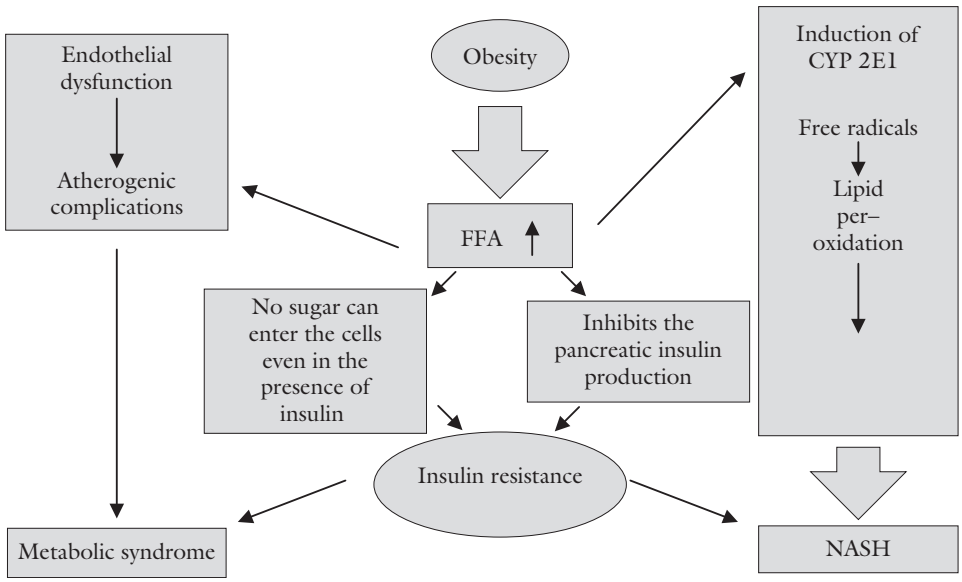
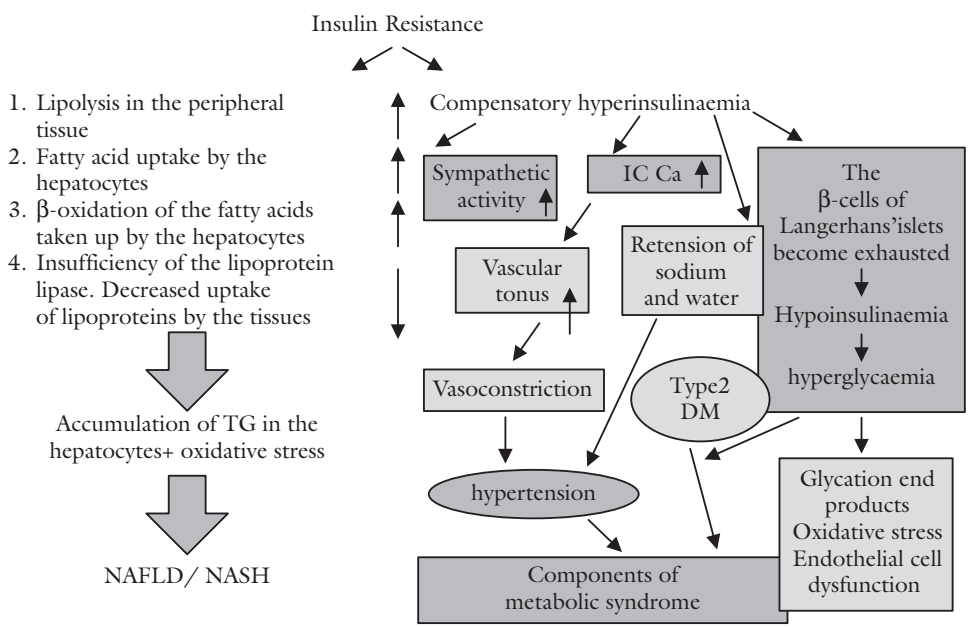


Fig. 2 Role of free fatty acids in the development of NASH and metabolic syndrome



IC = intracellular, Ca = calcium

Fig. 3 Insulin resistance as a main cause of fatty liver diseases and metabolic syndrome

Table 3 Laboratory studies in alcoholic and non-alcoholic liver damage [40]

