The therapeutic mechanisms and beneficial effects of ursodeoxycholic acid in the treatment of nonalcoholic fatty liver disease: a systematic review

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease, with an increasing prevalence in all regions of the world. Its spectrum includes hepatic steatosis (HS) and non-alcoholic steatohepatitis (NASH) with progression to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). NAFLD may represent the hepatic manifestation of the metabolic syndrome (MS), with a prevalence directly proportional to the prevalence of obesity and MS. The standard treatment for patients with NAFLD is lifestyle modification, which in medical practice has many limitations. To overcome them, numerous drugs with benefits in the prevention and treatment of the disease have been studied. Currently, the most used substances are vitamin E and Pioglitazone, with numerous benefits. Furthermore, new strategies and beneficial treatments are needed for the prevention of the disease, which is currently a priority in both the health and research fields. One of the most studied agents in the last decades has been ursodeoxycholic acid (UDCA), which is of great interest in the treatment of NAFLD due to its hepatoprotective effects.

Keywords: non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, therapy, ursodeoxycholic acid

Introduction

Worldwide, non-alcoholic fatty liver disease (NAFLD) is the most common cause of diffuse chronic liver disease [1]. Soon, NAFLD is expected to become the most common clinical form of the chronic liver disease progressing to end-stage cirrhosis and hepatocellular carcinoma (HCC), consequently becoming the main indication for liver transplantation [2,3]. NAFLD comprises a wide spectrum of diseases characterized by an accumulation of lipids in the liver, apart from other causes of secondary accumulation, such as chronic alcohol consumption, treatment with corticosteroids, methotrexate, amiodarone, and chronic hepatitis C virus infection. Hepatic steatosis (HS) represents the benign form of the disease, characterized by a simple accumulation of triglycerides in the liver. Non-alcoholic steatohepatitis (NASH) represents the severe form of the disease characterized by an accumulation of triglycerides with the appearance of inflammatory infiltrate and cell apoptosis, with an evolution towards fibrosis and, subsequently, cirrhosis [4-7]. The prevalence of the disease has increased rapidly in the last decade in western countries, currently the worldwide prevalence of NAFLD reaching 25%, with great potential to become the most common chronic liver disease in western countries, especially in patients with type 2 diabetes mellitus (T2DM), abdominal

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obesity, dyslipidemia and metabolic syndrome (MS) [8]. NAFLD is considered the hepatic manifestation of MS, being strongly associated with insulin resistance (IR), impaired glucose tolerance, T2DM, abdominal obesity, hypertriglyceridemia, and hypertension [9]. The prevalence of the disease is directly proportional with the prevalence of obesity and MS, this association being responsible for the increase in the number of cardiovascular and oncological diseases, as well as morbidity and mortality from liver causes [10-14]. To understand the clinical behavior of the disease, great efforts have been made in the last decades to explore the natural history of the disease [11]. This has allowed the development of diagnostic strategies with the ultimate goal of preventing the occurrence of hepatic and extrahepatic complications [15].

Currently, the gold standard for the diagnosis of the disease is the liver puncture biopsy (LPB) which reveals various structural changes, which vary from the simple deposition of triglycerides in the liver in the form of lipid droplets, to extensive forms of steatohepatitis that associate inflammatory infiltrates and a variable degree of fibrosis. Most patients present "non-progressive" forms of the disease, but a small part of them develop NASH with evolution to liver failure and HCC [8,16]. At this moment, there is no biological marker that can confirm the diagnosis of NAFLD or distinguish between HS, NASH, and cirrhosis [17]. A normal level of serum transaminases is found in 78% of the patients, although the main biological change in these patients is the increase of the serum level of transaminases that does not exceed more than four times the normal limit [8,18,19]. An increase in gamma-glutamyltransferase (GGT) levels can also be observed, which has been associated with increased mortality in several studies [20,21] and advanced fibrosis [22]. Alkaline phosphatase (ALP) may also be elevated in these patients, but it is rarely the only abnormality of liver involvement [23]. Advanced stages of the disease that evolve with cirrhosis also associate hyperbilirubinemia, hypoalbuminemia, thrombocytopenia, and a prolonged prothrombin time [24]. In healthcare facilities, the most accessible method of diagnosing HS is ultrasound examination, an accessible and less expensive method. The diagnosis of non-alcoholic liver disease is established after secondary causes of HS have been ruled out. Also, several validated scales are used for the diagnosis of the disease, such as the fatty liver index (FLI), the liver fibrosis index (FIB-4), and the non-alcoholic fatty liver fibrosis score (NFS) [25].

