Liver: Review

Digestive Diseases

Dig Dis 2019;37:63–68
DOI: 10.1159/000491429

Received: March 13, 2018
Accepted: June 21, 2018
Published online: July 17, 2018

Hepatoprotection by L-Ornithine L-Aspartate in Non-Alcoholic Fatty Liver Disease

Roger F. Butterworth[a] Ali Canbay[b]

[a]University of Montreal (St-Luc Hospital), Montreal, QC, Canada; [b]University of Magdeburg (University Hospital), Magdeburg, Germany

Keywords

L-ornithine L-aspartate · Non-alcoholic fatty liver disease · Non-alcoholic steatohepatitis · Hepatoprotection · Antioxidant · Hepatic microcirculation · Glutamine

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is the leading chronic hepatic condition worldwide and new approaches to management and treatment are limited. ***Summary:*** L-ornithine L-aspartate (LOLA) has hepatoprotective properties in patients with fatty liver of diverse etiology and results of a multicenter randomized clinical trial reveal that 12 weeks treatment with oral LOLA (6–9 g/d) results in a dose-related reduction in activities of liver enzymes and triglycerides together with significant improvements of liver/spleen CT ratios. A preliminary report described improvements of hepatic microcirculation in patients with non-alcoholic steatohepatitis (NASH) following treatment with LOLA. Mechanisms responsible for the beneficial effects of LOLA in NAFLD/NASH involve, in addition to its established ammonia-lowering effect, metabolic transformations of the LOLA-constituent amino acids L-ornithine and L-aspartate into L-glutamine, L-arginine, and glutathione. These metabolites have well-established actions implicated in the prevention of lipid peroxidation, improvement of hepatic microcirculation in addition to anti-inflammatory, and anti-oxidant properties. ***Key Messages:*** (1) LOLA is effective for the treatment of key indices in NAFLD/NASH. (2) Mechanisms other than LOLA's ammonia-lowering action have been postulated. (3) Further assessments in the clinical setting are now required.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as the leading chronic liver disease worldwide that is strongly associated with obesity and the metabolic syndrome. The pathological spectrum of NAFLD spans simple hepatic steatosis, non-alcoholic steatohepatitis (NASH) to liver fibrosis with subsequent development of cirrhosis leading in many cases to hepatocellular carcinoma [1].

Given the key role of the liver in the removal of excess ammonia, it is not surprising that patients with NASH are

KARGER

E-Mail karger@karger.com
www.karger.com/ddi

Roger F. Butterworth, PhD, DSc
University of Montreal (St-Luc Hospital)
45143 Cabot Trail, Englishtown, Nova Scotia
Montreal, QC B0C 1H0 (Canada)
E-Mail rb@enceph.com

Table 1. Effect of LOLA (oral formulation) on liver enzymes in 463 patients with fatty liver

	ASAT (U/l)	ALT (U/l)	γGT (U/l)
Start of LOLA treatment*	48.1±53.7	52.6±44.7	155.4±236.7
End of LOLA treatment	25.7±16.1	39.2±36.5	60.9±56.3
Difference, %	–46.60	–40.57	–60.82

* 9 g/d, 60 days, Hepa-Merz granules (Merz Pharmaceuticals GmbH).
LOLA, L-ornithine L-aspartate.

hyperammonemic [2] and hepatic accumulation of ammonia has been confirmed in both patients and animal models of fatty liver disease [3].

L-ornithine L-aspartate (LOLA) is a mixture of endogenous amino acids with the demonstrated capacity to increase ammonia removal by residual hepatocytes and skeletal muscle of patients with cirrhosis [4]. Recent reports suggest that LOLA has, in addition to its established role as an ammonia scavenger, a direct protective effect on the liver per se. In a groundbreaking report by Grüngreiff and Lambert-Baumann [5], 463 patients with fatty liver, 29% of whom were non-alcoholic, were treated with a range of doses of oral LOLA for periods of 30–90 days. Increased blood levels of liver enzymes (ASAT, ALT and y-GT) were significantly attenuated (by up to 70%) by LOLA treatment (Table 1) indicative of improving hepatic function and the effect was dose-related. Beneficial treatment outcomes were more pronounced in patients with fatty liver compared to patients with cirrhosis, and subgroup analysis revealed that only patients with clear abstinence from alcohol achieved optimal outcomes with respect to liver enzymes.