Laboratory test	alcoholic (ASH)	non-alcoholic (NASH)
Transaminases	high	max. 2–4 times the normal levels, rarely higher
AST : ALT	often > 1	often < (1) (mild) > 1 (severe)
Bilirubin	↑ or ↑↑	normal
AP	normal or ↑	normal or ↑
CDT	↑ with alcohol ≥ 60 g/day	normal
Albumin	normal or ↓	normal
MCV	↑	normal
ANA/SMA	sometimes	low titres
Transferrin saturation	↑	↑
Serum ferritin	↑	↑

Free fatty acids (FFA) have a decisive role in the development of insulin resistance by being transformed into acyl-CoA intracellularly *(Fig. 2)* [43]. In addition to the free fatty acids, TNF-α and hyperinsulinaemia also play a role in the activation of one of the mechanisms responsible for the negative control of signal transduction of insulin – namely the Ser/Thr-phosphorylation of insulin receptor and insulin-receptor-substrate (IRS) protein – that blocks the signal transduction processes of the insulin [44]. In this way muscle cells, hepatocytes, and adipocytes all become resistant to insulin. As a result of this, the glucose cannot enter the cells in spite of the presence of insulin, and cannot exert its effect on the metabolism. The result is that glycogen synthesis in the skeletal muscle, stimulated by the insulin, is clearly reduced due to the stalling of GLUT-4 [43].

Underlying the process, possible genetic defects or disorders of the capillary density are assumed in addition to the above-mentioned substances. It is important to note the mitochondrial disorders observed in NASH: The mitochondria are swollen, and ribbed; they lose their cristae, and often contain paracristal inclusions. The activity of MRC complexes is reduced in patients with NASH, showing a positive correlation with TNF-α levels, IR, and BMI values [46]. Inherited defects in the mitochondrial oxidative phosphorylation are supposed to exist in the offspring – diagnosed with IR – of patients with type 2 diabetes mellitus, leading to disorders in the intramyocellular fatty acid metabolism. The genes dependent on nuclear respiratory factor-1 (NRF-1) that code enzymes, that play crucial roles in the oxidative metabolism and mitochondrial function, are expressed to a lesser extent in diabetes mellitus and IR possibly due to the reduction of PGC1-expression (PPAR-γ co-activator 1-β and 1-α) [47]. Based on these observations, a connecting link between NASH and the metabolic syndrome also is formed by disorders of the mitochondrial function which is hereditary. It has also been demonstrated in NASH that IR is the main risk factor for transition into severe fibrosis [8]. Some authors recommend OGTT to be performed in the presence of non-alcoholic fatty liver in order to diagnose diabetes mellitus in its early stage [47–48].

In the metabolic syndrome FFA is released from the abnormal mesenteric tissue to a certain extent depending on the ratio of the β-3- and α-2 receptors in the given fat tissue, as β-3-adrenoreceptors and α-2-receptors are responsible for the induction and the inhibition of the lipolytic

process, respectively. Polymorphism of the β-3-receptor has been connected with IR and visceral obesity for a long time, most recently with NAFLD [5, 35, 49–51].

In recent years, more and more studies have been published that are searching for the relationship between NASH and the metabolic syndrome. By analyzing relations between NASH and obesity, as well as interconnections with insulin resistance, type 2 diabetes mellitus and disorders of lipid metabolism, several working groups have come to the conclusion, primarily on the basis of epidemiological studies, that NAFLD can be considered as the hepatic consequence of metabolic syndrome. Risk factors of primary NASH: obesity, insulin resistance or type 2 diabetes mellitus, dyslipidaemia, together with the hypertension, constitute the key components of the metabolic syndrome [5].

Insulin resistance (IR) is of decisive importance in how NASH is expressed in a patient, expecially in the presence of a reduced mitochondrial oxidation. According to recent research this involves increased lipolysis in the peripheral tissues, an increase in fatty acid uptake by the hepatocytes, the β-oxidation of the fatty acids taken up by the hepatocytes increases, and the uptake of lipoprotein by the tissues is reduced due to insufficient lipoprotein lipase *(Fig. 3)*. IR is much more severe in patients with NASH than in those diagnosed with 'simple' fatty liver [52–55].

Metadoxine can be an important remedy, because it avoids oxidative damage. It can also be an effective treatment for non-alcoholic fatty liver. Oxidative stress and lipid peroxidation play a role in the pathogenesis of both alcoholic and non-alcoholic fatty liver. Kupffer and dendritic liver cells are the main inducers of the inflammatory reaction, cytokine production, fibrogenesis and lipid accumulation (in conjunction with, or independent of, alcohol consumption) [35] .

