TANG HUMANITAS MEDICINE

Review

Natural products in the research of cholesterol gallstones

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ABSTRACT

Cholesterol gallstones are a digestive disease of high prevalence that has many risk factors; for this reason, research has focused mainly on how to prevent it rather than how to treat it. Many molecules of the hepatic, bile and intestinal systems are involved in the pathophysiology of this disease, making it very difficult to find a therapeutic target. The pharmacological treatment is limited, so when gallstones generate symptoms, medical treatment indicates gallbladder removal. Ursodeoxycholic acid is used to dissolve cholesterol stones, and ezetimibe and statins are other drugs with possible applications in the treatment of this disease. Given the small number of drugs that have been developed for treating this disease, the research of natural products becomes of paramount importance. Resources such as black radish, glucosinolates, fenugreek, capsaicin, curcumin, garlic, and onion, have all shown significant effects in the prevention and treatment of cholesterol gallstones. In this review, we made a synthesis of the scientific reports that deal with these natural products and that can serve as antecedents for finding a way to treat the most common disease of the gallbladder.

Keywords cholesterol gallstones, natural products, pathophysiology, prevention

INTRODUCTION

Cholesterol gallstones are a very common gastrointestinal disease in Western countries, China, U.S., Mexico, Argentina and Chile (Chen et al., 2012; Stinton and Shaffer, 2012). This disease causes high costs to health systems and millions of surgeries to remove the gallbladder (Portincasa et al., 2012; Reshetnyak, 2012). The treatment of this disease is a virtually paralyzed research focus, as, due to the increasing number of risk factors, preference has been given to preventing the development of gallstones. However, the pathophysiology of the disease is polygenic, involving biliary cholesterol secretion imbalances (ABC canalicular transporters: ABCB4, ABCB11, ABCG5/8 and protein NPC1L1) (Krawczyk et al., 2012; Portincasa and Wang, 2012; Wang et al., 2013), inflammatory reactions in the epithelium of the gallbladder (Maurer et al., 2009), mucin production (biliary sludge) (Chuang et al., 2012), and gallbladder motility disturbances (Lavoie et al., 2012; Portincasa and Wang, 2012). This makes it very difficult to search for a therapeutic target, as there are many points to be analyzed. The drugs used for the treatment of gallstones often have cholesterol-lowering properties (Lioudaki et al., 2011), but the only authorized drug is ursodeoxycholic acid. Statins and ezetimibe have properties for inhibiting the formation of gallstones, but their effects for the treatment are limited (Ahmed et al., 2011; Stein et al., 2012). Because of these limitations, the research of natural products is an alternative field that needs to be explored.

Important studies have shown that some natural resources have effects that serve to treat and inhibit cholesterol gallstones, as is the case of the black radish (*Raphanus sativus* L. *var niger*), which has the property of dissolving cholesterol gallstones in mice and lowering cholesterol and triglyceride levels in serum (Castro-Torres and Naranjo Rodríguez 2012, Castro-Torres et al., 2012). Fenugreek (*Trigonella foenum-graecum*) also exerts antilithiasic activity in rodents, with significant additional effects such as the reduction of plasma, liver and biliary cholesterol, and the reduction of LDL cholesterol and triglyceride levels (Reddy and Srinivasan, 2011); few natural products have shown a remarkable effect such as the dissolution of gallstones (antilithiasic agents) because studies are made with a preventive orientation (antilithogenic agents). Onion (*Allium cepa*) and garlic (*Allium sativum*) have the capacity to inhibit the formation of gallstones in mice by decreasing biliary cholesterol secretion (Vidyashankar et al., 2010). Capsaicin and curcumin also prevent the development of the disease in rodents fed a lithogenic diet (Shubha et al., 2011). Many of the drugs that are currently on the market were isolated from plants used in traditional medicine; therefore, it is necessary to use these resources in the study of cholesterol gallstones.

The traditional theory on the use of natural products reported in this article is scarce; most of them are used as condiments in many regions and, given these characteristics, there are few reports about their medicinal use. The black radish has not been subjected to ethnobotanical studies in the different countries where it has shown therapeutic properties, and neither has fenugreek. Research studies have started from

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traditional medicine data from each country, but these are not reported in complete ethnobotanical studies, so there is an open field for research on these subjects.

