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REVIEW

Silymarin as a Potential Hypocholesterolaemic Drug

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Summary

Silymarin, a mixture of flavonolignans from medicinal plant *Silybum marianum*, is used in supportive treatment of liver diseases of different etiology due to its hepatoprotective activity, which is considered to involve antioxidative and the membrane stabilizing effects. The liver plays an important role in regulation of metabolism of plasma lipoproteins, and liver injury is often reflected as a secondary dyslipoproteinaemia, which may lead to the development of atherosclerosis, particularly when associated with hypercholesterolaemia. This review summarizes the experimental evidence indicating that silymarin-induced protection of liver functions may be of benefit with regard to liver lipid metabolism related to the regulation of plasma lipoproteins. Moreover, some data suggest that silymarin could have a direct effect on liver cholesterol metabolism by inhibiting cholesterol biosynthesis. It is proposed that silymarin deserves to be studied as a potential hypocholesterolaemic agent.

Key words
Silymarin – Silybin – Liver lipids – Plasma lipoproteins – Cholesterol

1. Introduction

Silymarin is a mixture of flavonolignans from the fruits of *Silybum marianum* (L.) Gaertn. containing silybin (main constituent), isosilybin, silydianin and silychristin. Silybin (Fig. 1), as well as other three constituents, is a polyphenolic substance with limited solubility in water.

S. marianum has been known since ancient time and recommended in traditional European and Asiatic medicine mainly for treatment of liver disorders (for a review see Morazzoni and Bombardelli 1995). Nowadays, the hepatoprotective activities of silymarin, proposed to be based partially on its antioxidant properties, are sufficiently documented (Morazzoni and Bombardelli 1995) and silymarin became an ingredient of some phytopharmaceuticals (Legalon^R, Flavobion^R) used often in supportive therapy of mushroom (*Amanita*) liver poisoning and in chronic liver diseases, such as liver steatosis (Geller *et al.* 1993) and alcoholic liver disease (Salmi and Sarna 1982, Ferenci *et al.* 1989).

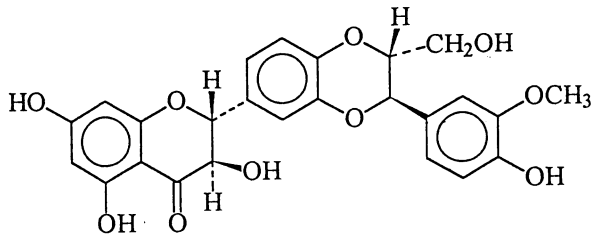

Fig. 1. *The chemical structure of silybin*

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Liver injury of different etiology is often accompanied by disorders of lipid metabolism in the liver and is reflected in altered plasma lipids and lipoproteins as secondary dyslipoproteinaemia. Hyperlipoproteinaemias with a significant increase of serum cholesterol and its carrier LDL are known to be associated with an increased risk of coronary heart disease (Mahley 1982, Steinberg 1989). Moreover, oxidatively damaged LDL may be of central importance in atherogenesis (Steinberg *et al.* 1989).

The important factor of hepatoprotective activity of silymarin and its constituents, related to lipid metabolism, is its ability to stabilize cell membranes. It can involve both antioxidant action of silymarin protecting membranes against lipid peroxidation and subsequent damage by free radicals (Mourelle *et al.* 1988, Letteron *et al.* 1990), and the influence of silymarin on qualitative and quantitative composition of membrane lipids (Schriewer *et al.* 1973, Muriel and Mourelle 1990, Mourelle and Franco 1991, Nassuato *et al.* 1991) resulting from changes of liver lipid metabolism. Therefore this review attempts to evaluate whether silymarin-induced liver protection is associated with improved lipoprotein metabolism. Other numerous effects of silymarin have been summarized in an excellent review by Morazzoni and Bombardelli (1995).

2. Role of the liver in lipoprotein metabolism

Plasma lipoproteins consist of core lipids, surface lipids and apolipoproteins. The core of lipoproteins contains a mixture of triacylglycerols and cholesteryl esters, while the surface consists of apolipoproteins and polar lipids, mainly cholesterol and phospholipids (Gotto *et al.* 1986). Depending on their composition, the lipoproteins have been divided into different classes – chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

