Toward the Definition of the Mechanism of Action of Silymarin: Activities Related to Cellular Protection From Toxic Damage Induced by Chemotherapy

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Silymarin, the active extract from milk thistle, has been extensively used in patients with liver disease of different etiology. Although silymarin is a complex of 7 flavonolignans and polyphenols, silibinin is usually regarded as the most active component. In vitro and in vivo studies indicate that silymarin and silibinin protect the liver from oxidative stress and sustained inflammatory processes, mainly driven by Reactive Oxygen Species (ROS) and secondary cytokines. Oxidative stress and inflammation are also involved in cellular damage of many other tissues and their role in the development and toxic reactions in patients receiving cancer therapies is established. The protective effects of silymarin and silibinin, demonstrated in various tissues, suggest a clinical application in cancer patients as an adjunct to established therapies, to prevent or reduce their toxicity. Here we discuss the possible mechanism of the protective action of silymarin and silibinin, focusing on cancer therapies as agents causing cellular damage.

Keywords: ***silymarin; silibinin; free radicals; cytokines; liver damage; chemotherapy; cancer***

Silymarin, the active extract from milk thistle, has been extensively used for a long time, with an excellent safety profile, in patients suffering from liver diseases of different etiology (see, for review, Saller et al,[1] Rambaldi et al,[2] and Rainone[3]). Silymarin has been reported to be one of the herbal preparations most frequently used by cancer patients on a voluntary basis.[4] In many countries a substantial percentage of patients regularly complement chemotherapy with Complementary and Alternative Medicine (CAM) and silymarin is reported to be one of the herbal preparations most frequently taken by cancer patients on a regular basis to prevent or alleviate chemotherapy-induced hepatotoxicity, with figures of approximately 7% of all the patients taking herbal remedies.[4-6]. Although silymarin is a safe complex, with a low probability of interference with the pharmacokinetics of established and effective cancer therapies at doses lower than 5 g/d,[7] clinicians should accurately consider the chemical composition of the silymarin complex used by a given patient as well as the relative concentration of its components. Clinical studies on silymarin hepatoprotection have been controversial, and its effects are considered uncertain. The clinical studies have been subjected to numerous reviews and reassessments,[1-3] possibly because of the chemical differences among silymarin preparations administered, a factor likely related to the difficulty in establishing the effective dose.

The standardized silymarin extract, obtained from the seed of *Silybum marianum* (L.) Gaertn. (Asteraceae), contains approximately 65% to 80% of flavonolignans, with small amounts of flavonoids, and approximately 20% to 35% of fatty acids and polyphenolic compounds. Definitions and details on the chemical structure of the silymarin complex and of silibinin, still regarded as the most active antioxidant and antineoplastic component of the extract, are provided by Kroll et al in this issue. It is worth noting that other flavonolignans in the silymarin complex besides silibinin are endowed with specific pharmacologic activities. Thus, the administration of the silymarin complex or of a purified component could be differentially relevant to the desired action and expected outcome.[8-10] To this end, the capability of silymarin and silibinin to interfere with promotion and progression of carcinogenesis and its reported anticancer activity (see, for review, Moon

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et al[11] and Aggarwal and Shishodia[12]) deserves particular attention. When silymarin or silibinin is used as an adjunct to cancer therapies to exert hepatoprotection, questions remain on the possible synergistic or additive action with different cytotoxics; indeed, synergistic actions of silymarin and silibinin were reported with doxorubicin, cisplatin, and carboplatin.[13-15] Analyzing the huge amount of published evidence on silymarin and silibinin, we suggest that a unifying mechanism of action emerges, likely related to early scavenging and antioxidant activity on Reactive Oxygen Species (ROS)–induced oxidative stress and sustained inflammation in tissues. Through this basic action, silymarin and silibinin substantially interfere with Nuclear Factor (NF)-κB–controlled transcriptional processes involved in tissue damage as well as in cellular proliferation.[16] With regard to the latter, silymarin and silibinin support processes driving cell growth arrest and apoptosis and oppose the ones promoting abnormal cell accumulation. With regard to the former, cellular damage induced by various agents is reduced by silymarin and silibinin and can be estimated by evaluating many different readouts of biological and clinical relevance.

