Biochemical bases of the pharmacological action of the flavonoid silymarin and of its structural isomer silibinin

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The flavonoid silymarin and one of its structural components, silibinin, have been well characterized as hepato-protective substances. However, little is known about the biochemical mechanisms of action of these substances. This review deals with recent investigations to elucidate the molecular action of the flavonoid. Three levels of action have been proposed for silymarin in experimental animals: a) as an antioxidant, by scavenging prooxidant free radicals and by increasing the intracellular concentration of the tripeptide glutathione; b) regulatory action of the cellular membrane permeability and increase of its stability against xenobiotic injury; c) at the nuclear expression, by increasing the synthesis of ribosomal RNA by stimulating DNA polymerase I and by exerting a steroid-like regulatory action on DNA transcription. The specific hepatoprotective action of silibinin against the toxicity of ethanol, phenylhydrazine and acetaminophen is also discussed. It is suggested that the biochemical effects observed for the flavonoid in experimental models may settle the basis for understanding the pharmacological action of silymarin and silibinin.

Key words: free radical scavenger action, hepatoprotective flavonoids, natural antioxidants, silibinin, silymarin.

INTRODUCTION

Flavonoids are plant products belonging to the family of the benzo-gamma-pyrones and are mostly abundant in the photosynthetic cells of higher plants (Havsteen, 1983). More than 500 different types of flavonoids are now known, being ubiquitous both in the plant and in the animal kingdoms. Flavonoids, when incorporated into the alimentary chain may also be present in insects, molluscs, reptiles, and even mammals (Middleton, 1984). For centuries, a number of different therapeutic and curative properties have been ascribed to flavonoids and many of them have been incorporated to the popular folk medicine. Flavonoids such as quercetin (Beretz et al, 1982), taxifolin (Vladutiu et al, 1986) and silymarin (Vogel, 1968) have been used as pharmacological principles, either as such, or mixed in several chemically complex preparations. Of these flavonoids, the flavonolignane silymarin, introduced as a "hepatoprotective" agent a few years ago (Koch and Tschemy, 1983), is the best known because of its well defined therapeutic and prophylactic properties.

Silymarin which is extracted from the seeds and fruits of the milk thistle Silybum marianum, Gaertner (Compositae), is a mixture of three structural isomers, silibinin, silidianin, and silichristin (Koch et al, 1980). The structures of the isomers of silymarin were elucidated by Wagner et al (1965) and by Pelter and Hansel (1968). Figure 1 shows the chemical structure of the three isomers composing silymarin. A feature that distinguishes silymarin from other flavonoids is

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that its isomers are always substituted by coniferyl alcohol (Wagner et al, 1974). Of the isomers composing silymarin, silibinin (formerly named silybin) shows the higher pharmacological potency when compared to those of silidianin and silichristin (Lecompte, 1975). Medically, silymarin and silibinin have been defined as cytoprotective substances (Stockinger et al, 1976) and specifically as hepatoprotective principles (Vogel et al, 1977). The flavonoid is used in the clinical treatment of several hepatopathies where degenerative necrosis and functional impairment are involved (Lecompte, 1975a). Silymarin is also an effective antidote against intoxication by the mushroom Amanita phalloides (Choppin and Desplaces, 1979).

Silymarin also shows hepatoprotective effects against the intoxication with phalloidin (Vogel, 1981), galactosamine (Barberino et al, 1977), thioacetamide (Schriewer et al, 1973), halothane (Janiak, 1974), and carbon tetrachloride (Dubin et al, 1976).

Although silymarin has been incorporated to the pharmacopeia of many countries as Legalon® or Hepaton®, being prescribed for the treatment of a great variety of diseases, little is known about the biochemical basis of its mechanism of action. Formerly, silymarin was described as a “membrane stabilizing substance” by Schriewer and Rauen (1971), referring to its possible antioxidant capacity.

The present review deals with the efforts of different researchers, including ourselves, towards the elucidation of the protective action of silymarin and silibinin at the cellular level. Results discussed here were obtained from different in vivo and in vitro experimental models, such as whole animals, perfused organs, cells tissue homogenates and isolated nuclei. Although no definitive conclusions may be drawn about the molecular action of silymarin (or silibinin), a general hypothesis for the mechanisms of action of the flavonoid may be proposed.