Autoantibody positive patients showed lower antioxidant capacity: lower SH-group concentration, decreased total antioxidant status, and elevated chemiluminescence intensity. Abnormal IL-6 concentrations were detected in most subjects belonging to both patient groups, but not significantly higher IL-6 concentrations were measured in autoantibody negative patients. The explanation for higher IL-6 concentration in NOSA-negative patients with better antioxidant capacity, as well as the relation between cytokine production and oxidative stress in NAFLD needs further investigation. Through our most recent work [56] we could conclude that non-alcoholic fatty liver disease undoubtedly has immune-mediated components in its pathogenesis, but further investigations are still needed to understand their significance and role in it, as well as their effects on activity and progression of NAFLD. We suppose that similar to alcoholic liver disease the aldehyde products of lipid peroxidation, such as malondialdehyde can react with proteins and form stable protein adducts. These are very immunogenic and capable of inducing immune response, resulting in generation of antibodies [57–58]. NAFLD with autoantibody positivity may have a worse prognosis because of the impaired antioxidant status. Our results can confirm that antioxidants (like metadoxine) may also have beneficial effects on the progression of this widespread disease of uncertain pathogenesis. The pathogenesis of NAFLD undoubtedly has an immune-mediated component. We suppose that the appearance of autoantibodies in NAFLD is triggered by free radical reaction, but further investigations are needed to fully understand the significance, role and the exact mechanisms of NOSA production in NAFLD.

Unfortunately, proven treatment modalities for NAFLD do not exist [59]. Weight reduction, lipid-lowering drugs, blood sugar regulation, and conventional antidiabetic medications can be effective, but current treatment options for treating NASH are limited. Currently, treatment of NAFLD is focused on modifying risk factors (obesity, diabetes mellitus, hyperlipidaemia). Many therapeutic approaches have been attempted with varying degrees of success [60]. What remains

clear is that combination, multimodality therapy is required for the effective treatment of NAFLD. According to the beneficial effect of metadoxine, as an antioxidant, it could be recommended as a supplementary drug within this treatment [35, 61].

Experimental Studies

The protective effect of metadoxine during acute alcohol poisoning in the liver as well as other organs has been seen. In acute alcohol intoxication, the level of reduced glutathione decreases first, then that of oxidated glutathione increases, and glutathionereductase activity decreases [12–14, 16]. Evidence is accumulating which suggests that intermediates of oxygen reduction may be associated with the development of alcoholic disease. In addition, free radical-induced perturbation of the oxidant/antioxidant balance in cells is widely recognized as the main causative factor of age-related disorders. *Calabrese et al.* [62] investigated the effects of 25 months of ethanol consumption on the antioxidant defense system in different organs of rats in comparison with normal aging, in the absence and presence of treatment with metadoxine. They demonstrated that aged rats underwent a significant perturbation of the antioxidant defense system, as indicated by depletion of reduced glutathione (GSH) content, and increases in oxidized GSH and free radical-induced luminescence associated with a decrease of GSH reductase and an increase of GSH transferase activities. These modifications, observed particularly in the liver and brain with respect to other organs, were enhanced by long-term alcohol exposure, and interestingly, significantly reduced after metadoxine supplementation. In another study the team demonstrated the effectiveness of metadoxine in the management of all those pathological conditions in which a severe imbalance of cellular redox state seems to take place as a result of the generation of free radical species [63].

Annoni et al. [64] evaluated the protective activity of pyridoxol-L,2-pyrrolidon-5-carboxylate (metadoxine) against CCl_4 intoxication in rats, especially in relation to liver fibrosis. After 6 consecutive weeks of CCl_4 treatment, the animals developed liver fibrosis and inflammation as revealed by histological analysis which also included semiquantitative scoring of these features. In addition the serum levels of the immunoreactive prolyl hydroxylase (SIRPH), an enzyme involved in the hydroxylation of the procollagen molecule, were significantly higher in the CCl_4 treated group of animals than in controls. On the contrary, animals treated with CCl_4 and metadoxine (200 mg/kg i.p.) had less severe liver fibrosis and normal SIRPH levels. On the basis of these results the authors suppose that metadoxine may be an effective pharmacological tool for preventing the progression of liver disease to cirrhosis in rats exposed to CCl_4 [64].