In this article, we review briefly the main cellular actions exerted by silymarin and silibinin in different cell types, with particular regard to the actions likely related to a protection from cancer therapy–induced toxicity, contributing to the already existing framework supporting silymarin administration in cancer patients.

Cellular Actions and Molecular Targets of Silymarin and Silibinin

A large body of evidence indicates that the ability of silymarin to interfere with redox status and intracellular transduction mechanisms can explain most of the described observations. Consistent with the pleiotropic toxicity exerted by free radicals and taking into account free radical scavenging action as the core of cellular activities exerted by silymarin, it has been repeatedly observed that both normal cells and cancer cells are sensitive to silymarin action. We thus discuss in the following section both action on normal cells and some of the observations reported in cancer models, focusing on the results that more likely are related to the proposed mechanism of action.

Antioxidant Action

ROS and Reactive Nitrogen Species (RNS) are constantly generated in living systems as products of metal-catalyzed reactions, mitochondria-catalyzed electron transport reactions, immune reactions during inflammation, irradiation (ie, ultraviolet light, X-rays, and gamma rays), and chemicals.[17] ROS are beneficial to living cells at low levels but harmful at high concentrations, particularly in rapidly proliferating cells, such as cancer cells, basal keratinocytes, and gastrointestinal epithelia. At high concentrations, ROS damage cell structures, including lipids, membranes, proteins, and nucleic acids. This damaging process is termed *oxidative stress*. The cellular and tissue levels of ROS are tightly regulated by the antioxidant defense systems, that is, the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), and glutathione reductase (GR). The activities of these enzymes contribute to the elimination of superoxide ($O_2\cdot$) and hydroxyl (·OH) radicals. Nonenzymatic compounds such as α-tocopherol, β-carotene, ascorbate, and glutathione also exert antioxidant protection. Redox homeostasis is maintained when transient mild increases in ROS are well balanced by the antioxidant system, but redox signaling imbalance may be caused by a sustained increase in ROS generation or a defective or deficient antioxidant system.[18]

Silymarin treatment has been observed to recover toward control values both expression and activity of the antioxidant enzymes, which were significantly diminished after alloxan intoxication in rats.[19] Additionally, silibinin inhibited leukotriene formation with an IC_{50} of 15 μM in human platelets, white blood cells, and endothelial cells via the 5-lipoxygenase pathway, detected as leukotriene (LT)B4 and LTC4/D4/E4/F4 formation. This enzyme is strongly sensitive to redox status. The inhibition of arachidonic acid metabolism via the cyclooxygenase pathway, detected as prostaglandin E_2 secretion, required up to 4-fold higher silibinin concentrations compared with inhibition of the 5-lipoxygenase pathway, most likely because cyclooxygenase activity is not regulated by redox status as tightly as the former.[20] Silymarin administered to patients with chronic alcoholic liver disease significantly enhanced the low SOD activity measured in the patients' erythrocytes and lymphocytes. Furthermore, silymarin therapy markedly increased the serum activity of GSHPx. The resulting beneficial antioxidant consequences were confirmed by a considerable decrease in serum malondialdehyde concentration.[21]

Antioxidant Activity in the Setting of Liver Toxicity

Ionizing radiation and chemotherapy induce an abnormally large production of ROS in living cells, resulting in chronic oxidative stress that disturbs signal transduction and gene expression in such a way that pathologic conditions can occur. In particular, hepatotoxicity and oral/gastrointestinal mucositis are frequently observed in the treatment of cancer.[22-24] Silymarin and

Table 1. Protection of Liver Cells From ROS-Induced Toxicity by Silymarin and Silibinin