An important role in regulation of serum lipoprotein levels is played by the liver as an organ of synthesis, secretion, uptake and degradation of some serum lipoproteins (Havel 1986). VLDL and precursors of HDL are formed from lipids and apolipoproteins in the liver and secreted into the blood circulation (Gotto *et al.* 1986). After the triacylglycerol-rich VLDL has entered the blood, it is available for hydrolysis by lipoprotein lipase, an enzyme bound to the vascular surface. LDL are the final products of intravascular VLDL lipolysis carrying mainly cholesterol, and their level in the blood is regulated by the activity of LDL receptors (Goldstein and Brown 1987, Steinberg 1989) of different tissues including the liver. Another liver receptor – a low density lipoprotein-related receptor (LRP) became known as a chylomicron remnant receptor (Herz *et al.* 1988) and the lipoprotein receptor family is still extending (Schoonjans *et al.* 1996). The liver also seems to be involved in the binding of some apolipoprotein specific HDL subpopulations as an end-point of a pathway transporting cholesterol from peripheral tissues into the liver (reverse cholesterol transport) (Eisenberg 1984). Receptor-mediated endocytosis is a major mechanism by which lipoproteins are taken up by the liver, where they undergo lysosomal hydrolysis and they are further metabolized.

Although this brief outline has recapitulated only the most important aspects of the role of the liver in plasma lipoprotein metabolism, it is evident, that protection of the liver against xenobiotic injury could be of benefit in the prevention of disorders of lipoprotein metabolism. It is undoubtedly favourable, when a hepatoprotective drug exerts a further, direct effect on processes leading to the normalization of lipoprotein metabolism.

3. Influence of silymarin on liver lipids

It has been proposed that the hepatoprotective action of silymarin is based on three principles: 1. the antilipoperoxidative underlain by ability of silymarin to scavenge free radicals and to increase the intracellular content of reduced glutathione, 2. the regulatory towards membrane permeability and leading to an increase of membrane stability against xenobiotic injury, and 3. the regulatory towards nuclear expression by exerting steroid-like effect (Valenzuela and Garrido 1994).

The influence of silymarin on membrane permeability is closely associated with qualitative and quantitative changes of membrane lipids, cholesterol and phospholipids (Muriel and Mourelle 1990, Mourelle and Franco 1991). This suggests that silymarin can also affect other lipid compartments in the liver with possible consequences for the secretion as well as uptake of lipoproteins.

a) Cholesterol

Silybin, one of silymarin constituents, was found to inhibit incorporation of cholesterol precursors 1-^{14}C-acetate and 2-^{14}C-mevalonate into the postmitochondrial supernatant of rat liver homogenates (Schriewer and Rauen 1977). Nassuato *et al.* (1983) have proposed that silybin could decrease liver synthesis of cholesterol *de novo*, since they found decreased concentrations of biliary cholesterol. Later, these authors demonstrated *in vitro* dose-dependent inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key enzyme of cholesterol synthesis, by silybin dihemisuccinate (Nassuato *et al.* 1991). However, they failed to show a decrease of HMG-CoA reductase activity after silybin dihemisuccinate administration to the rat *in vivo*. In

spite of this, it has been suggested that the inhibitory effect of silybin on HMG-CoA reductase *in vivo* can not be definitively excluded, since silybin could be released from binding to the reductase during the isolation procedure (Nassuato *et al.* 1991), and the effective inhibition of HMG-CoA reductase *in vitro* suggests a direct interaction between silybin and the enzyme. The inhibitory effect of silybin on cholesterol biosynthesis in hepatocytes *in vitro* was subsequently confirmed by others (Gebhardt 1993, Kren and Gebhardt 1996). Inhibition of HMG-CoA reductase activity is considered to underlie the hypocholesterolaemic effect of statins (mevastatin, lovastatin, simvastatin) (Tobert 1987), substances of natural origin used successfully in the treatment of primary hypercholesterolaemias.

b) Phospholipids

It has been reported that silymarin and silybin decrease the synthesis and turnover of phospholipids in the rat liver (Montanini *et al.* 1977). On the other hand, silybin was able to counteract the ethanol-induced inhibition of phospholipid synthesis in rats (Castigli *et al.* 1977) and to antagonize the decreased incorporation of 2-^{3}H glycerol into lipids of isolated rat hepatocytes induced by ethanol (Corazzi *et al.* 1982). Moreover, stimulation of phosphatidylcholine synthesis and an increase of choline phosphate cytidyltransferase activity by silybin was found in the normal rat liver as well as in the liver intoxicated with galactosamine (Schriewer and Weinhold 1979). Silybin antagonized the CCl_4-induced increase of liver lyso-phosphatidylcholine and activity of serum phospholipase A, but it failed to normalize the increase of cardiolipin and the decrease of phosphatidylcholine and phosphatidylethanolamine (Vengerovskii *et al.* 1987). These results suggest that the interferences of silymarin or silybin with phospholipid metabolism are complicated and the specific effects on different types of phospholipids are probably involved in different models of liver damage.