Metadoxine tests on hepatocytes and dendritic liver cell cultures demonstrated their protective properties against the cytopathogenic effects of ethanol and acetaldehyde [65]. In the case of HepG2 cells, metadoxine prevents GSH depletion from ethanol and acetaldehyde, and reduces cell damage from lipid peroxidation. Metadoxine reduces the collagen synthesizing effect of acetaldehyde on dendritic liver cells, as well as TNF production. Metadoxine can positively influence the course of early stage liver disease by preventing the disruption of redox homeostasis. It also reduces the induction of TNF, thus blocking the first two steps in liver damage. On the basis of the results it can be supposed that metadoxine prevents glutathione depletion and the increase in lipid peroxidation damage caused by ethanol and acetaldehyde in HepG2 cells. In hepatic stellate cells, metadoxine prevents the increase in collagen and attenuated TNF-alpha secretion caused by acetaldehyde. Thus, metadoxine could be useful in preventing the damage produced

in the early stages of alcoholic liver disease as it prevents the redox imbalance of the hepatocytes and prevents TNF-alpha induction, one of the earliest events in hepatic damage [65].

Alcoholic steatosis is the earliest and most common response to heavy alcohol intake, and may precede more severe forms of liver injury. Accumulation of fat, largely triglyceride, in hepatocytes results from the inhibition of fatty acid oxidation and excessive oxidative stress involving CYP2E1. In a recent study *Ki et al.* [66] evaluated the therapeutic effects of metadoxine, garlic oil or their combination on alcoholic steatosis in animal experiment. Feeding rats an alcohol-containing diet for 4 weeks elicited an increase in hepatic triglyceride content and induced CYP2E1. The concurrent administration of metadoxine and garlic oil to rats during the last week of the diet feeding efficaciously abrogated both fat accumulation and CYP2E1 induction as compared to the individual treatment at higher doses. Histopathology confirmed the ability of metadoxine and garlic oil combination to inhibit lipid accumulation. Blood biochemistry verified improvement of liver function in rats treated with metadoxine and garlic oil. Alcohol administration resulted in a decrease in AMP-activated protein kinase-alpha phosphorylation, which was restored by metadoxine and garlic oil treatments. Recovery of AMPK activity by metadoxine and garlic was supported by an increase in acetyl-CoA carboxylase phosphorylation. Hepatic fatty acid synthase expression was markedly decreased after alcohol consumption, which correlated with a decrease in AMPK activity and a commensurate increase in lipid content. Combined metadoxine and garlic oil treatments caused restoration of the fatty acid synthase level. These results demonstrate that the combination of metadoxine and garlic oil effectively treats alcoholic steatosis with CYP2E1 inhibition, which may be associated with the recovery of AMPK activity, promising that this combination may constitute an advance in the development of candidates for a new clinical therapy for alcoholic steatosis [66].

In an experiment to evaluate liver-beneficial properties of metadoxine, not related with alcohol metabolism, bioactivation of external toxins or antioxidant mechanisms, the chronic bile duct ligation model (in Wistar rats) was used and results were compared with the effects of colchicine. Both compounds showed similar antifibrotic properties; metadoxine was more effective in preserving glycogen. Besides its antioxidant effects and its ability to induce alcohol metabolism, metadoxine possesses important antifibrotic and antinecrotic properties, while maintaining energy stores efficiently [67].

In our laboratory with my coworkers *(Váli et al.)*, we used an ischaemic model for analysing the effects of metadoxine in hepatic ischaemia-reperfusion. Based on these data it can be concluded that metadoxine can protect the liver from the oxidative damage caused by ischaemia-reperfusion. The beneficial properties can be detected even in the blood. Metadoxine may protect the liver from the injury caused by ischaemia-reperfusion through the restoration of redox homeostasis. Protection against necrosis is a well-defined aim of liver surgery, whereas the inhibition of apoptosis may have negative effects on patients undergoing surgery for hepatic cancer. Our short-term model can give new perspectives of the metadoxine treatment [68].