Model/Disease	*Preparation*	*Dose*	*Actions*	*Reference*
Acetaminophen hepatotoxicity	Primary rat hepatocytes (from Wistar rats)	25 μM silymarin	↓ DNA strand breaks	42
CDDP hepatotoxicity	Wistar albino rats	100 mg/kg intraperitoneal silymarin (pretreatment)	↓ ALT and AST; ↑ GSH, SOD, GSHPx; ↓ MDA; ↓ NO in liver	43
Methotrexate, methotrexate and ethanol, methotrexate and acetaminophen	Hep G2, NHPH	0.5 mmol/L silymarin	↓ Cytotoxicity; ↑ cell viability; ↑ GSH; ↓ TNF production; ↓ IL-6, IL-8	44
Diethylnitrosamine hepatotoxicity	Wistar albino rats	50 mg/kg oral silymarin	↓ AST, ALT; ↓ MDA (lipid peroxidation); ↑ SOD, GSH, CAT; ↑ GST and GR	45
Ionizing radiation hepatotoxicity	Wistar albino rats	70 mg/kg oral or 50 mg/kg intravenous silymarin for 7 days	Restoration of alkaline phosphatase activity; ↓↑ ALT and AST; ↓ γ-glutamyl transpeptidase (pre-RT exposure); ↑ GSH (pre-RT exposure); ↑ GR (pre-RT exposure); ↑ GSHPx	46
Ethanol hepatotoxicity	C57BL/6 mouse	200 mg/kg silymarin by gavage	↓ TNF production; ↓ ALT activity; ↓ lipid peroxidation; ↑ GSH	47
T-cell–dependent hepatitis (ConA 20 mg/kg intravenously)	BALB/c mice + primary mouse splenocyte	25-100 mg/kg intraperitoneal silibinin; Legalon® SIL	↓ Plasma ALT and AST; ↓ cytokines in splenocytes; ↓ DNA fragmentation; ↓ hepatic TNF, IFN-γ, IL-4, IL-2; ↓ hepatic NF-κB activation; ↑ hepatic IL-10	48

CDDP = cisplatin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GSH = reduced glutathione; SOD = superoxide dismutase; GSHPx = glutathione peroxidase; MDA = malondialdehyde; NO = nitric oxide; Hep G2, NHPH = Normal Human Primary Hepatocytes; TNF = tumor necrosis factor; IL = interleukin; CAT = catalase; RT = ionizing radiation; GR = glutathione reductase; ConA = Concanavalin A; SIL = Legalon SIL; IFN = interferon; NF = nuclear factor.

silibinin exert antioxidant activity and support redox homeostasis in several in vitro and in vivo models of oxidative stress–induced hepatocellular injury. Both silymarin and silibinin have been demonstrated to affect redox status, lipid peroxidation, and proinflammatory cytokine expression and release. These observations have been previously reviewed in detail (see Saller et al[1]), and we have selected some representative examples, summarizing the main results in Table 1.

Hepatotoxic cellular events triggered by chemotherapy are very similar to the ones triggered by acetaminophen, antibiotics, antipsychotics, and antidepressants.[22,25] In particular, antimetabolites, such as methotrexate, 6-mercaptopurine, and the parent compound azathioprine, are frequently responsible of hepatotoxicity, requiring dose reductions. The most common pattern of toxicity is hepatocellular injury, attributable directly to membrane and cell function damage or indirectly to immune-mediated damage. The common final mechanism of hepatotoxicity can be summarized as follows: (a) depletion of reduced glutathione (GSH), leading to increased toxicity of free radicals and ROS and causing cellular necrosis; and (b) increased release of cytokines, including tumor necrosis factor (TNF), interleukin (IL)-6, and IL-8, causing cellular apoptosis. Silymarin was effective in counteracting all the parameters of oxidative stress. The free radical scavenging and antioxidant properties of silymarin and silibinin are demonstrated by (a) protection from lipid peroxidation, detected as reduced malondialdehyde content; and (b) restoration and potentiation of the endogenous antioxidant enzymes (SOD, CAT, and GSHPx) in oxidative stress conditions, leading to increased intracellular

concentration of GSH. Moreover, silymarin supports the nonenzymatic antioxidant system through maintenance of effective levels of α-tocopherol, β-carotene, and ascorbate, resulting in an increased cell viability and preserved functionality in several cell lines and animal models. Interestingly, the silymarin complex seems to be stronger than silibinin as an antioxidant, suggesting that the mixed components, as well as their oxidized metabolites in the mixture,[26] are required for optimal redox control.