Black radish and glucosinolates

Raphanus sativus L. *var niger* (black radish) is a plant of the cruciferous family, widely used in Mexican traditional medicine for the treatment of pigment and cholesterol gallstones (Castro-Torres et al., 2012). Its use is widespread in many countries, such as Germany, USA, Hungary, Iran, France, Mexico, Egypt, and China. Significant ethnobotanic uses could only be verified in a preclinical study that used female C57BL/6 mice in which gallstones were induced by a diet enriched in cholesterol and cholic acid. After the mice generated gallstones they were administered black radish juice concentrate, diluted in water 10 and 100 times (0.1 ml/10 g mouse weight) for 6 days. With the treatment of juice concentrate diluted 10 times in water there were significant effects of dissolution of gallstones and lowering of cholesterol/triglyceride levels in serum ($p < 0.05$), compared with the group that received no treatment and that had gallstones (Castro-Torres et al., 2012). The study is preliminary and the action mechanism has not been described, but when the stones are formed, cholesterol is crystallized and wrapped in a glycoprotein matrix; therefore, the effects that could disintegrate the stones must necessarily occur in the gallbladder bile.

Secondary metabolites responsible for the antilithiasic effect must have affinity to transport cholesterol, because they disintegrate the stones, and given that the lipid does not accumulate in the plasma, there must necessarily be some elimination or accumulation pathway. In another study, methanolic extract of black radish at doses of 10, 100, and 1000 mg/kg inhibited cholesterol gallstones formation in mice; this extract decreased the expression of Abcg8 and Abcg5, the main biliary cholesterol transporters. On the other hand, mice that received methanolic extract (10 and 100 mg/kg) showed a lower expression of Abcb11 as compared to lithogenic diet group. Aqueous extract of black radish also inhibits cholesterol gallstones formation; this extract increased the expression of Abcb4 in mice (Castro-Torres et al., 2013).

The majority of the metabolites of black radish are glucosinolates, which exert significant therapeutic effects that can be used in the prevention and treatment of gallstones (Castro-Torres et al., 2013). The chemical structure of these metabolites contains nitrogen, sulfur and a carbohydrate; they are produced according to the needs of each plant. The most studied glucosinolates are glucoraphanin and glucoraphasatin, together with their respective hydrolysis products, raphasatin and sulforaphane. Their therapeutic effects have not been assessed in biological models for gallstones, but in models that involve imbalances present in this gastrointestinal disorder. Glucoraphasatin and raphasatin exert antioxidant effects in cell lines and *in vivo* models. Raphasatin is a potent inducer of enzymatic detoxification in liver cell lines (HepG2) at a concentration of 10 μM, and the antioxidant mechanism facilitates the increased expression of phase II metabolic enzymes: quinone reductase, hemoxigenase and thioredoxin reductase (Hanlon et al., 2007). Although these enzymes have not been associated with gallstones, its antioxidant capacity may be important for the prevention of this disease, because when the cholesterol concentration increases in either liver or gallbladder, reactive oxygen species are produced, damaging liver and biliary tissues. In addition, in gallstone disease there is a lack of antioxidant nutrients, and the concentration of glutathione, the main antioxidant in the body, is reduced. In Wistar rats fed a high-fat diet (2% cholesterol, 0.5% cholic acid and 20% fat) the blood antioxidant system becomes altered, but after being treated orally for 9 days with black radish juice (diluted 10 times in water ad libitum), rich in glucosinolates, the concentration of glutathione peroxidase, an important antioxidant, increases (Lugasi et al., 2005). The major metabolite of black radish is glucoraphasatin, likely to be involved in the therapeutic effects. Another important glucosinolate is glucoraphanin; its product is sulforaphane, an isothiocyanate with antioxidant capacity. A study evaluated various diets of lyophilized extracts of broccoli (*Brassica oleracea* var. *italica*), rich in glucoraphanin (4 μmol of glucoraphanin/g of diet) and sulforaphane (0.7 μmol of sulforaphane/g of diet), in Syrian hamsters treated with a hypercholesterolemic diet for 7 weeks. The diets were administered to provide 20 μmol of each glucosinolate per day, which produced the significant effect of decreasing hepatic cholesterol levels. The mechanism of action involved a decrease in the expression of the proteins binding to the sterol response element (SREBP-1 and SREBP-2) and of the fatty acid synthase, FAS (Rodríguez-Cantú et al., 2011). The decrease of hepatic cholesterol prevents a large secretion of biliary cholesterol and the development of gallstones; it can also prevent the development of hypercholesterolemia. These secondary metabolites, present in many plants of the family Brassicaceae, could in the future be employed for the prevention and treatment of gallstones.