I. ANTIOXIDANT PROPERTIES OF SILIBININ

Flavonoids are generally recognized as good antioxidant compounds (Fraga et al, 1987). The presence of hydroxyl groups in different positions of their benzene rings makes feasible hydrogen abstraction for neutralizing free radicals (Nieto et al, 1993). Silibinin, when assayed as the hydro-soluble silibinin-2,3'-dihydrogensuccinate sodium salt, shows a potent inhibitory effect against the oxidation of a linoleic acid:water emulsion when catalyzed by Fe²⁺ (Valenzuela and Guerra, 1986). The observed antioxidant
action is even more efficient than that of two well known synthetic antioxidants, butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) (Valenzuela et al., 1986). However, when the antioxidant effect of the flavonoid is assayed against complex oxidizable systems, such as the NADPH-Fe^{2+}-ADP or the ter-butyldihydroperoxide (TBH)-induced hepatic microsomal peroxidation, different results are obtained. Sili­binin exhibits a good concentration-dependent inhibitory effect of the microsomal peroxidation induced by NADPH-Fe^{2+}-ADP (Valenzuela and Guerra, 1986), a well known hydroxyl free radical forming-system (Svingen et al., 1979), when the peroxidation is measured either as accumulation of thiobarbituric acid reactive substances (TBARS) or as spontaneous chemiluminescence (QL) (Valenzuela and Guerra, 1986). However, sili­binin is unable to inhibit the peroxidation induced by TBH to the same microsomal preparation (Valenzuela and Guerra, 1986). Therefore, it seems that sili­binin may only scavenge low molecular weight free radicals, as the hydroxyl free radical, being unable to neutralize more bulky free radicals as the ter­butoxy free radical (Valenzuela and Guerra, 1986).

II. PROTECTIVE EFFECT OF SİLİBININ IN BIOLOGICAL MODELS WHERE OXIDATIVE STRESS IS INDUCED

Oxidative stress is described as the structural and/or functional damage produced on a tissue by the uncontrolled formation of prooxidant oxygen free radicals (Sies, 1986). Generally, oxidative stress is developed when the prooxidant action of an inducer (enzyme, xenobiotic or metal) exceeds the antioxidant capacity of the cellular defense system, surpassing its homeostatic capability and eventually leading to their death (Weiss et al., 1982). Many xenobiotics have been characterized as oxidative stress-inducers, being the effects of carbon tetrachloride (CCl₄) (Comporti, 1985), TBH (Minotti, 1989), ethanol (Valenzuela et al., 1980), acetaminophen (Mason and Fischer, 1986) and phenylhydrazine (Valenzuela et al., 1977) among the best characterized. Erythrocytes obtained from rats treated with silymarin show a high resistance against the hemolytic effect of phenylhydrazine (Valenzuela et al., 1987) and against the lytic effect of osmotic shock (Valenzuela et al., 1985a). This latter effect suggests that silymarin might act by increasing the stability of the erythrocyte membrane. However, the molecular interaction of the flavonoid with erythrocyte membranes obtained from rats treated with silymarin has not yet been assayed. Interestingly the stimulatory action of the flavonoid on the permeability of rat bone-marrow cells to labelled substrates in vitro has been demonstrated. The permeability of isolated rat bone marrow cells to ^3^H-uridine is greatly enhanced when animals are previously treated with silymarin (Garri­do et al., 1988). This stimulatory effect is observed both in the acid-soluble fraction (cytoplasmatic ^3^H-uridine) and in the acid-insoluble fraction (RNA), suggesting that the flavonoid enhances the transport of the radioactive precursor to the cytoplasm and its incorporation into the nucleic acids.

Liver perfusion can be a valuable experimental tool to assay both the effect of oxi­dative stress-inducers and the protective action of free radical-scavenger substances (Valenzuela and Guerra, 1985). Perfusion of isolated rat livers with a solution containing phenylhydrazine results in an increase in the oxygen consumption of the organ and in the release of TBARS to the caval perfusate (Valenzuela and Guerra, 1985). Such stress is accompanied by a substantial reduction of the glutathione (reduced form, GSH) content of the liver (Valenzuela et al., 1985b). GSH has been identified as an important protective biomolecule against chemically-induced oxi­dative stress (Videla and Valenzuela, 1982).