Currently, the most effective therapy appears to be weight loss, which is a key step in both NAFLD treatment and cardiovascular disease pathogenesis [26]. The drugs currently accepted for the treatment of SH intervene at different stages of the pathogenesis, including the correction of IR. The main therapeutic options are Pioglitazone, statins, antioxidant and liver cytoprotective drugs, such as UDCA, vitamin E, omega-3 polyunsaturated fatty acids, and obeticholic acid [27-34]. The effects of UDCA in NAFLD can be explained by the numerous antioxidant mechanisms on dyslipidemia and the reduction of the risk of cardiovascular disease by the protective effects on iron-dependent oxidative reactions and hydroxide radicals. It also inhibits lipid peroxidation products and prevents oxidative stress induced by reactive oxygen species (ROS) [35-43]. Recently, it has been shown that physiological concentrations of ROS increase with arterial intima thickening, and atherosclerosis is stimulated by the peroxidative glutathione redox state [39,40]. Given the lack of an approved treatment for NAFLD, in recent years numerous authors have studied the effects of UDCA in the treatment of non-alcoholic liver disease. In this regard, we aimed to present the main studies that investigated the pathogenic mechanisms and the beneficial impact of UDCA treatment among patients with NAFLD.

Material and methods

The systematic review was conducted and reported according to Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA).

Sources and search strategies

The most relevant articles for our review were obtained from databases such as PubMed, PubMed Central and ScienceDirect using keywords such as "Non alcoholic fatty liver disease", "Non-alcoholic steatohepatitis", "treatment", "ursodeoxycholic acid" using also AND and OR Boolean.

Eligibility criteria

To choose the best articles on our research topic, we took into account a series of inclusion criteria, such as: (i) any article that included the treatment strategy of fatty liver disease; (i) the latest treatment guidelines; (i) articles that included general data about NAFLD and NASH; (i) the most relevant studies and their results about the effects of UDCA treatment in NAFLD; (i) articles written in English. The exclusion criteria were: (i) articles that included alcoholic fatty liver disease; (i) any work without abstract; (i) studies without material and methods.

Screening

The screening process started with the filtering of a large number of articles from the selected databases, and then review articles or research articles were selected. Next, a list of articles was composed in Microsoft Excel, from which only the articles with titles and summaries were selected in accordance with our theme and finally the inclusion and exclusion criteria were applied.

Results

In total, we identified a number of 920 articles from the selected databases. Of these, 440 were eliminated after applying filters, such as review articles and research articles. A number of 480 articles remained, of which 257 were selected from Pubmed and PubMed Central and 223 from

ScienceDirect. Subsequently, 20 articles were removed because they were duplicates. The screening imposed the selection of the most relevant titles for our topic, eliminating another 320 articles. Next, 33 articles were eliminated because we did not have access to their summaries or because they were not related to our question. We also removed 30 articles from the 107, because of inaccessible full text and 47 articles after applying inclusion/exclusion criteria. Finally, the remaining 30 articles contributed to the creation of the review (Figure 1).