In the clinical setting, the free radical scavenging and antioxidant properties of silymarin may support the tolerance of cancer patients to the increasing oxidative stress generated in healthy cells and tissues by oncological therapies. This could prevent or reduce onset and progression of chemotherapy-induced toxicity, known to be a limiting factor requiring dose reduction and even time off from therapy administration.[27] If this is the case, silymarin could allow patient compliance with courses of treatment. In this view, orally administered Siliphos® at a dose of 5.1 mg/kg/d has been demonstrated to exert a protective action on chemotherapy-induced hepatotoxicity in acute lymphoblastic leukemia patients, when used as supportive therapy during a course of antineoplastic maintenance therapy.[28] Furthermore, in a 34-year-old woman with promyelocytic leukemia who was repeatedly unable to comply with courses of treatment because of antimetabolite-induced liver toxicity, normal liver aminotransferase level and completion of maintenance chemotherapy with methotrexate and 6-mercaptopurine were obtained by adjunctively administered 800 mg of silymarin over 4 months.[29]

Finally, on the basis of experimental studies and pathophysiologic considerations, one might expect that application before or early after the toxic insult could result in a stronger protective effect. Recent results supporting this view have been obtained by the Strickland group (GT Strickland, November 2006, personal communication), working in patients with acute viral hepatitis, where silymarin-treated patients experienced more rapid resolution of symptoms and signs as well as earlier normalization in laboratory test results in comparison to an active vitamin placebo preparation.

Protection From Oxidative Stress in Other Cell Types

Beside liver cells, many other cells have proven to be sensitive to the protective action against toxic agents, including kidney cells, immune system cells, neurons, endothelial cells, fibroblasts, and keratinocytes, as shown in Table 2. Experimental silymarin and silibinin concentrations are similar to those studied in liver cells, supporting the notion that a common mechanism is likely operative in different cell types, where ROS-induced damage is relevant to toxicity. The possible application of these findings is strongly dependent on the local concentration of silibinin in different organs and may be supported also by development of new formulations, specifically designed for different pathologic targets.

Table 2. Protection of Different Cell Types Exerted by Silymarin and Silibinin In Vitro

Cell Type	*Dose*	*Challenge*	*Reference*
Mesangial cells	50-200 μmol/L silymarin	TNF-α, IL-1β	49
Monocytes	5-20 μg/mL silymarin	β-thalassemia	50
Endothelial cells	6.25-25 mg/L silibinin	Hydrogen peroxide	51
Dopaminergic neurons as mesencephalic mixed neuron-glia cultures	20-80 μmol/L silymarin	LPS-induced neurotoxicity	52
Keratinocytes and fibroblasts	0.7-34 mg/L silymarin, silibinin, other components	Hydrogen peroxide	53

TNF = tumor necrosis factor; IL = interleukin; LPS = lipopolysaccharide.

TNF-Dependent Kinase Inhibition

TNF strongly activates c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK or MEK). Human histiocytic lymphoma U-937 cells were pretreated with silymarin at different concentrations (10-100 μM) for 2 hours and stimulated with TNF. Silymarin was dose-dependently able to counteract the approximately 7-fold TNF-induced activation of JNK. MEK, known to also activate JNK, is similarly activated by TNF induction. This MEK activation was also found to be inhibited by silymarin in a dose-dependent manner.[16]

Anti-Inflammatory and Anticarcinogenic Action

A number of in vitro and in vivo studies have analyzed the action of silymarin and silibinin as anticancer agents. Comprehensive reviews on chemopreventive and anticancer activity on skin cancer cell lines and animal models[30] and on anticancer activity on prostate cancer cell lines and animal models[31] have been previously published, and the subject is further discussed in this issue by Deep and Agarwal.