When rats are previously treated in vivo with sili­binin (50 mg/kg iv), a significant reduction in the phenylhydrazine-stimulated oxygen consumption of the liver is observed (Valenzuela and Guerra, 1985). In addition, as a result of the flavonoid administration, the release of TBARS to the perfusing solution is substantially reduced, where as the GSH content of the tissue remains unchang­ed (Valenzuela and Guerra, 1985). The antioxidative effect of sili­binin has also been observed in rats acutely intoxicated either with ethanol (Videla et al., 1982) or with
acetaminophen (Campos et al, 1989). Both xenobiotics have been characterized as hepatic lipid peroxidation-inducers and as drastic liver GSH-depletors (Videla et al, 1980; Wendel et al, 1979). Treatment of rats with either silymarin (Valenzuela et al, 1985b) or silibinin (Campos et al, 1988) protects animals against the hepatic oxidative stress induced by an acute intoxication with ethanol (5 g/kg b w) or with acetaminophen (50 mg/kg ip). In addition, silibinin treatment attenuates the substantial increases in the plasmatic levels of the enzymes glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase (Garrido et al, 1989), as well as the activity of gamma-glutamyl transpeptidase (Muriel et al, 1994), observed after acetaminophen intoxication (Mitchell et al, 1973). The levels of these enzymes are generally considered to be good markers of the hepatic function (Black, 1980).

It has been reported that acetaminophen, when administered in high concentrations, exhibits antioxidant properties upon both in vivo and in vitro experimental models (DuBois et al, 1983). Isolated rat hepatocytes incubated with acetaminophen (over 5 mM) show a drastic reduction in their GSH content, lipid peroxidation being not observed (TBARS values significantly lower than controls) (Garrido et al, 1991). When acetaminophen-treated hepatocytes are also incubated with silibinin, a potentiation of the antilipoperoxidative effect of acetaminophen is observed, as well as a protective effect on the GSH depletion induced by the drug (Garrido et al, 1991). This paradoxical behaviour is explained as follows: acetaminophen-induced lipid peroxidation has been related to the prooxidative action of the electrophilic reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) (Corcoran et al, 1980), which is formed when acetaminophen undergoes oxidative metabolism at the microsomal cytochrome P-450 system (Potter et al, 1987). NAPQI may react with cellular macromolecules, eventually leading to cell death (Tsokos-Kuhn et al, 1988). Silibinin, as several flavonoids (Beyeler et al, 1988), is a competitive and reversible inhibitor of the microsomal cytochrome P-450 system (Bindoli et al, 1977). Thereby, in the presence of silibinin, the microsomal transformation of acetaminophen to NAPQI should be inhibited; this inhibition may in turn induce an increase in the intracellular concentration of the non-metabolized acetaminophen molecules, avoiding the GSH consumption by the drug metabolite (NAPQI), which reacts with GSH forming conjugation adducts (Garrido et al, 1991). This effect may explain the protective action observed for the flavonoid on the hepatocyte GSH depletion when cells are incubated with acetaminophen. Moreover, nonmetabolized acetaminophen molecules may be acting themselves as free radical scavengers, enhancing the antioxidative action of silibinin.

Carbon tetrachloride-induced liver cirrhosis has been used as another biological model to assess the hepatoprotective action of silibinin in rats (Mourelle et al, 1989). It is known that chronic treatment of rats with CCl₄ produces liver fibrosis and a set of biochemical and histological changes that closely resemble most aspects of human portal cirrhosis (Guengerich, 1990). Using this toxicological model, Muriel and Mourelle (1990) demonstrated that silibinin may prevent the changes in the phospholipid composition of the hepatic membranes induced by CCl₄ intoxication, preserving the functional and the structural integrity of these membranes. The activities of alkaline phosphatase and gamma-glutamyl transpeptidase, two membrane enzymes whose activities reflect the functional state of the hepatic membrane (Meister and Tate, 1976), are also restored following the treatment of CCl₄-intoxicated rats with silibinin (Muriel and Mourelle, 1990). Mourelle and Favari (1988) have also demonstrated that silibinin improves the metabolic disposition of aspirin (e.g., deacetylation by plasma and tissue esterases) studied in rats with liver fibrosis induced by chronic treatment with CCl₄. An interesting feature of silibinin (and also of silymarin) is its regulatory action on the GSH content of different organs. When rats receive silymarin (ip) or silibinin (iv), significant increases in GSH contents of liver, intestine and stomach are observed. However, the tripeptide concentration in the lungs, spleen and kidneys remains unchanged (Valenzuela et al, 1989). This organ-specific action of the flavonoid may be due conceivably to differences in
those metabolic characteristics of each organ related to GSH synthesis and turnover, and/or to specific effects of silibinin upon hepatic, gastric and intestinal cells (Valenzuela et al, 1989). Pharmacokinetic studies of silymarin show that the plasma half-life of the flavonoid (in human and rats) is relatively short, being the liver the main target organ (Mennicke, 1976), where silymarin accumulates (Vogel and Trost, 1975). Over 80% of silymarin and its metabolites are excreted by the biliary tract as glucuronide and sulfoglucuronide conjugates (Lorenz et al., 1984). The flavonoid initiates a cyclic transportation via entero-hepatic circulation, because the gut flora splits the silymarin conjugates (Vogel and Trost, 1975). As result of this, the liver, the stomach and the intestine are the tissues bearing the higher silymarin concentrations, and also the tissues where the flavonoid induces large increases in GSH concentrations.