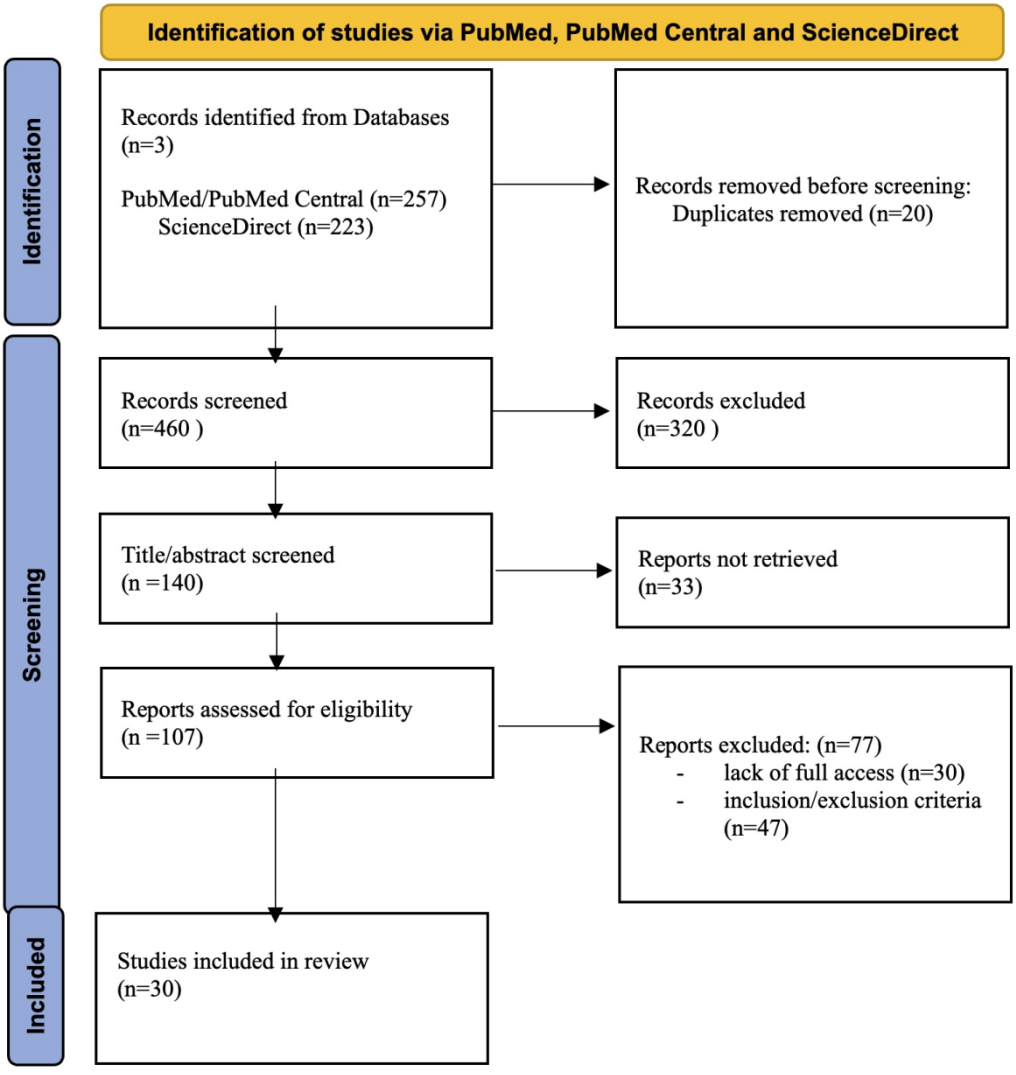

Figure 1. PRISMA flow diagram for systematic review.

Discussion

The pathogenic basis of UDCA treatment in NAFLD

Initially, the pathogenesis of metabolic liver disease was conceived in the form of the "two-hit" theory [44]. The "first hit" involves the accumulation of lipids at the level of hepatocytes, an accumulation that favors IR, the key element in the development of HS, and the "second hit" is the consequence of the action of cytokines, pro-inflammatory adipokines, but also of oxidative stress, mitochondrial dysfunction, and the endoplasmic reticulum. All these pathogenic mechanisms result in hepatocytic damage, inflammation, and fibrosis.

In recent years, numerous authors [45,46] have reported that steatohepatitis is associated with an unfavorable outcome and for this reason, SH and NASH are considered two different entities. Moreover, after the description of the progressive form of NAFLD, represented

by NASH, from a pathogenic point of view, the "multiple hits" theory was proposed [47]. This concept suggests that the various pathogenic mechanisms that cause liver damage proceed in parallel and not consecutively. Therefore, all these events favoring disease progression to steatohepatitis represent possible therapeutic targets. The mechanisms involved in the development of steatohepatitis emphasize IR and systemic inflammation, both being the main elements in the progression of SH to steatohepatitis.

Molecularly, IR is the result of the involvement of genetic and non-genetic mechanisms initiating a vicious cycle that predisposes inflammation, hypercoagulability, and atherogenesis. Vascular IR develops at first, then liver and adipose IR are established. All these changes explain the high cardiovascular risk in patients with IR [48].

Tumor necrosis factor-α (TNF-α) plays an important role in the development of the inflammatory state, which in turn causes apoptotic, fibrogenic reactions and regulates IR [49]. Moreover, oxidative stress is considered to be an important stage in the development of steatohepatitis, being the consequence of mitochondrial dysfunction that generates ROS species and favors the dysfunction of DNA, lipid, and protein membranes [50,51]. Oxidative stress of the endoplasmic reticulum in adipose tissue and liver stimulates de novo lipogenesis, lipid storage, and synthesis of cytokines and adipokines, with a role in the progression of steatohepatitis [52].

In recent years, the role of the intestinal microbiota in the pathogenesis of NASH has generated particular interest, as changes in the intestinal composition could generate an increase in intestinal permeability, but also the translocation of bacterial endotoxins that favor IR and systemic inflammation [53]. The most studied endotoxin is lipopolysaccharide (LPS), which stimulates hepatic infiltration with neutrophils in subjects with NASH [54].

Bile acids are key regulators of glucose homeostasis through several pathways that share the regulation of both glucose and cholesterol metabolism. In conditions of T2DM, the composition of bile acids is altered and a reduction in bile secretion in the intestine is observed. Also, patients with NAFLD can develop a state of hyperinsulinemia that stimulates lipogenesis and the deposition of hepatic lipids involved in the development of liver diseases [55,56]. Also, many authors support the relationship between changes in cholesterol homeostasis and the hepatic accumulation of free cholesterol, an important stage in the pathogenesis of NASH [57,58] Moreover, the accumulation of free cholesterol at the level of the endoplasmic reticulum membrane will cause the decrease of its fluidity and the appearance of cellular stress and apoptosis [59,60].