LOLA in the Treatment of NAFLD/NASH

LOLA manifests hepato-protective properties in patients with fatty liver of diverse etiology as summarized above. In order to address this issue directly in relation to NAFLD/NASH, a multicenter open label, multi-dose, randomized controlled trial comprising 72 such patients was undertaken in order to assess the efficacy of LOLA administered orally for a 12-week period. Patients were diagnosed with NASH according to the 2010 edition of "The Guidelines for the treatment of NASH": liver-spleen CT ratio of less than 1 through CT scan, ALT 1.3 times higher than the normal upper limit, aged between 18 and 65 years. After 12 weeks of oral LOLA treatment, a significant dose-related reduction in ALT was observed together with significant decreases in concentration of triglycerides. Liver/spleen CT ratios also improved significantly following LOLA treatment [6].

In a subsequent trial, 78 patients with NASH all of whom manifested disorders of hepatic microcirculation were studied using the technique of polyhepatography, a modified technique for the noninvasive estimation of intrahepatic blood flow. Changes produced by LOLA consisted of increased resistance and abnormalities of waveform and amplitude (sinusoidal level). Improvements of hepatic microcirculation were observed in all patients even in the presence of 0–1 stage fibrosis [7].

Mechanisms Involved in Hepatoprotection by LOLA in NAFLD/NASH

The pathogenesis of NAFLD has not been fully elucidated. However, the "two-hit hypothesis" has gained considerable attention where the initial hit relating to altered lipid metabolism results in fat accumulation. The second hit relates to a series of factors including oxidative stress and inflammation.

The principle mechanism of action of LOLA that underpins its ammonia scavenger properties in chronic liver disease involves the removal of ammonia via 2 distinct mechanisms, namely, the synthesis of urea (L-ornithine is a metabolic intermediate in the urea cycle) by periportal hepatocytes and the synthesis of glutamine via the enzyme glutamine synthetase (GS), an enzyme located in both the perivenous hepatocytes and skeletal muscle. It is well established that flux through the GS pathway is reduced by up to 50% in liver biopsy samples from patients with histologically-proven steatosis and raised serum transaminases and bilirubin [8]. These findings are consistent with the significant loss of the high-affinity ammonia-removing pathway involving GS located in the perivenous hepatocytes.

Studies in recent years have identified a series of mechanisms involving LOLA's effects on sarcopenia as well as hepatic intermediary metabolism, oxidative stress, and lipid peroxidation that could play a role in the hepatoprotective effects of LOLA in NAFLD/NASH.

Sarcopenia

Defined as a progressive loss of skeletal muscle mass, strength, and function, sarcopenia is a risk factor for the development of NAFLD. Mechanisms relating sarcopenia to NAFLD include proinflammatory factors with the potential to result in liver injury [9]. It has been reported that LOLA treatment in patients with cirrhosis [10, 11] or experimental animals with chronic liver failure [12] results in the restoration of muscle proteostasis and significant improvements of muscle function. It is possible that the apparent hepatoprotective properties of LOLA in patients with NAFLD are mediated, at least in part, via mechanisms involving improvements of skeletal muscle function. Further evaluations of this possibility are ongoing.