Table 3 summarizes some of these findings. It is interesting to mention that many observations seem likely related to the proposed mechanism of action of silymarin and silibinin.

Table 3. Selected Activities of Silymarin and Silibinin Observed In Vivo in Rodent Cancer Models

Model/Disease	*Strain*	*Dose*	*Actions*	*Reference*
Stage I and stage II skin tumor	SENCAR mouse	3-12 mg of topical silymarin	↓ Lipid peroxidation; ↓ carcinogen-induced proliferation; ↓ DNA synthesis in tumor cells; ↓ skin edema and epidermal hyperplasia; ↑ prevention of tumor promotion	54
Skin photocarcinogenesis*	SKH-1 hairless mouse	1% silibinin in the diet	↓ Proliferation index; ↑ p53 positive cells; ↑ Cip1/p21; ↑ Kip21/p27; ↓ CDK-cyclin kinase activity; ↓ Akt activation; ↑ caspase-3 positive cells; ↓ MAPK in healthy skin cells; ↑ MAPK in tumorigenic skin cells (eg, induced apoptosis)	55
Human prostate cancer** (DU-145 tumor subcutaneously)	Athymic (BALB nu/nu) mouse	0.05%-0.1% silibinin in the diet	↓ Tumor volume and weight; ↑ IGFBP-3	56
Human non–small-cell lung cancer (NSC A549 xenograft)	Athymic (BALB nu/nu) mouse	200 mg/kg silibinin (per oral gavage)	↓ Tumor weight; ↓ proliferation index; ↓ tumor microvessel density; ↓ NF-κB activation; ↓ COX-2; ↑ apoptosis; ↑ doxorubicin efficacy	57
Urethane-induced lung tumor	A/J male mouse	0.033%-1% (wt/wt) silibinin in the diet	↓ Tumor numbers/mice; ↓ tumor volume; ↓ proliferation; ↓ cyclin D1; ↓ tumor microvessel density; ↓ VEGF and bFGF; ↓ COX-2	58
Azoxymethane-induced colon cancer	F3444 rats	100-1000 ppm silymarin in the diet; 40-400 mg/kg silymarin by gavage	↓ Aberrant crypt foci number and volume; ↑ phase II detoxifying enzymes (glutathione S-transferase, quinolone reductase); ↓ colonic β-glucuronidase activity; ↓ prostaglandin E_2	59
Azoxymethane-induced colon cancer	F3444 rats	5000 ppm silymarin in the diet (5 g/kg body weight)	↓ Aberrant crypt foci number	60

CDK = cyclin-dependent kinase; MAPK = mitogen-activated protein kinase; IGFBP = insulin-like growth factor binding protein 3; NF = nuclear factor; COX = cyclooxygenase; VEGF = Vascular Endotelial Growth Factor; bFGF = basic Fibroblast Growth Factor.

In general terms, silymarin and silibinin interfere with the NF-κB–controlled transduction cascade. ROS, acting as second messengers, cause sustained NF-κB activation through TNF-induced and interleukin-1–induced expression. NF-κB (see, for review, Dobrovolskaia and Kozlov [32]) is an inducible and ubiquitously expressed DNA binding protein, acting as a transcription factor for genes involved in inflammation, cell survival, differentiation, and growth. Proinflammatory agents, carcinogens, and tumor promoters such as toxic metals, ultraviolet radiation, phorbol esters, asbestos, alcohol, lipopolysaccharide, TNF, okadaic acid, ceramide, and benzo(a)pyrene are also considered NF-κB–activating agents.[32] In unstimulated cells, NF-κB is sequestered in the cytoplasm by interaction with inhibitory protein 1 kappa B alpha (IkBα). On activation from oxidative stress, NF-κB dissociates from IkBa, and IkBa is degraded. NF-κB translocates to the nucleus and, through kinase phosphorylation, drives the activation of genes supporting inflammation. The inflammatory response is typically accompanied by stimulation of cytokines and chemokines and the expression and release of growth and angiogenesis factors. Indeed, sustained NF-κB activation and chronic inflammation constitute a risk factor with a variety of epithelial cancers because the oxidant cellular microenvironment is permissive for genetic instability and proliferation, with transformed cells escaping apoptosis,[18] as is the case for prostate, cervix, esophagus, stomach, liver, colon, pancreas, and bladder malignancies. Consistent with their antioxidant activity, silymarin and silibinin were demonstrated to inhibit NF-κB activation through suppression of IkBa phosphorylation and degradation, decrease of p65 subunit nuclear translocation, and NF-κB–dependent reporter gene transcription. Silymarin, in a 10- to 100-μM range, was demonstrated to dose-dependently inhibit the activation of NF-κB and related kinases. Silymarin concentrations were about 100-fold lower than salicylate concentrations, suggesting that such a potent action can be exerted at concentrations substantially free of toxic effects.[16, 33]