Histopathological basis of UDCA treatment in NAFLD

The histopathological aspects found in patients with NAFLD, in most cases, cannot be distinguished from those found in alcoholic liver disease, so the pathologist must rely on the attending physician to exclude alcohol-induced liver disease [61]. Histopathologically, for diagnostic purposes, NAFLD is divided into HS (predominantly macrovesicular steatosis with or without the association of non-specific inflammation) and NASH.

From a pathological point of view, NASH is characterized by: macrovesicular steatosis, predominantly lobular inflammation, hepatocytic ballooning, apoptotic bodies, and Mallory-Denk bodies (MDB). In evolution, patients can also associate a certain degree of fibrosis, which is not necessary for diagnosis.

Steatosis, the common histopathological feature of the NAFLD spectrum, is a nonspecific lesion that can be seen in many liver diseases. To be considered significant, steatosis must affect more than 5% of total hepatocytes [62]. In NAFLD, the appearance of steatosis is macrovesicular [63], with hepatocytes showing cytoplasm of a foamy appearance, an appearance observed in single or grouped hepatocytes, never with a diffuse distribution. In the early stages, SH is located in zone 3, but as the disease progresses, the steatosis can be evenly distributed throughout the liver acinus, or it can take on an irregular appearance [64].

In NASH, the inflammatory infiltrate is mixed, mainly with a lobular distribution, composed of a mixture of CD8+, CD4+ lymphocytes, and Kupffer cell aggregates [65]. Another characteristic of NASH is portal inflammation dominated by CD8+ T lymphocytes and macrophages, which is associated with disease progression to fibrosis [66-68]. The degree of portal inflammation correlates with the intensity of the ductal reaction.

A ductal reaction is a reactive canalicular lesion that affects the bile ducts at the level of the port space, being located at the interface of this space. This reaction occurs secondary to hepatocyte regeneration and proliferation of hepatic progenitor cells (CHP). CHPs are bipotential cells capable of proliferation and differentiation into either hepatocytes to replace injured cells or cholangiocytes. CHP activation followed by the ductal reaction are common cellular responses to chronic hepatocyte injury and precede the onset of progressive portal fibrosis. Therefore, reducing the ductal inflammatory reaction is an important goal in the therapeutic management of NASH [67].

The major characteristic observed in patients with NASH is hepatocyte ballooning, its presence being associated with an increased risk of progression to cirrhosis [69]. Apoptotic bodies represent another variety of lesions that can be found in NASH, which, however, do not represent a histopathological feature for this disease, as they can also be observed in viral hepatitis [70]. Also, the histopathological examination of patients with NASH can reveal megamitochondria, which can also be found in patients with alcoholic steatohepatitis [71].

Iron deposition was frequently observed in biopsy specimens from patients with NAFLD, with iron being deposited in both hepatocytes and reticuloendothelial cells. The significance of iron accumulation is currently unclear, but numerous studies support that reticuloendothelial iron is associated with an advanced stage of fibrosis [72,73].

In progress, NASH associates liver fibrosis, present perisinusoidal and pericellular changes at the level of zone 3, with a "chicken wire" appearance, a typical appearance found in the histopathological examination of patients with steatohepatitis. As the disease progresses, fibrosis also occurs in the port space, which in the absence of treatment evolves into fibrosis and cirrhosis [74]. A meta-analysis that included ten histopathological studies of patients with NASH demonstrated that the presence of inflammation in the initial stages and age are independent predictive factors of the progression of steatohepatitis to advanced fibrosis [68]. Another recent study associated the presence of portal inflammation, hepatocyte ballooning, and fibrosis with increased mortality in these patients [75].

Clinical and biological basis of UDCA treatment in NAFLD

Cholestasis is determined by the alteration of bile synthesis and secretion and/or bile flow in the bile ducts [76]. The symptoms of cholestasis syndrome are generated by the accumulation of products excreted by the liver through bile in the blood.

One of the most common symptoms is steatorrhea characterized by a loss of more than 10g of lipids in the feces after consumption of 70g/day [77] as a result of inadequate postprandial bile secretion in the small intestine. All these events lead to malabsorption syndrome for fat-soluble vitamins [78,79]. Manifestations of the malabsorption syndrome are also determined by vitamin A deficiency, which associates with night vision deficiency, vitamin E deficiency, which is manifested by hyporeflexia or ataxia, vitamin K deficiency, which predisposes to coagulation disorders, and vitamin D deficiency, which is manifested by disorders of the musculoskeletal system [77-79]. In evolution, chronic liver diseases that associate cholestasis can evolve to the stage of portal hypertension with the appearance of ascites, encephalopathy, and upper digestive hemorrhage [77]. The clinical examination reveals jaundice and pruritus without grating lesions, xanthomas, and xanthelasmas [76,80].