Glutamine

Treatment of experimental chronic liver disease with LOLA results in a significant threefold increase of plasma glutamine resulting from a 2-step reaction involving the transamination of L-ornithine to glutamate, the obligate substrate for GS [13]. Intravenous infusions of LOLA likewise result in significant increases of plasma glutamate and restoration of glutamine in patients with chronic liver disease [14]. Restoration of the synthesis of glutamine in liver may represent an important step implicated in the hepatoprotective properties of LOLA in NAFLD/NASH in view of the observations that administration of glutamine results in improvement of hepatic injury caused by a range of insults including ischemia/reperfusion injury and that resulting from chronic alcohol ingestion [15, 16]. More recently, reports from 2 studies in experimental NAFLD/NASH demonstrate significant hepatoprotective effects of glutamine [17, 18]. In the first of these studies, NAFLD was induced by a high fat diet and oral treatment with glutamine resulted in reduced expression of hepatic markers of oxidative stress and inhibition of NFkB p65 accompanied by improvement in hepatic steatosis. In the second study, the hepato-protective effects of oral glutamine supplements on the development of Western-style diet-induced NASH were associated with protection against lipid peroxidation in the liver. Moreover, glutamine supplements were associated with significantly less proinflammatory activity (Fig. 1).

Glutathione

Another important product of LOLA-derived glutamate, namely, glutathione (GSH), is a potent antioxidant that has the requisite properties for the control of oxidative damage and treatment with LOLA has been shown to correct the loss of GSH in the serum of animals with liver failure resulting from toxic liver injury [19]. Taken together, these findings offer a cogent explanation for the findings of a hepatoprotective effect of LOLA, namely, the antioxidant properties of 2 of its metabolic products (glutamine and the antioxidant GSH).

The use of antioxidants for the treatment of NAFLD/NASH has been proposed and assessed on several occasions so far with somewhat mixed results. Initial trials with vitamin E in patients with NAFLD resulted in improvements in transaminase levels. However, the effects on histological improvement remain equivocal. Initial clinical trials with the lipid-lowering antioxidant probucol have so far yielded disappointing results [20]. Further studies are clearly required.

Nitric Oxide

It has been suggested that sinusoidal perfusion alterations in steatosis lead to compression of the sinusoidal spaces and consequent disturbances of the hepatic microcirculation [21]. Accordingly, increased production or release of the vasoactive modulator nitric oxide (NO) could provide an effective novel strategy for the prevention and treatment of NAFLD [22]. Indeed, results of 2 studies have provided evidence consistent with this possibility. In the first study, the administration of the hepato-selective NO donor compound [O(2)-vinyl-1(pyrrolidine-1-yl) diazen-1-ium-1,2-diolate], V_PYRRO/NO was shown to be protective against high fat-induced liver steatosis. It was suggested that such liver-targeted NO donors that are free of systemic hypotensive effects represent a promising therapeutic strategy for NAFLD [22].

In the second study that is also directly related to NO, the administration of L-arginine, the substrate for nitric oxide synthase, was found to improve microvascular