Growth Factor Receptors and Transcription Factors

Silymarin and silibinin can inhibit growth factor receptor–mediated mitogenic and cell survival signaling, particularly as related to the activation of tyrosine

kinases, and consequently alter cell cycle regulators. These growth factor–related transmembrane glycoproteins with intrinsic tyrosine kinase activity (a family of key enzymes involved in normal and abnormal cellular regulation) when overexpressed lead to mutation, hybrid gene formation, amplification, and perturbation of transcriptional processes. PDGFR, EGFR, Bcr-Abl, and KIT are examples of tyrosine kinases overexpressed in most human cancers. These tyrosine kinases control the cell cycle, migration, metabolism, proliferation, differentiation, and survival through the phosphorylation of various target molecules. Counteracting their activating signals could be a simple but effective way to reduce the impact of their cancer growth–related transcriptional processes. Silymarin or silibinin corrects the imbalance between cell survival and apoptosis through interference with cell cycle regulator expression, down-modulating the carcinogenetic antiapoptotic gene activity, on one hand, and supporting the proapoptotic gene activity, on the other.

This positive activity of silymarin has been documented in different human cancer cells and animal models. With regard to the transduction system involved in this action, it has been shown, using rat glioma cell lines modified in the expression of epidermal growth factor receptor (EGFR), that the presence of EGFR is necessary and sufficient to observe toxicity in response to silibinin.[34] Silymarin or silibinin is particularly effective in inhibiting EGFR signaling with suppression of cyclin-dependent kinase expression (ie, CDK4) and up-regulation of the CDK inhibitors $p21^{CIP1}$ and $p27^{KIP1}$, with concomitant increase in their binding to CDKs. The net result is a potent G1 arrest in EGFR-overexpressing tumor cells, such as hormone refractory LNCaP cells. Exposure of prostate, breast, cervical, and epidermoid carcinoma cell lines to silymarin alone primarily promoted growth arrest at the G1 or G2 checkpoints, rather than inducing apoptosis. Interestingly, when the EGFR-positive epidermoid carcinoma line A431 was exposed to increasing silymarin doses, a biphasic response was observed. The lower doses were able to drive growth arrest through Extracellular Signal-Regulated Kinases (ERK1/2) inhibition, whereas the higher doses led to apoptosis through the MAPK/JNK pathway.[35]

The well-defined scavenging and antioxidant activity of silymarin primarily functions to maintain the healthy cellular phenotype. In this way, silymarin or silibinin is able to prevent or reduce epigenetic phenomena that could initiate, and even more, promote and progress, carcinogenesis through free radicals and ROS generation. ROS are known to affect both directly and indirectly the amount of mitogenic stimuli activating receptors with tyrosine kinase activities, such as EGFR and insulin-like growth factor receptor. Therefore, one can propose a priority order of silymarin activity and mechanism of action, relevant to its possible use as an adjunct or complementary support to cancer therapies.