Conclusions

According to the literary data, metadoxine has several beneficial effects within the living organisms *(Fig. 4)*. It is able to increase the ATP concentration in the liver, and to restore the redox balance. Furthermore it can inhibit an increase of triglycerides and fatty acid esthers in the hepatic cells, modify the apoptotic and necrotic processes as well as prevent the TNF-alpha induction

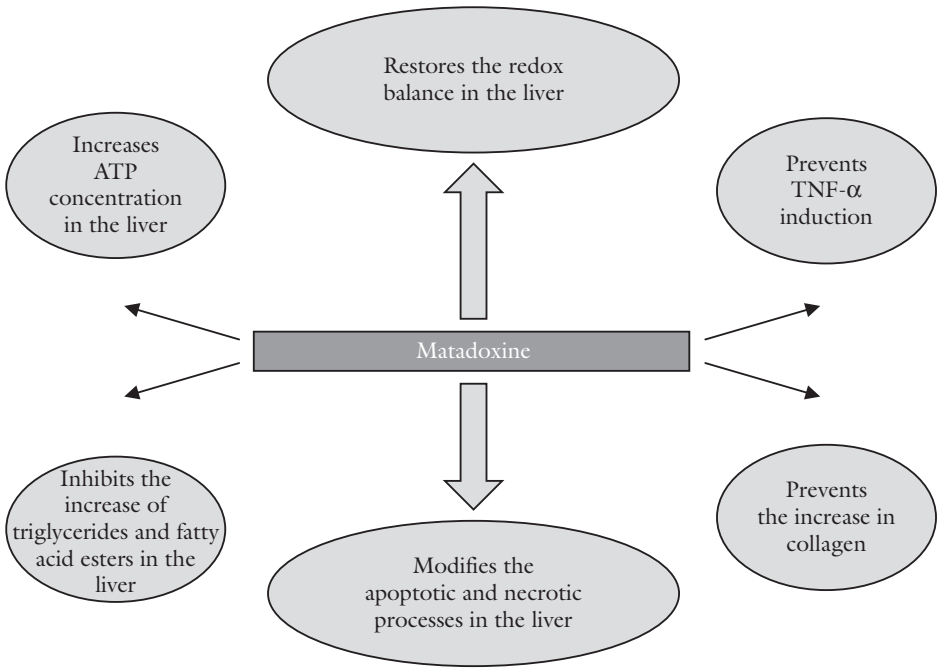


Fig. 4 Main effects of metadoxine in living organisms

which counters the increase of collagen in liver tissue. On the basis of the multifocal effect of metadoxine it can be recommended as a supplementary drug for use in the treatment of cases of alcoholic and non-alcoholic fatty liver diseases.

Acknowledgment

This paper is closely associated to and written under the framework provided by the EU Project entitled *"COST B35 Action: Lipid Peroxidation Associated Disorders: LPO".*

References

[1] *Adams, L. A., Lindor, K. D.:* Nonalcoholic fatty liver disease. Ann. Epidemiol., 2007, *17,* 863–869.
[2] *Akbar, D. H., Kawther, A. H.:* Non-alcoholic fatty liver disease and metabolic syndrome: what we know and what we dont't know. Med. Sci. Monit., 2006, *12,* 23–26.
[3] *Targher, G., Bertolini, L., Padovani, R. et al.:* Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diab. Care, 2007, *30,* 1212–1218.
[4] *Bogdanova, K., Poczatkova, H., Uherkova, L. et al.:* Non-alcoholic fatty liver disease (NAFLD) – a novel common aspect of the metabolic syndrome. Biomed. Pap. Med. Fac. Univ. Papacky Olomouc Czech. Repub., 2006, *150,* 101–104.