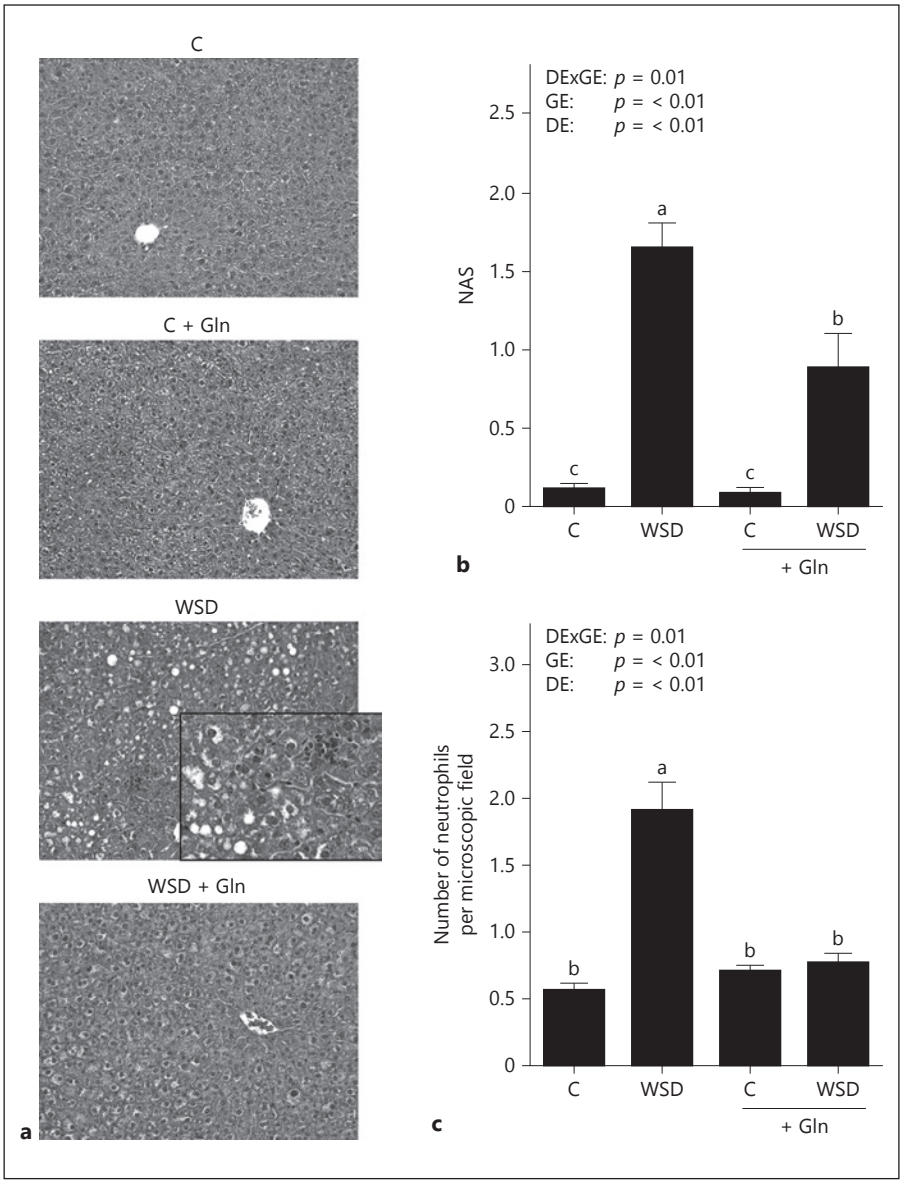
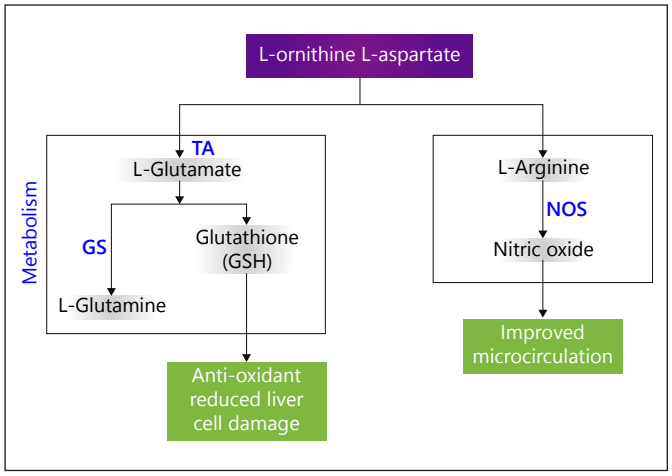


Fig. 1. Effects of Gln on liver histology, NAFLD scores, and proinflammatory activity associated with a WSD. Indices of liver damage in female mice fed a control diet or a WSD with or without supplemental Gln for 6 weeks. **a** Representative photomicrographs of liver sections (hematoxylin and eosin staining; original magnifications: 200× and 400×). **b** Evaluation of liver histology using the NAS. **c** Number of neutrophils in the liver tissue. Values are means ± SEMs, $n = 7–8$. Means without a common letter differ, $p < 0.05$. Gln, glutamine; WSD, Western-style diet; C, control; DE, diet effect; GE, glutamine effect; DEXGE, interaction between diet and glutamine; NAS, non-alcoholic fatty liver disease activity score. From Sellmann et al. [18] with permission.

perfusion in fatty liver [23]. These findings of a beneficial effect of L-arginine are of particular interest in view of the results of studies in experimental chronic liver disease that clearly demonstrated that treatment with LOLA led to a significant 2.5-fold increase of plasma L-arginine [13]. Increases of circulating L-arginine have also been reported in patients with cirrhosis following treatment with LOLA [14]. These findings suggest a mechanism whereby LOLA has the potential to improve the microcirculatory disturbances in NAFLD/NASH, namely, by supplying increased concentrations of LOLA-derived L-arginine that is available for NO synthesis (Fig. 2).