III. EFFECT OF SILIBININ AT NUCLEAR LEVEL

In addition to the antilipoperoxidative and GSH sparing effects described for silibinin, Machicao and Sonnenbichler (1977) and Sonnenbichler et al (1980), have carried out a series of experimental protocols demonstrating several effects of silibinin at the nuclear level. Silibinin increases the synthetic rate of the ribosomal RNA species 5.8S, 18S and 28S by about 20% (Sonnenbichler and Zetl, 1984). This stimulation was observed in rat liver, hepatocyte cultures and in isolated liver nuclei via activation of the DNA-dependent RNA polymerase I (Sonnenbichler and Zetl, 1985). Subsequently, the formation of mature ribosomes is stimulated and, as an important consequence, the protein biosynthesis in the liver is increased as well (Sonnenbichler and Zetl, 1985). Sonnenbichler et al (1986) have also demonstrated that the flavonoid increases DNA replication in the liver of rats partially hepatectomized. This effect evidences the liver cell regenerating capacity ascribed to the flavonolignane derivative (Hahn et al., 1968), supporting the clinical reports on this subject (Fintelmann and Albert, 1980). Stimulation of ribosomal RNA synthesis and DNA replication appear to be specific for silibinin, because the flavonoid shows a larger stimulatory effect than 35 other flavonoid derivatives tested (Sonnenbichler and Zetl, 1988).

By comparing the structure of different flavonoids, using space filling atomic models, it has been proposed that these natural vegetable substances have some similarities to sterols (Sonnenbichler and Zetl, 1988). Silibinin can compete specifically with the estradiol receptor site at concentrations higher than 2 x 10^{-7} M, which means a somewhat reduced affinity of the flavonolignane as compared to the pure hormone (Sonnenbichler and Zetl, 1988). The kinetics of the influence of estradiol on ribosomal RNA synthesis is indeed similar to that of silibinin (Sonnenbichler and Zetl, 1988). To explain this, it has been hypothesized that silibinin might interact as a regulator directly with polymerase I (Sonnenbichler and Zetl, 1985). One of the subunits of the enzyme might bear a receptor site for the steroid hormone which could be substituted by silibinin (Sonnenbichler and Zetl, 1988). As stated before, it is known from clinical findings that silibinin enhances the regenerative capacity of liver tissue after intoxication (Fintelmann and Albert, 1980). From the biochemical point of view, regeneration represents an increase in protein synthesis, which has been demonstrated for silibinin, but also an increase in proliferation and DNA synthesis. Protein and RNA syntheses are prerequisites for DNA synthesis. Therefore, it seems reasonable that DNA synthesis might also be influenced by the flavolignane derivative, as stated by Sonnenbichler and Zetl (1986).

FINAL CONSIDERATIONS

Although the biochemical mechanism(s) of action of the flavonoid silymarin (or silibinin) is (are) not yet well understood, some considerations may be drawn from the above description. The “hepatoprotective action” of the flavonoid may be mainly ascribed to its free radical scavenger properties. This effect is reflected in the membrane stabilizing and GSH-sparing actions described for the
flavonoid, thus providing effective protection against the toxicity induced by a number of xenobiotics. The flavonoid may also act at the nuclear level, enhancing the synthesis of ribosomal RNA and the cellular regeneration. The steroidal-like behaviour of the flavonoid on the control of DNA expression has also been proposed. Figure 2 summarizes the main proposed biochemical actions of silymarin.

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