[5] *Fehér, J., Németh, E., Lengyel, G.:* Non-alcoholic steatohepatitis (NASH) – is it a part of the metabolic syndrome? Arch. Med. Sci., 2005, *1,* 37–47.
[6] *Felicioli, R., Saracchi, I., Flagiello, A. M. et al.:* Effects of pyridoxine-pyrrolidon-carboxylate on hepatic and cerebral ATP levels in ethanol treated rats. Int. J. Clin. Pharmacol. Ther. Toxicol. ,1980, *18,* 277–280.
[7] *Baldacci, M., Catalani. R., Banoli, C. et al.:* Effects of pyridoxine-pyrrolidone-carboxylate on hepatic adenosine triphosphate levels in rals. Bol. Soc. Ital. Biol. Sper., 1982, *58,* 1643–1649.
[8] *Marchi, S., Pol1oni, A., Costa, F. et al.:* Liver triglyceride accumulation and chronic ethanol administration: a possible proteclive role of metadoxina and ubiquinone. Int. J. Tissue React., 1990, *12,* 247–250.
[9] *Felicioli, R., Saracchi, I., F1agiello, A. M. et al.:* Effects or pyridoxine-pyrrolidon-carboxylate on hcpatic and cerebral ATP levels in ethanol treated rats. Int. J. Clin. Pharm. Ther., 1980, *18,* 277–280.
[10] *Santoni, S., Corradini, P., Zocchil, M. et al.:* Metadoxinc in alcohol related pathology. Clin. Ther., 1989, *130,* 115–122.
[11] *Pár, A.:* Treatment of alcoholic liver diseases. Abstinence, nutritional support, drug therapy, liver transplantation. (In Hungarian) Orv. Hetil., 2000, *141,* 827–833.
[12] *Hagymási, K., Blázovics, A., Lengyel, G. et al.:* Oxidative damage in alcoholic liver disease. Eur. J. Gastroenterol. Hepatol., 2001, *3,* 49–53.
[13] *Hagymási, K., Blázovics, A., Lengyel, G. et al.:* Investigation of redox homeostasis of liver in experimental and human studies. (In Hungarian) Acta Pharm. Hung., 2004, *74,* 51–63.
[14] *Hagymási, K., Blázovics, A.:* Antioxidants in liver protection. (In Hungarian) Orv. Hetil., 2004, *145,* 1421–1425.
[15] *Caballeria, J., Pares, A., Bru, C. et al.:* Metadoxine accelerates fatty liver recovery in alcoholic patients: results of a randomized double-blind, placebo-control trial. Spanish Group for the Study of Alcoholic Fatty Liver. J. Hepatol., 1998, *28,* 54–60.
[16] *Váli, L., Blázovics, A., Fehér, J.:* The therapeutic effect of metadoxine on alcoholic and non-alcoholic steatohepatitis. (In Hungarian) Orv. Hetil., 2005, *146,* 2409–2414.
[17] *Diaz Martinez, M. C., Diaz Martinez, A., Villamil Salcedo, V. et al.:* Efficacy of metadoxine in the management of acute alcohol intoxication. J. Int. Med. Res., 2002, *30,* 44–51.
[18] *Vedrov, N. N., Gnezdilov, N.:* Metadoxyl in combined treatment or alcohol damage to the liver. Klinicheskaia Meditsina, 2001, *79,* 56–58.
[19] *Marchi, S., Polloni, A., Costa, F. et al.:* Liver triglyceride accumulation after chronic ethanol administration: a possible protective role of metadoxina and ubiquinone. Int. J. Tissue React., 1990, *12,* 247–250.
[20] *Bono, G., Sinforiani, E., Merlo, P. et al.:* Alcoholic abstinence syndrome: short-term treatment with metadoxine. Int. J. Clin. Pharm. Res., 1991, *11,* 35–40.
[21] *Pares, X., Moreno, A. I., Peralba, J. M. et al.:* Action of metadoxine on isolated human and rat alcohol and aldehyde dehydrogenases. Effect on enzymes in chronic ethanol-fed rats. Method Find. Exp. Clin., 1991, *13,* 37–42.
[22] *Santoni, S., Corradini, P., Zocchi, M. et al.:* Metadoxine in alcohol-related pathology. (In Italian) Clin Ter., 1989, *130,* 115–122.
[23] *Corsini, G., Gelso, E., Giuliano, G.:* Effects of metadoxine on main biohumoral changes induced by chronic alcoholism. Clin. Ther., 1992, *140,* 251–257.
[24] *Calabrese, V., Bombaci, G, Calderone, A. et al.:* Effects of metadoxine on cellular free fatty acid ethyl esther in ethanol treated rats. Int. J. Tissue React., 1993, *15,* 235–243.
[25] *Addolorato, G., Ancona, C., Capristo, E. et al.:* Metadoxine in the treatment of acute and chronic alcoholism: a review. Int. J. Immunopathol. Pharmacol., 2003, *16,* 207–214.
[26] *Shpilenya, L. S., Mozychenko, A. P., Gasbarrini, G. et al.:* Meladoxine in acute alcohol intoxication: a double blind, randomized, placebo conlrolled study. Alcohol Clin. Exp. Res., 2002, *23,* 340–346.
[27] *Lü, Y., Kang, Z. S., Liu, Y. et al.:* Pharmacokinetics of metadoxine for injection after repeated doses in healthy volunteers. Chin. Med. J., 2007, *120,* 166–168.
[28] *Stickel, F., Hoehn, B., Schuppan, D., Seitz, H. K.:* Review article: Nutritional therapy in alcoholic liver disease. Aliment. Pharmacol.Ther., 2003, *18,* 357–373.