Biologically, the markers of cholestasis syndrome are ALP and GGT which are located at the plasma membrane of hepatocytes and are released into the circulation as a result of the action of bile acids. Cholestasis syndrome can also associate with conjugated hyperbilirubinemia as a result of the inability of the liver to excrete bile [81]. On the other hand, the biological markers of hepatocellular cytolysis, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are located in the cytoplasm and show elevated values.

Effects of UDCA treatment on NAFLD

The standard treatment for patients with NAFLD emphasizes lifestyle change. Because this approach has many limitations, the use of multiple drugs has been proposed.

First-line drugs are vitamin E and Pioglitazone, both of which have positive effects on serum transaminases, inflammation, and lipid accumulation. Despite these effects, vitamin E has no effects on fibrosis and long-term mortality and morbidity, and Pioglitazone has negative effects on weight. Other drugs, such as Metformin, UDCA, statins, Pentoxifylline, and Orlistat, have also been studied, but with partially positive results. The pathogenesis of steatohepatitis is complex, thus the association of several products that act in different stages of pathogenesis has been proposed, requiring extensive studies with long-term results [82].

Ursodeoxycholic acid (3α, 7β-dihydroxy-5β-colonic acid; UDCA) is a hydrophilic bile acid that is currently increasingly used in the treatment of cholestatic disorders [83,84]. The first effects of UDCA in patients with liver diseases were reported in Japan in 1961 [85]. Later, a series of studies on the use of UDCA in primitive biliary cirrhosis (PBC) and primitive sclerosing cholangitis (PSC) was presented [86]; moreover, currently, UDCA is the only drug approved for the treatment of PBC [87]. In cholestatic syndromes, the main mechanisms underlying the positive effects of UDCA are increasingly being studied. Experimental studies suggest three main mechanisms: stimulation of hepatobiliary secretion, protection of hepatocytes against bile acid-induced apoptosis, and protection of cholangiocytes against bile acid cytotoxicity. All these mechanisms may be important in individual cholestatic diseases or different stages of chronic cholestatic liver diseases [88,89].

In recent decades, UDCA has been of interest for the treatment of NAFLD patients due to its hepatoprotective effects [90]. The beneficial effects of UDCA treatment have been studied by numerous authors [91-94], that demonstrated that UDCA intervenes in the process of hepatocytic apoptosis, which is a characteristic of patients with NASH, but also reduces the serum level of TNF-α, which is increased in these patients and aggravates IR. Due to these beneficial effects, the use of UDCA in these patients has numerous benefits on disease progression.

The experimental studies of Ozcan and Kars [95,96] demonstrated that UDCA conjugated with taurine (TUDCA) can influence endoplasmic reticulum stress and improve muscle and liver sensitivity to insulin in obese subjects with IR. Since the cellular mechanisms of these effects are still unknown, further research is needed.

Table I. Characteristics and summary of included studies.