c) Triacylglycerols and fatty acids

Data about the influence of silymarin on liver metabolism of triacylglycerols are rare in spite of the fact that perhaps the most often used model for testing of hepatoprotective substances is the intoxication of liver with CCl_4, which is associated with liver steatosis. Silybin has been demonstrated to partially antagonize the CCl_4-induced increase of total lipids and triacylglycerols in the rat liver (Vengerovskii *et al.* 1987, Mourelle *et al.* 1989) and probably to activate beta-oxidation of fatty acids (Vengerovskii *et al.* 1987). Petronelli *et al.* (1981) proposed that silymarin could decrease the triacylglycerol synthesis in the liver. This effect has also been suggested for probucol, a phenolic substance used as a hypolipidaemic drug with an additional antioxidative effect (Parthasarathy *et al.* 1986, Barnhart *et al.* 1989), because probucol prevented the progression of liver steatosis (Yoshida *et al.* 1995).

d) Biliary lipids

Nassuato *et al.* (1983, 1991) have reported, that silybin decreased biliary cholesterol concentration and the cholesterol saturation index in rats and in man, while biliary phospholipids mildly decreased and the concentration of bile acids was not changed. The effect on cholesterol concentration was ascribed to decreased liver synthesis of cholesterol *de novo* (Nassuato *et al.* 1991).

4. Influence of silymarin on plasma lipids and lipoproteins

The treatment with silymarin decreased the levels of plasma cholesterol and LDL-cholesterol in hyperlipidaemic rats (Rui 1991). In normal rats, silybin did not decrease the plasma cholesterol, but it reduced the levels of plasma phospholipids (Nassuato *et al.* 1983) which, in the plasma, are mainly transported in HDL. Unfortunately, data about serum cholesterol levels are lacking in rats with assumed inhibited activity of HMG-CoA reductase in the liver after silybin dihemisuccinate treatment (Nassuato *et al.* 1991).

In a clinical study, silymarin (Silliver) decreased the level of plasma triacylglycerols and pre-beta lipoproteins in patients with hyper-lipoproteinaemias IV and IIb, but it had no effect on cholesterol level (Petronelli *et al.* 1981). Somogyi *et al.* (1989) did not confirm the effect of silymarin (Legalon^R) on plasma triacylglycerols but, on the other hand, they did find a moderate decrease of total and HDL cholesterol after silymarin treatment of patients suffering from hyperlipoproteinaemia II. This was accompanied by a significant decrease of apolipoproteins A-I and A-II, so that HDL appear to be relatively enriched with cholesterol. It is known that the treatment of hypercholesterolaemia with antioxidant probucol is also associated with a decrease of HDL-cholesterol and apolipoproteins A (Eder 1982, Dachet *et al.* 1985). However, the probucol-induced decrease of HDL appears to result even in improved reverse cholesterol transport (Bagdade *et al.* 1990), and probucol has been demonstrated to slow the progression of atherosclerosis (Carew *et al.* 1987). The possible favourable influence of silymarin on atherosclerosis has been proposed in man (Somogyi *et al.* 1989) and in rabbits fed a high-cholesterol diet (Schneider *et al.* 1990).

Data about plasma lipids and lipoproteins in model experiments of liver damage have shown that silymarin is able to normalize an increase of plasma total lipids induced by CCl_4 (Vengerovskii *et al.* 1987) and to antagonize a decrease of serum free fatty acids induced by thioacetamide (Siblíková *et al.* 1985). However, silymarin did not normalize the fall of serum

triacylglycerols in the thioacetamide model (Siblíková *et al.* 1985). In paracetamol-induced damage of hepatocytes, silymarin improved the binding of LDL to rat hepatocytes (Singh *et al.* 1992), an event important for decreasing plasma LDL.

5. Antilipoperoxidative activity of silymarin

Free radicals are recognized to play an important role in the mechanisms of many pathological processes including atherosclerosis. Lipid peroxidation is initiated by the interaction of free radicals of various origin with unsaturated fatty acids of lipids followed by a reaction with oxygen. The consequences of lipid peroxidation involve a wide spectrum of damages. With regard to lipoprotein metabolism, the lipoperoxidation of LDL may be of crucial importance in atherogenesis (Steinberg *et al.* 1989). Furthermore, degeneration of cell membranes caused by lipid peroxides may contribute to the development of other disorders of lipoprotein metabolism both in the liver and peripheral tissues.

Silymarin is considered to act as a chain-breaking antioxidant by scavenging free radicals which induce lipid peroxidation (Letteron *et al.* 1990, Mira *et al.* 1994, Pietrangello *et al.* 1995). Other important antioxidative effects of silymarin are based on its influence on enzyme system associated with glutathione (Valenzuela *et al.* 1989) and superoxide dismutase (Lang *et al.* 1993).