[29] *Corlez-Pinto, H., Chalham, J., Chacko, V. I. et al.:* Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA, 1999, *282,* 1659–1664.
[30] *Guerrini, I., Gentili, C., Nelli, G., Guazzelli, M.:* A follow up study on the efficacy of metadoxine in the treatment of alcohol dependence. Subst. Abuse Treat. Prev. Policy, 2006, *18,* 35.
[31] *Díaz Martínez, M. C., Díaz Martínez, A., Villamil, S. V. et al.:* Efficacy of metadoxine in the management of acute alcohol intoxication. J. Int. Med. Res., 2002, *30,* 44–51.
[32] *Vonghia, L., Leggio, L., Ferrulli, A. et al.:* Acute alcohol intoxication. Intern. Med., 2008, *19,* 561–67.
[33] *Fehér, J., Lengyel, G.:* Pathogenesis and clinics of non-alcoholic steatohepatitis. (In Hungarian) Eur. J. Gastroenterol. Hepatol., 2002, *6,* 185–191.
[34] *Loria, P., Lonardo, A., Leonardi, F. et al.:* Non-organ-specific autoantibodies in nonalcoholic fatty liver disease. Dig. Dis. Sci., 2003, *48,* 2173–2181.
[35] *Fehér, J., Lengyel, G.:* A new approach to drug therapy in non-alcoholic steatohepatitis (NASH). J. Int. Metab. Res., 2003, *31,* 537–551.
[36] *McCullough, A. J.:* Update on nonalcoholic fatty liver disease. J. Clin. Gastroeneterol., 2002, *34,* 255–262.
[37] *Sanyal, A. J.:* AGA technical review on nonalcoholic fatty liver disease. Gastroenterology, 2002, *123,* 1705–1725.
[38] *Farrell, G. C., Larter, C. Z.:* Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology, 2006, *43 (Suppl. 2),* S99–S112.
[39] *Duvnjak, M., Lerotié, I., Barsié, N. et al.:* Pathogenesis and management issues for nonalcoholic fatty liver disease. World J. Gastroenterol., 2007, *13,* 4539–4550.
[40] *James, O. F., Day, P.:* Non-alcoholic steatohepatitis (NASH): a disease or emerging identity old importance. J. Hepatol., 1998, *29,* 495–501.
[41] *Day, C. P.:* Non-alcoholic steatohepatitis (NASH): where are we now and where we going. Gut, 2002, *50,* 585–588.
[42] *Fehér, J., Lengyel, G., Szabó, Gy.:* Carbohydrate-deficient transferrin as a marker of alcohol consumption. Hung. Med. J., 2007, *1,* 73–82
[43] *Beck-Nielsen, H.:* Insulin resistance: organ manifestation and cellular mechanisms. Ugeskrift for Laeger, 2002, *164,* 2130–2135.
[44] *Capeau, J.:* Insulin signaling: mechanisms altered in insulin resistance. Med. Sci., 2003, *19,* 834–839.
[45] *Perez-Carreras, M., Del Hoyo, P., Martin, M. A. et al.:* Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. Hepatology, 2003, *38,* 999–1007.
[46] *Petersen, K. F., Dufour, S., Befroy, D. et al.:* Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N. Engl. J. Med., 2004, *350,* 664–671.
[47] *Sargin, M., Uygur-Bayramicli, O., Sargin, H. et al.:* Association of nonalcoholic fatty liver disease with insulin resistance: is OGTT indicated in nonalcoholic fatty liver disease? J. Clin. Gastroenterol., 2003, *37,* 399–402.
[48] *Seeley, R. J., D'Alessio, D. A., Woods, S. C.:* Fat hormones pull their weight in the CNS. Nat. Med., 2004, *10,* 454–454.
[49] *Patti, M. E., Butte, A. J., Crunkhorn, S. et al.:* Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc. Nat. Acad. Sci. USA, 2003, *100,* 8466–8471.
[50] *Fraenkel, E., Lazúrová, I., Fehér, J.:* Role of lipid peroxidation in non-alcoholic steatohepatitis. Orv. Hetil., 2004, *145,* 611–618.
[51] *Bugianesi, E., Manzini, P., D'Antico, S. et al.:* Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology, 2004, *39,* 179–187.
[52] *Haque, M., Sanyal, A. J.:* The metabolic abnormalities associated with non-alcoholic fatty liver disease. Best Pract. Res. Clin. Gastroenterol., 2002, *59,* 709–731.
[53] *Hsiao, T. J., Chen, J. C., Wang, J. D.:* Insulin resistance and ferritin as major determinants of nonalcoholic fatty liver disease in apparently healthy obese patients. Int. J. Obes. Relat. Metab. Disord., 2004, *28,* 167–172.