Induction of Apoptosis

Silibinin can induce apoptosis of endothelial cells and inhibit angiogenesis because it was shown to suppress the growth and to induce apoptosis of human umbilical vein endothelial cells. With regard to human leukemia, escaping apoptosis and cell survival action of the constitutively activated Akt pathways have been described. Silibinin inhibited constitutive NF-κB activation, consistent with a significant decrease in its nuclear level of p65 subunit, and it activated caspase 3 and caspase 9, all playing parts in the induction of endothelial apoptosis.[36] Silymarin has been recently demonstrated to inhibit the activities of Akt significantly in the human chronic myeloid leukemia cell line K562, accompanied by caspase activation, inhibition of proliferation, and apoptosis induction.[37]

Sites of Silymarin Detection

Pharmacokinetic studies of silymarin have analyzed the presence of silibinin in the blood or in target organs, on the basis of the availability of adequate high-pressure liquid chromatography methods and on the assumption that this substance is the main component of silymarin. Kroll et al discuss in this issue the implications involved in such practice and some relevant results. We would like to add here that it should be very useful to analyze and consequently consider the presence of the other active components present in silymarin preparations. Furthermore, Kroll et al clearly spell out the difference between low- and high-dose studies, likely used for different clinical applications. In any case, after oral silymarin administration to humans, silibinin is measurable in the blood, the levels depending on the dose and the tested formulation. The analysis of formulation studies, though, is not within the scope of this article. In principle, the basis of ongoing work is to find a method to increase oral bioavailability of silymarin, increasing the lipid compatibility property of the preparations. Focusing the attention on dosages usually used in patients with liver disease and a formulation extensively studied, Table 4 summarizes some data obtained in healthy volunteers after administration of silymarin and of a preparation containing silibinin and phosphatidylcholine, known as silipide. The data indicate that higher values of silibinin can be measured after administration of similar amounts

Table 4. Selected Pharmacokinetic Parameters of Silibinin After Administration of Silymarin and Silipide in Healthy Human Volunteers

	Preparation and Dosage	
	Silymarin (360 mg of Silibinin Equivalent)	*Silipide (360 mg of Silibinin Equivalent)*
C_{max}, ng/mL	102	298
T_{max}, h	1.4	1.6
Mean residence time, h	3.5	3.6
$AUC_{0\text{-}12\ h}$, ng/mL·h	257	881

Peak Concentration (C_{max}), Peak Time (T_{max}), Area Under the Plasma Concentration Time Course (AUC). Data taken from Barzaghi et al.[61]

Table 5. In Vivo Silibinin Content in Tissues After 5 Weeks of Dietary Administration of 0.5% Silymarin

Tissue	*Silibinin*
Skin, μg/g tissue	3.1
Skin tumor, μg/g tissue	6.5
Liver, μg/g tissue	13.7
Lung, μg/g tissue	7.7
Mammary gland, μg/g tissue	5.9
Spleen, μg/g tissue	4.4
Plasma, μg/mL	10.0

Data taken from Singh et al.[38]

Table 6. In Vivo Levels of Silibinin After Administration of Diets With Different Concentrations of Silibinin

Experimental Design	*Prostate, μg/g Tissue*	*Plasma, μg/mL*
Diet containing 0.05% silibinin for 60 days after tumor implantation	3.7	7.1
Diet containing 0.1% silibinin for 60 days after tumor implantation	4.6	12.8
Diet containing 0.05% silibinin 3 weeks before and 6 weeks after tumor implantation	ND	6.7
Diet containing 0.1% silibinin 3 weeks before and 6 weeks after tumor implantation	ND	10.2

ND = not determined. Data taken from Singh et al.[39]

of silibinin if this active principle is administered together with phospholipids, with similar patterns of disposition. Similar values have been reported by other authors as well (see, for review, Saller et al[1]), but in any case, blood levels of silibinin are much lower than those observed in recent animal studies treated with dietary supplementation (see below). The relevance of these findings with regard to clinical use of silymarin is questionable because only 1 component is detected and liver concentrations cannot be estimated.