Lipid peroxidation of LDL is inhibited by some lipid soluble antioxidants, such as vitamin E (Coffey *et al.* 1995, Shaish *et al.* 1995), beta-carotene (Shaish *et al.* 1995) and a hypolipidaemic drug probucol (Parthasarathy *et al.* 1986, Barnhart *et al.* 1989). The antioxidative activities of lipid soluble silymarin suggest that silymarin could inhibit lipid peroxidation of LDL, but this has not yet been studied as far as we know. However, some studies have indicated that flavonol quercetin inhibits oxidative modifications of LDL *in vitro* (Whalley *et al.* 1990, Negre-Salvayre and Salvayre 1992) probably by radical scavenging and by protection of alpha-tocopherol in plasma lipoproteins against oxidation (Whalley *et al.* 1990). In this context, it is interesting that polyphenols (tannins) of red wine also inhibit LDL oxidation (Frankel *et al.* 1993) and this could explain the reduced risk of cardiovascular disease in drinkers of red wine (French paradox). A clinical study (Zutphen Elderly Study) dealing with the relation of diet intake of antioxidative flavonoids to the risk of coronary heart disease showed that the intake of flavonoids correlates inversely with the incidence of myocardial infarction as well as with the mortality from coronary heart disease. The inhibition of LDL oxidation has been suggested as a probable mechanism of flavonoid action (Hertog *et al.* 1993). Recently, it has been reported that quercetin and its derivatives decrease the plasma level of cholesterol and the atherogenic index in hypercholesterolaemic rats (Igarashi and Ohmuma 1995).

6. Bioavailability of silymarin

A problem associated with peroral administration of silymarin concerns the low bioavailability of silymarin constituents due to their limited solubility in water (Morazzoni *et al.* 1992, 1993). Till now, attention to improve the bioavailability has been paid to silybin, the main constituent of silymarin. It has been shown that the bioavailability of silybin in a complex with phosphatidylcholine (Silipide) is about ten times higher than that of silybin as a component of silymarin (Morazzoni *et al.* 1993) due to enhanced gastrointestinal absorption (Morazzoni *et al.* 1992). Other authors reported that the silybin-phosphatidylcholine complex (IdB 1016, Silipide) was more active as radical scavenger (Carini *et al.* 1992, Comoglio *et al.* 1995) similarly as another more soluble form of silybin, silybin dihemisuccinate (Mira *et al.* 1994). Because of the lower stability of silybin dihemisuccinate, glycosylated derivatives of silybin are being studied at present (Kren and Gebhardt 1996).

However, it has been shown that silymarin is more active as a chain-breaking antioxidant than silybin (Letteron *et al.* 1990). Subsequently, other silymarin constituents – silychristin, silydianin and isosilybin have been proved to inhibit lipid peroxidation (Bosisio *et al.* 1992), and isosilybin appears to be the most bioavailable of the silymarin flavonolignans (Morazzoni *et al.* 1993).

Recently, it has been reported that perorally administered silymarin complexed with phosphatidylcholine has combined antioxidant (decrease of malondialdehyde) and metabolic (fall of cholesterol) effects in the livers of rabbits fed a high-cholesterol diet (Drozdzik *et al.* 1996). The finding that silymarin decreased the liver cholesterol content supports the hypothesis that silymarin could act as a hypocholesterolaemic drug, since the drug-induced hypocholesterolaemic effect (statins, resins) is underlain mainly by a decrease of liver cholesterol content, which stimulates the uptake of serum LDL due to the increased activity of liver LDL receptors (Goldstein and Brown 1987, Steinberg 1989).

Conclusion

Till now, silymarin has been widely studied as a hepatoprotective agent stabilizing cell membranes. Since this effect is caused, in addition to inhibition of lipid peroxidation, by altered lipid composition of membranes, the data related to the metabolism of liver lipids and plasma lipoproteins were summarized with the aim of assessing, whether silymarin deserves to be studied as a hypocholesterolaemic drug.

A hypocholesterolaemic effect of silymarin can be expected on the basis of experimental evidence showing that silybin inhibits liver HMG-CoA reductase activity *in vitro*, and silymarin 1. improves the binding of LDL to rat hepatocytes, 2. decreases the liver cholesterol content in rabbits fed a high-cholesterol diet, and 3. decreases plasma cholesterol and LDL-cholesterol levels in hyperlipaemic rats. These aspects are promising and should encourage further studies concerning the influence of silymarin on lipoprotein metabolism in experimental animals as well as in man, in spite of the present ambiguous results in the treatment of human hypercholesterolaemia based, however, on a small number of studies only.

Attention should be paid to the study of activities of separate flavonolignans from *S. marianum* as well as to the use of their more suitable bioavailable forms, since some contradictory results may be based on the insufficient bioavailability of silymarin flavonolignans.

Further studies of the silymarin effects on lipoprotein metabolism could lead to the development of a phytopharmaceutical preparation with combined antioxidant and hypocholesterolaemic properties.

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