Author	Total number of patients	Inclusion criteria	Drug and dose	Study duration	Result
Laurin J et al. Hepatology, 1996 [33]	40 patients	NASH confirmed by LBP	24 patients received a dose of 13-15 mg/kg/day UDCA and 16 patients with hypertriglyceridemia received 2g/day Clofibrate	12 months	Serum ALP, GGT, ALT, and HS significantly improved after UDCA treatment compared to Clofibrate treated patients who did not show any improvement.
Lindor et al. Hepatology, 2004 [102]	166 patients	NASH confirmed by LBP	86 patients received a dose of 13-15mg/kg/day UDCA and 80 patients received a placebo	24 months	No significant improvements were reported between the two groups of patients in the degree of steatosis, necroinflammation, or fibrosis.
Dufour et al. Randomized Controlled Trial, 2006 [34]	88 patients	NASH confirmed by LBP	15 patients received a dose of 12-15 mg/kg/day UDCA and vitamin E 400 IU twice a day; 18 patients received UDCA and a placebo; 15 patients only placebo therapy	24 months	In patients treated with UDCA/vitamin E, the serum level of AST, and ALT significantly decreased and the histopathological activity index improved.
Hong Qian et al. J Gangdong Med College, 2007 [103]	52 patients	NASH confirmed by LBP	26 patients received a dose of 15-20mg/kg/day UDCA in three doses and 26 patients Essentiale Forte two capsules three times a day	6 months	A significant reduction in plasma levels of AST, ALT, GGT, TG, TC, and CRP was observed in both patient groups. However, TG and TC levels decreased more significantly in UDCA-treated patients.
Leuschner et al. Hepatology, 2010 [35]	185 patients	Proven NASH based on modified Brunt score and NAS score	94 patients received a high dose of 23-28 mg/kg/day UDCA and 91 patients received a placebo	18 months	No significant histopathological improvements were reported in the two groups. However, UDCA improved GGT levels.
Ratziu et al. Hepatology, 2011 [30]	126 patients	NASH evidenced by LBP and elevated ALT	62 patients received a high dose of 28-35mg/kg/day UDCA and 64 patients received a placebo	12 months	The serum ALT level was significantly reduced (18.3%) after the high dose of UDCA compared to the placebo group (1.6%).
Mueller et al. J Hepatol, 2015 [104]	40 patients	NAFLD with morbid obesity	19 patients received a double dose of 20mg/kg/day UDCA and 18 patients were controls,	3 weeks	The group of patients treated with UDCA showed a decrease in the serum level of ALT, GGT, free fatty acids, TC and HDLc and an increase in the level of TG.
Oliviera et al. Arq Gastroenterol, 2019 [105]	53 patients	NASH evidenced by LBP	26 patients used NAC(1.2g)+UDCA(15mg/kgc)+MTF(800-1500mg/day) 13 patients: UDCA(20mg/kg)+MTF(800-1500mg) 14 patients: NAC(1,2g) + MTF(850-1500mg)	48 weeks	There were no significant changes in biochemistry and histology in the three groups of patients. In the intragroup analysis, the degree of steatosis, ballooning, the NAFLD activity score and the ALT level were changed at the end of the NAC+MTF treatment.
Nadinskaia et al. World J Gastroenterol, 2021 [106]	174 patients, 121 men and 53 women	NAFLD proved by abdominal ultrasound, FLI, and noninvasive fibrosis scores	15mg/kg/day of UDCA	6 months	Gender differences were observed; men showed a significant decrease in serum ALT, AST, and GGT levels at both 3 months and 6 months, while women showed a decrease in only ALT levels in both periods.
Fouda et al. Eur Rev Med Pharmacol Sci, 2021 [107]	102 patients	Diagnosed with NAFLD	Vitamin E 800 mg/day, UDCA 750 mg/day and Pentoxifylline 800 mg/day	3 months	The three drugs improved the serum level of transaminases and inflammatory markers.
Seo et al. Gastroenteroly Report, 2022, [108]	Mouse models	Mice with NAFLD injected with thioacetamide and fed a high-fat diet	Both low (15m/kg) and high (30mg/kg) doses of UDCA and the combination of Ezetimib/Rosuvastatin (1mg/kg) were used.	3 weeks	The simultaneous administration of UDCA and RSV/EZE significantly decreased the accumulation of collagen but also the serum level of ALT. Histopathologically, the combination decreased the number of apoptotic cells.

NAFLD: non-alcoholic fatty liver disease; HS: hepatic steatosis; NASH: non-alcoholic steatohepatitis; LBP: liver biopsy puncture; UDCA: ursodeoxycholic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyltransferase; ALP: alkaline phosphatase; TG: total triglycerides, TC: total cholesterol; HDLc: high-density lipoprotein; CRP: C reactive protein; NAS: fatty liver activity score; FLI: fatty liver index; NAC: N-acetylcysteine; MTF: Metformin; RSV: Rosuvastatin; EZE: Ezetimibe.

The presence of liver fibrosis in patients with NAFLD is the main determinant of the prognosis of the disease, which requires an aggressive pharmacological approach [97].

Currently, in the USA, no pharmacological agent has regulatory approval, the central management focusing on reducing obesity by improving the lifestyle that involves a diet rich in monounsaturated acids but also surgical or endoscopic interventions [98].

Over time, scientific societies have developed several guidelines for the management of patients with NAFLD [99].

The best known societies involved in the management of the disease are the American Association for the Study of Liver Diseases (AASLD), which published practice guidelines in 2018 and 2023, and also the European Association for the Study of the Liver [EASL], the European Association for the Study of Diabetes [EASD] and the European Association for the Obesity Study [EASO] which published guidelines in 2016.

The 2016 EASL-EASD-EASO recommendations were for the use of Pioglitazone which can be used in the treatment of patients with NAFLD/NASH and diabetes, while glucagon-like peptide 1 (GLP-1) agonists and UDCA did not present sufficient evidence to demonstrate their use [99].

Also, the 2018 AASLD issued recommendations similar to EASL-EASD-EASO for Pioglitazone, GLP-1 and UDCA, this one additionally including vitamin E that can be used in non-diabetic patients [100].

Later, in the new AASLD guide from 2023, they were accepted as a GLP-1 treatment line with its representatives Liraglutide which proved its effects on steatosis, steatohepatitis, but also on fibrosis, while Semaglutide only improved NASH, without significant effects on fibrosis. Also in the 2023 guide, UDCA, Metformin, polyunsaturated fatty acids, Ezetimib and Silymarin were recommended but without strong evidence on the histology of the disease, they offer cytoprotection and intervention on immunity [101].