When silymarin is absorbed, a consequent high concentration in the liver and the bile has been repeatedly measured in various experimental and clinical conditions. To this end, a distribution study like the one performed by Rajesh Agarwal's group in mice with established skin tumors[38] seems to us particularly relevant because in this case, the tissue values were established after prolonged treatment. Silymarin was added to the diet (0.5% w/w) for 5 weeks, and blood and organs were collected and analyzed for silibinin concentration. Several organs showed detectable concentrations of silibinin, as indicated in Table 5; interestingly, the highest concentration was observed in the liver.

Another important study examining plasma and tissue levels has been published by the same group in nude mice bearing a human prostate carcinoma xenograft.[39] In this case, silibinin was used instead of silymarin. The data are reported in Table 6 and indicate that plasma levels associated with significant reduction in tumor volume are achievable with administration of a relatively small amount of the active compound. Interestingly, the plasma values of silibinin observed in the 2 studies were quite similar, regardless of the substance added to the diet and the concentrations used.

Regarding the relationships between tissue concentrations and administered dosage, a pilot study in patients diagnosed for colorectal adenocarcinoma demonstrated the ability of silibinin to concentrate in malignant colorectal tissue, when oral silibinin conjugated with soy phosphatidylcholine (silipide, Indena, Milan, Italy) was administered daily at dosages of 360, 720, or 1440 mg over a 7-day period before colorectal resection.[40] Administration of this silibinin preparation at a dose of 360 mg gives plasmatic levels comparable to those obtained with 420 mg of silymarin complex (ie, Legalon®, Madaus GmbH, Cologne, Germany), for which excellent safety is established.[1-3] Further studies over a long time period will clarify whether, according to the hypothesis, silibinin acts as a chemopreventive agent, through the up-regulation of insulin-like growth factor binding protein 3 in colonic mucosa, thus sequestering carcinogenic levels of insulin-like growth factor from tissue milieu.

Taken together, these data indicate that silymarin/silibinin administration at nontoxic levels may indeed result in tissue concentrations of silibinin potentially active toward different types of toxic challenge, thus potentially expanding the use of the

substance outside liver application. Nevertheless, liver concentrations are higher than those obtained in the same experiment in other tissues, thus confirming the rationality of the long-established application in this particular organ.

Conclusion

Silymarin exerts powerful actions at cellular and subcellular levels, explaining the effects observed in many in vitro and in vivo models and in particular in experimental models of liver disease. Recent evidence indicates that the observed cellular activities not only may be important with regard to hepatic damage but indeed may be linked to other consequences, in particular on tumor cells and on cellular damage induced in many tissues by chemotherapy and radiotherapy.[41] Clinical efficacy in cancer patients has not been studied extensively and is now an active area of investigation, with the possibility of applying different readouts for efficacy depending on the trial design. As a consequence, clinicians using silymarin in cancer patients should consider all the available evidence, either supportive or not, as discussed in this journal issue. The data reviewed here support the notion that between the multiple possible applications, oral administration of silymarin in patients receiving chemotherapy is potentially of great relevance and quite rational on the basis of the available evidence. This is true in particular for chemotherapy regimens inducing liver damage. Indeed, the liver shows high levels of active principles after oral administration. It is a site where many agents exert their toxic effects, and hepatic cells are clearly sensitive to silymarin's protective actions. Considering experimental studies and pathophysiologic considerations, one might expect that application of the active agent before or early after the toxic insult can result in a strong protective effect. In the case of chemotherapy, the time course of liver damage is known, and thus silymarin can be given before and during administration of the toxic agents, fully exploiting its potential activity.

Considering the established safety demonstrated by the long-standing use of silymarin, the basis of actions exerted at the cellular level by silymarin and its constituents, its pharmacokinetic properties, and the existing clinical data, it can be expected that the application of silymarin in patients undergoing chemotherapy for various types of cancer will improve outcome. The ultimate goal of such an adjunctive treatment will be the reduction of side effects and, even more relevant, the possibility of full exploitation of chemotherapy regimens, which, without any effective adjunctive treatment, could be limited in dose administration and completion by the onset of unacceptable toxicity.

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