[54] *Pagano, G., Pacini, G., Musso, G. et al.:* Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: Further evidence for an etiologic association. Hepatology, 2002, *35,* 367–372.
[55] *Valenti, L., Fracanzani, A. L., Dongiovanni, P. et al.:* Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology, 2002, *122,* 274–228.
[56] *Hagymási, K., Lengyel, G., Nagy, E. et al.:* Impaired antioxidant status in non-organ specific autoantibody positive patients with nonalcoholic fatty liver disease. Hung. Med. J., 2008, *2,* 563–570.
[57] *Dupont, I., Lucas, D., Clot, P. et al.:* Cytochrome P4502E1 inducibility and hydroxyethyl radical formation among alcoholics. J. Hepatol., 1998, *28,* 567–571.
[58] *Tuma, D. J.:* Role of malondialdehyde-acetaldehyde adducts in liver injury. Free Rad. Biol. Med., 2002, *32,* 303–308.
[59] *Torres, D. M., Harrison, S. A.:* Current treatments in nonalcoholic steatohepatitis. Curr. Treat. Opt. Gastroenterol., 2007, *10,* 425–434.
[60] *Mehta, K., Van Thiel, D. H., Shah, N. et al.:* Nonalcoholic fatty liver disease: pathogenesis and role of antioxidants. Nutr. Rev., 2004, *60,* 289–293.
[61] *Lheureux, P., Penaloza, A., Gris, M.:* Pyridoxine in clinical toxicology: a review. Eur. J. Emerg. Med., 2005, *12,* 78–85.
[62] *Calabrese, V., Calderone, A., Ragusa, N. et al.:* Effects of Metadoxine on cellular status of glutathione and of enzymatic defence system following acute ethanol intoxication in rats. Drugs Exp. Clin. Res., 1996, *22,* 17–24.
[63] *Calabrese, V., Randazzo, G., Ragusa, N. et al.:* Long-term ethanol administration enhances age-dependent modulation of redox state in central and peripheral organs of rat: protection by metadoxine. Drugs Exp. Clin. Res., 1998, *24,* 85–91.
[64] *Annoni, G., Contu, L., Tronci, M. A. et al.:* Pyridoxol L,2-pyrrolidon-5 carboxylate prevents active fibroplasia in CCl4-treated rats. Pharmacol. Res., 1992, *25,* 87–93.
[65] *Gutierrez-Ruiz, M., Bucio, L., Correa, A. et al:* Metadoxine prevent damage produced by ethanol and acetaldehyde in hepatocyte and hepatic stellate cells in culture. Pharm. Res., 2001, *44,* 431–436.
[66] *Ki, S. H., Choi, J. H., Kim, C. W. et al.:* Combined metadoxine and garlic oil treatment efficaciously abrogates alcoholic steatosis and CYP2E1 induction in rat liver with restoration of AMPK activity. Chem. Biol. Interact., 2007, *169,* 80–90.
[67] *Muriel, P., Deheza, R.:* Fibrosis and glycogen stores depletion induced by prolonged biliary obstruction in the rat are ameliorated by metadoxine. Liver Int., 2003, *23,* 262–268.
[68] *Váli, L., Fehér, J., Szentmihályi, K. et al.:* Metadoxine modifies both apoptotic and necrotic processes in hepatic ischaemia-reperfusion, Falk Symposium, Berlin, 2008.