Numerous studies have been conducted to highlight the beneficial impact of UDCA in patients with NAFLD. The results and effects of UDCA treatment in patients with NAFLD are shown in table I.

Studies in the literature have used UDCA both alone and in combination with other drugs.

Monotherapy significantly improved liver function by improving ALT, AST, and GGT levels [32,102,109-111], but also reduced the degree of steatosis and fibrosis [32,98]. For NASH, the effects are biologically beneficial.

The effects of UDCA in animals were studied by Castro and colleagues [112] who reported the improvement of SH and the inflammatory process by inhibiting the miR-34a/SIRTI1/p53 pathway. Although this mechanism is not yet clear, the study by Beuers [113] concluded that UDCA protects the hepatocytes as a result of inhibiting the absorption of toxic bile salts in the small intestine.

Over time, existing studies in the literature have demonstrated that UDCA has anti-inflammatory, antioxidant and anti-fibrotic effects, even in patients with NASH. However, the effects on histology and the improvement of liver function in NASH patients still remain unclear.

In 2020, Zhang et al. [114] starting from the increased prevalence of NAFLD in the general population [115,116] and from the need to develop therapeutic methods to help prevent NAFLD [7], they performed a meta-analysis on a number of randomized controlled trials (RCTs) that have emerged to verify the role of UDCA in NAFLD. In this meta-analysis, they analyzed the impact of UDCA treatment on ALT, AST, GGT, ALP, bilirubin and total albumin levels. They showed that the UDCA was indeed beneficial in lowering the ALT levels among NAFLD patients which promotes the disease recovery [114].

To evaluate the effectiveness of the treatment, Lin's most recent study from 2022, which included 8 studies and a total of 655 participants, showed that UDCA has effects on the blood concentration of ALT, GGT, but without any significant effect on anthropometric and histopathological characteristics. In conclusion, studies are needed to prove the effectiveness of the treatment in patients with NASH [117].

The development of new strategies and treatments for the prevention of NAFLD is a priority in both the research and health fields [7]. Current clinical trials focus on the pharmacological effects on NAFLD of GLP-1 receptor agonists and sodium glucose cotransporter-2 (SGLT2) inhibitors [118].

GLP-1 stimulates the secretion of insulin at the level of pancreatic beta cells, suppresses the motility of the upper gastrointestinal tract with a beneficial effect on weight loss by inducing the feeling of satiety in patients with T2DM [119]. For GLP-1 analogues two randomized studies of phase II showed a histological resolution, but the effects on liver fibrosis are still unclear, requiring additional studies [120,121].

Regarding SGLT2 inhibitors, they block the reabsorption of glucose filtered by the glomeruli at the level of the proximal renal tubules and thus cause a decrease in blood sugar. Their use in NAFLD is supported by numerous reports indicating that SGLT2 inhibitors improve liver function by decreasing steatosis. Instead, effects on the improvement of inflammation, ballooning and fibrosis have been reported in a small number of patients, requiring additional studies on histology [122].

In 2022, Elhini and colleagues [123] starting from a series of previous studies carried out on patients with diabetes [124-127] that highlighted the beneficial role of Empagliflozin (EMPA), an SGLT2 with hypoglycemic effect [128,129], which improves any aspects of the

MS and from antioxidant, anti-inflammatory, and anti-apoptotic properties [130] of UDCA aimed to evaluate the differences between EMPA and UDCA in terms regarding safety and efficacy as adjunctive therapy in LFC regression and fibrosis in T2DM patients with NAFLD. The authors noted that in diabetic patients with EMPA would present a better glycemic profile, and serum triglycerides reduction, but also a greater liver steatosis regression than UDCA. On the other hand, UDCA improved IR and liver fibrosis scores more than EMPA. Both drugs were comparable in decreasing liver enzymes and BMI. Finally, the authors concluded that these findings to suggest that both EMPA and UDCA could be used safely and effectively for NAFLD patients with diabetes [123].

Also, another antidiabetic drug used in the treatment of NAFLD is Pioglitazone, which acts on the nuclear receptor (gamma receptor activated by the peroxisome proliferator). Recently, a study by Pepa et al. evaluated the effects of treatment with Pioglitazone and sulfonylurea (Glibenclamide, Glicazide and Glimepiride) for 1 year in patients with NAFLD and diabetes poorly controlled with Metformin. The doses used were 26mg/day Pioglitazone, 5mg/day Glibenclamide, 36mg/day Glicazide and 2.6mg/day Glimepiride. Pioglitazine treatment improved NAFLD and IR indices, something that sulfonylureas failed to produce. In conclusion, Pioglitazone treatment has significant effects on steatosis, liver inflammation, systemic resistance and adipose tissue to insulin in patients with diabetes [131]