Fenugreek

Fenugreek (*Trigonella foenum-graecum*) is a plant with a distinctive aroma used as a condiment, especially its seeds and leaves (Srinivasan, 2013). One study evaluated the effect of different diets enriched with fenugreek on the treatment of mice with gallstones. The rodents developed gallstones after being fed a high cholesterol diet for 10 weeks; after that time, the mice were treated with two diets containing powdered fenugreek (6% and 12%) for 10 days. The diet supplemented with 6% fenugreek produced a gallstone incidence of 54.6% in mice (n = 12), while the diet with 12% fenugreek generated the lowest incidence of lithiasis (40.5%); the results of the different diets were compared with the group with gallstones (incidence 100%) (Reddy and Srinivasan, 2011). In this paper the authors report an antilithogenic study, but in fact they show an antilithiasic effect because they treated diseased mice, instead of trying to prevent the development of the disease. Fenugreek-enriched diets significantly reduced ($p < 0.01$) plasma cholesterol, triglycerides and LDL cholesterol levels; for example, treatment with 6% fenugreek showed significant results with respect to plasma cholesterol (178.5 ± 11.4 mg/dL *versus* 352.0 ± 15.9 mg/dL in mice with gallstones) and LDL cholesterol (128.6 ± 8.50 mg/dL *versus* 309.1 ± 15.4 mg/dL in mice with gallstones). The diet with 12% of fenugreek also had significant effects for the decrease of this type of lipids. Regarding biliary lipids, fenugreek has the capacity to significantly reduce cholesterol and phospholipid levels, increasing the content of bile acids (229.4 ± 16.3 mM with the 6% fenugreek diet *versus* 173.2 ± 11.7 mM for the lithiasis group) (Reddy and Srinivasan, 2011). The latter bile solutes are precursors of bile salts, which, in abundance, can decrease biliary cholesterol by emulsifying the lipid and preventing its crystallization. The antilithiasic effects may be due to the capacity of bile salts to capture cholesterol from gallstones and dissolve them. However, the action mechanism used by fenugreek to increase the bile acid content must be the most important; a study that could contribute to its understanding is that of the expression of the bile salt export pump

(ABCB11/BSEP) found in the canalicular membrane of hepatocytes.

Garlic and onion

Garlic (*Allium sativum*) and onion (*Allium cepa*) are two internationally used condiments; their importance for gallstone disease stands out among their nutritional and therapeutic properties. The prevention of lithiasis using these two condiments was studied in albino mice; they were added in natural state and dehydrated to a diet rich in cholesterol and bile salts. The treatments contained 0.6% of garlic and 2% of onion and were administered for 10 weeks. After this time, the formation of gallstones in mice was inhibited in a range of 15-39%, with the additive effect of lowering biliary cholesterol, which explains that, in the absence of imbalances in the secretion towards the gallbladder, the development of gallstones is prevented (Vidyashankar et al., 2010). The different diets decreased serological cholesterol in the following range: 33, 29, 31 and 34% for treatments with raw garlic, dehydrated garlic, raw onion and dehydrated onion, respectively. There was also a decrease in hepatic cholesterol, a result that was complemented with the study of the expression of HMGCoA-reductase and of the limiting enzymes in the biosynthesis of bile acids cholesterol 7α-hydroxylase and sterol 27-hydroxylase (Vidyashankar et al., 2010). The lithogenic diet decreased the expression of the enzyme HMGCoA-reductase, but this does not necessarily mean that plasma cholesterol can decrease, as it may be a biological response of the liver to the high concentration of cholesterol arriving through the enterohepatic circulation from the lithogenic diet. The liver regulates its biosynthesis, limiting the enzymatic functioning when the amount of cholesterol is high. The garlic and onion treatments increased the expression of this limiting enzyme in cholesterol biosynthesis, with percentages of expression of 92, 44, 61 and 35% with raw garlic, dehydrated garlic, raw onion and dehydrated onion, respectively (Vidyashankar et al., 2010). This result may seem contradictory with the cholesterol-lowering effect reported for these natural products; however, this is not so, because garlic and onion increase the expression of the enzymes that limit the metabolism of bile acids, and this indicates that cholesterol is converted to these solutes, which are precursors of the bile salts responsible for transporting excess cholesterol and water-insoluble solutes. The results of this work are very interesting, because the therapeutic effect must be related to these important enzymes of the metabolism of cholesterol and bile acids.

Capsaicin and curcumin

Capsaicin is a chemical compound present in plants with pungent properties of the genus *Capsicum (*Manjunatha and Srinivasan, 2007). The pungent taste of these plants is the reason for their use in the gastronomy of different countries. Curcumin is a yellow colorant from turmeric (Murakami, 2013), grown mainly in India.