Summary

Evidence from clinical trials supports the thesis that LOLA has hepatoprotective properties in patients with NAFLD/NASH. Such evidence includes the ability of LOLA to attenuate raised levels of liver enzymes including ALT and to reduce serum triglycerides. In addition, LOLA treatment results in significant improvements of liver/spleen CT ratios. Possible mechanisms responsible for the beneficial effects of LOLA include increased conversion of the constituent enzymes of LOLA to glutamine, L-arginine, and GSH. Both glutamine and GSH have hepatoprotective properties against the effects of



Color version available online

Fig. 2. Mechanisms proposed to account for the hepatoprotective effects of LOLA in NAFLD. LOLA, L-ornithine L-aspartate; NO, nitric oxide; GS, glutamine synthetase; NOS, nitric oxide synthase; TA, transaminase.

oxidative stress and lipid peroxidation in experimental NAFLD/NASH. L-arginine is known to improve the associated microcirculatory disturbances associated with these disorders via increased synthesis of NO.

It has been proposed that the application of a novel morphological method for the detection of increased hepatic concentrations of ammonia that was shown to correlate well with the severity of chronic liver disease could afford a useful method for the prediction of patient outcome in fatty liver disease [3]. Further elucidation of ammonia-related signaling pathways and their intermediates identified in this report has the potential not only for the identification of pathophysiological mechanisms in these disorders but also for the design of novel therapeutic strategies and the provision of biomarkers for risk stratification in relation to NAFLD/NASH. Further studies are now required in order to confirm the pertinence of these mechanisms with regard to the pathogenesis of NAFLD/NASH in the patient population and to assess the efficacy of LOLA in well-designed controlled clinical trials.

Acknowledgments

R.F.B.: Study funded in part by The Canadian Institutes of Health Research (CIHR).

Disclosure Statement

Neither author has any conflict of interest to declare.