Another randomized double-blind study included 120 patients with NAFLD who were randomly divided into 4 groups and received treatment with Metformin, Melatonin, UDCA and placebo for 3 months, studied the effects of these preparations in patients with NAFLD. The results study demonstrated that the group of patients treated with Metformin and Melatonin and a low-calorie diet for 3 months had beneficial effects on steatosis, the serum level of ALT, ALP and TG compared to the group of patients treated with UDCA and the hypocaloric diet. However, treatment with UDCA and Metformin improved the serum concentration of triglycerides and serum glucose, respectively, but without effects on fibrosis, AST, glycated hemoglobin, LDL or HDL. All these results demonstrate that treatment with Metformin and Melatonin plus a hypocaloric diet has significantly better benefits on NAFLD compared to UDCA [132].

The meta-analysis by Sánchez-García et al. [133] in 2018 showed a significant decrease in fasting plasma glucose, glycated hemoglobin (HbA1c) and insulin concentrations after UDCA therapy, suggesting a positive impact of UDCA on glucose homeostasis. The authors tried to identify the biological mechanisms that explain these effects. They showed that weight loss can be associated with the beneficial effects of UDCA on glycemic control and insulin sensitivity, according to studies by [134,135]. They also indicated that UDCA decreases serum levels of TNF-α [94], a pro-inflammatory cytokine that inhibits insulin signaling and its biological actions [136] suggesting a possible metabolic pathway that could improve insulin sensitivity.

Tsuchida's study [137] found that UDCA treatment decreases hepatic insulin resistance by reducing hepatic glucose production, leading to improvement in hyperglycemia and hyperinsulinemia. On the other hand, analysis of the study by [138] indicated that UDCA increases glucagon-like peptide-1 secretion through TGR5 signaling and decreases circulating glucose concentrations through this mechanism. However, the underlying mechanisms by which UDCA improves glucose metabolism are unclear and remain to be elucidated in further studies.

Also, 24-Norursodeoxycholic acid (norUDCA), a C_{23} homologue of UDCA [139,140] had unique therapeutic properties in an in vivo model of cholestasis [141,142]. Since 2011, Beraza noted that the administration of norUDCA could also downregulate lipogenic and apoptotic pathways in the genetic NASH mouse mice, thus remarkably alleviating steatosis [143]. Steinacher et al showed that norUDCA is a promising new approach in the treatment of cholestatic and metabolic liver diseases [144]. Recently, Traussnigg et al. showed a dose-dependent reduction in serum ALT in patients treated with norUDCA compared with a placebo in a phase 2, multicenter, double-blind clinical trial [145]. He showed that norUDCA undergoes cholehepatic shunting, resulting in ductular targeting, bicarbonate-rich hypercholeresis, and cholangiocyte protection. Furthermore, it showed anti-fibrotic, anti-inflammatory, and anti-lipotoxic properties in several animal models. Later, Marcihiani et al were confirmed that norUDCA alleviated liver steatosis and fibrosis in a NAFLD/NASH model induced by the Western diet [146].

Currently, for the treatment of NAFLD, there are numerous therapeutic agents in studies that intervene in the process of initiation and evolution of the disease.

Recently, Marchianò's study [147] studied the effects of BAR502, a farnesoid X receptor (FXR) and GPBAR agonist that is in advanced clinical stages with important effects on reducing steatosis and fibrosis in rodent models of NAFLD and NASH. The study compared the effects of BAR502 both alone and in combination with UDCA in mouse models of NAFLD/NASH fed a chow diet for 10 weeks. The results of the study emphasized the role of the combined therapy BAR502 and UDCA which offered protection against liver damage induced by the western diet, reversed the pro-atherogenic profile but also reduced the expression of inflammatory markers. Also, the combined therapy intervened from the histopathological point of view, reducing the degree of steatosis, ballooning, inflammation, and fibrosis. This study highlights the

importance and potential role of combination therapy in NAFLD, combination therapy that intervenes in all key disease processes.

The effects of UDCA on steatohepatitis remain unclear due to significant differences between the studies performed in terms of protocols of conduct, inclusion and exclusion criteria, duration of studies and treatment, and also its combinations with different therapeutic agents. Therefore, no additional research is currently available to support or refute UDCA treatment in patients with NASH [148].

Conclusions

Studies in the literature indicate that UDCA is useful in steatohepatitis, especially in combination with other drugs. However, the current evidence regarding the benefits of UDCA in the treatment of NAFLD is not sufficient for this preparation to be approved by the health authorities. Furthermore, further studies are needed to demonstrate the impact of UDCA on liver function and histopathological changes in patients with NASH. Therefore, currently, the main line of treatment for NASH is lifestyle change.

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