In a study aimed to the prevention of gallstones, OUTB/Swiss albino mice were exposed to a lithogenic diet for 10 weeks. The treatments with 0.015% capsaicin, 0.2% curcumin and a mixture of both compounds (0.015% capsaicin/0.2% curcumin) were concomitant with the lithogenic diet (Shubha et al., 2011). These treatments prevented the formation of gallstones in mice: 50%, 66% and 56%, respectively. Capsaicin and curcumin inhibit total cholesterol and LDL cholesterol levels in serum without altering the content of HDL. Moreover, these chemicals have the property of decreasing liver and biliary cholesterol, making improving the content of phospholipids and bile salts (Shubha et al., 2011). This work also studied the antioxidant activity, an important aspect in gallstone disease, as the content of cholesterol can generate reactive oxygen species that damage the gallbladder tissue. The catalase activity was not changed by the lithogenic diet or by the administration of capsaicin and curcumin, but the activity of the enzyme glutathione reductase decreased 42% due to gallbladder lithiasis, whereas the treatment with the mixture of capsaicin/curcumin increased the activity of this enzyme by 2.5 compared with its activity in untreated mice (control). Capsaicin and its mixture with curcumin, increased glutathione S-transferase levels by 20.7% and 42% respectively (Shubha et al., 2011). These components of peppers and chillies have important effects for the prevention of gallstones, and the antioxidant pathway may be involved in it.

DISCUSSION

The research of natural products that could be used in the treatment and prevention of cholesterol gallstones is very important. Because this disease is a public health problem, there is a need for agents that are able to dissolve gallstones; therefore, in our opinion, natural products that have antilithiasic effects are more important than those with antilithogenic effects. In some countries where there are many risk factors for developing lithiasis, preventive methods are priorities in research. It is necessary to mention that some natural and pharmacological products may have effects that can prevent and treat cholesterol gallstones, because, if we look at the pathophysiology of the disease, we find imbalances in the transport of biliary lipids, mainly of cholesterol, the key molecule that needs to be targeted for either treating or preventing gallstones. Pharmacological agents already exist on the market, such as ursodeoxycholic acid, ezetimibe and statins, that have proved to be antilithogenic agents in basic and clinical studies; the mechanism of action of these drugs reduces cholesterol levels. Statins have not been effective in treating gallstones, while ursodeoxycholic acid and ezetimibe have demonstrated antilithiasic effects. Fenugreek and black radish are natural products that have also shown preventive and curative effects. The medical algorithm for treating biliary lithiasis clearly indicates that cholecystectomy is the indicated therapy when symptoms appear, but if they do not appear, there is still the possibility of trying to dissolve the stones with a pharmacological agent. This is overlooked in many societies, because, after the detection of gallstones by ultrasound and with no presence of symptoms, cholecystectomy is also indicated, ignoring the possibility of initiating a pharmacological or alternative therapy.

Research in natural products for this disease is scarce and not very advanced in the molecular aspect. The effects reported so far are preliminary, especially considering that biliary lithiasis is a polygenic disease, but if curative treatments are going to be developed, new research should be based on the physico-chemical imbalance that occurs within the gallbladder, because that is the place where the mechanism of action to disintegrate gallstones must work. The chemical processes by which cholesterol crystallizes have not been understood yet, nor is yet known which processes could be applied to change its state and dissolve its aggregates. Biliary secretion is also very important for determining a treatment, as is the analysis of the proteins that transport the three biliary lipids; however, no such studies have been reported dealing with the use of natural products. Clinical trials cannot be done yet, as basic research is still incomplete. The phytochemical aspect is also important for this discussion, because the evaluation of plant extracts still

needs to identify the components or the active ingredient that can serve to treat gallstones, after which studies should be made on the possible mechanism of action, without forgetting all aspects related to toxicity. Cholesterol gallstones are a constantly growing disease. If a preventive therapy is to be found, several aspects must be studied, such as intestinal cholesterol absorption, transport and metabolism of liver and biliary cholesterol, as well as the secretion of other biliary lipids through its ABC transporter proteins; thus, the research of natural products with preventive properties should not forget the study of all these stages in the formation of cholesterol gallstones.

CONCLUSION

The research of natural products has led to significant results regarding the search for a treatment of cholesterol gallstones and the development of preventive methods in risk populations. It is necessary to expand the current phytochemical and molecular research in order to know the secondary metabolites and the pharmacological targets involved in the prevention or treatment of these gallbladder problems.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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