References

1 Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F: The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol 2013;58:593–608.
2 Felipo V, Urios A, Montesinos E, et al: Contribution of hyperammonemia and inflammatory factors to cognitive impairment in minimal hepatic encephalopathy. Metab Brain Dis 2012;27:51–58.
3 Gutierez-de-Juan V, Lopez de Davalilo S, Fernandez-Ramos D, et al: A morphological method for ammonia detection in liver. PLoS One 2017;12:e0173914.
4 Butterworth RF, Kircheis G, Hilger N, McPhail MJW: Effect of L-ornithine L-aspartate for the treatment of hepatic encephalopathy and hyperammonemia in cirrhosis: systematic review and meta-analysis of randomized controlled trials. J Clin Exp Hepatol 2018, https://doi.org/10.1016/j.jceh.2018.05.004.
5 Grüngreiff K, Lambert-Baumann J: Efficacy of L-ornithine L-aspartate granules in chronic liver diseases. Med Welt 2001;52:219–26.
6 Tian LY, Lu LG, Tang CW, Xie Y, Luo HS, Tan SY, Pang Z, Zhang YL, Gong LB, Li YM, Chen SH, Shi JP: Aspartate-ornithine granules in the treatment of non-alcoholic steatohepatitis: a multiple-dose parallel controlled clinical trial. Zhonghua Gan Zang Bing Za Zhi 2013;21:528–532.
7 Ermolova T, Ermolov S: Correction of intrahepatic microcirculation disorders by L-ornithine L-aspartate in chronic liver disease patients. J Hepatol 2018;68(suppl 1):S585–S586.
8 Kaiser S, Gerok W, Häussinger D: Ammonia and glutamine metabolism in human liver slices: new aspects on the pathogenesis of hyperammonaemia in chronic liver disease. Eur J Clin Invest 1988;18:535–542.
9 Bhanji RA, Narayanan P, Allen AM, Watt KD: Sarcopenia in hiding: the risk and consequence of underestimating muscle dysfunction in non-alcoholic steatohepatitis. Hepatology 2017;66:2055–2065.
10 Reynolds N, Downie K, Smith K, Kircheis G, Rennie MJ: Treatment with L-Ornithine L-Aspartate (LOLA) infusion restores muscle protein synthesis responsiveness to feeding in patients with cirrhosis. J Hepatol 1999;30(suppl 1):3.
11 Pasha Y, Leech R, Violante IR, Cook N, Crossey MME, Taylor-Robinson SD: The brain-muscle axis in minimal hepatic encephalopathy (MHE): a placebo-controlled, longitudinal double-blind trial with L-ornithine L-aspartate (LOLA) – preliminary results. J Clin Exp Hepatol 2017;7:S1–S21.
12 Kumar A, Davuluri G, Silva RNE, Engelen MPKJ, Ten Have GAM, Prayson R, et al: Ammonia lowering reverses sarcopenia of cirrhosis by restoring skeletal muscle proteostasis. Hepatology 2017;65:2045–2058.
13 Rose C, Michalak A, Pannunzio P, Therrien G, Quack G, Kircheis G, et al: L-ornithine L-aspartate in experimental portal-systemic encephalopathy: therapeutic efficacy and mechanism of action. Metab Brain Dis 1998;13:147–157.
14 Staedt U, Leweling H, Gladisch R, Kortsik C, Hagmüller E, Holm E: Effects of ornithine aspartate on plasma ammonia and plasma amino acids in patients with cirrhosis. A double-blind, randomized study using a four-fold crossover design. J Hepatol 1993;19:424–430.
15 Stangl R, Szijártó A, Ónody P, Tamás J, Tátrai M, Hegedus V, Blázovics A, Lotz G, Kiss A, Módis K, Gero D, Szabó C, Kupcsulik P, Harsányi L: Reduction of liver ischemia-reperfusion injury via glutamine pretreatment. J Surg Res 2011;166:95–103.
16 Peng HC, Chen YL, Chen JR, Yang SS, Huang KH, Wu YC, Lin YH, Yang SC: Effects of glutamine administration on inflammatory responses in chronic ethanol-fed rats. J Nutr Biochem 2011;22:282–288.

17 Lin Z, Cai F, Lin N, Ye J, Zheng Q, Ding G: Effects of glutamine on oxidative stress and nuclear factor-κB expression in the livers of rats with non-alcoholic fatty liver disease. Exp Ther Med 2014;7:365–370.
18 Sellmann C, Jin CJ, Degen C, De Bandt JP, Bergheim I: Oral glutamine supplementation protects female mice from non-alcoholic steatohepatitis. J Nutr 2015;145:2280–2286.
19 Najmi AK, Pillai KK, Pal SN, Akhtar M, Aqil M, Sharma M: Effect of L-ornithine L-aspartate against thioacetamide-induced hepatic damage in rats. Ind J Pharmacol 2010;42:384–387.
20 Adams LA, Angelo P: Treatment of non-alcoholic fatty liver disease. Postgrad Med J 2006; 82:315–322.
21 Ramalho FS, Fernandez-Monteiro I, Rosello-Catafau J, Peralta C: Hepatic microcirculatory failure. Acta Cir Bras 2006;21:48–53.
22 Kus K, Walczak M, Maslak E, Zakrzewska A, Gonciarz-Dytman A, Zabielski P, Sitek B, Wandzel K, Kij A, Chabowski A, Holland RJ, Saavedra JE, Keefer LK, Chlopicki S: Hepatoselective Nitric Oxide (NO) Donors, V-PYRRO/NO and V-PROLI/NO, in non-alcoholic fatty liver disease: a comparison of anti-steatotic effects with the biotransformation and pharmacokinetics. Drug Metab Dispos 2015;43:1028–1036.
23 Ijaz S, Yang W, Winslet MC, Seifalian AM: The role of nitric oxide in the modulation of hepatic microcirculation and tissue oxygenation in an experimental animal model of hepatic steatosis. Microvasc Res 2005;70:129–136.