

A Presurgical Study of Oral Silybin-Phosphatidylcholine in Patients with Early Breast Cancer

Matteo Lazzeroni¹, Aliana Guerrieri-Gonzaga¹, Sara Gandini², Harriet Johansson¹, Davide Serrano¹, Massimiliano Cazzaniga¹, Valentina Aristarco¹, Antonella Puccio¹, Serena Mora¹, Pietro Caldarella³, Gianmatteo Pagani³, Giancarlo Pruneri^{4,5}, Antonella Riva⁶, Giovanna Petrangolini⁶, Paolo Morazzoni⁶, Andrea DeCensi^{1,7,8}, and Bernardo Bonanni¹

Abstract

Silybin-phosphatidylcholine is an orally bioavailable complex of silybin, a polyphenolic flavonolignan derived from milk thistle, endowed with potential anticancer activity in preclinical models. The purpose of this window of opportunity trial was to determine, for the first time in early breast cancer patients, the breast tissue distribution of silybin. Twelve breast cancer patients received silybin-phosphatidylcholine, 2.8 g daily for 4 weeks prior to surgery. Silybin levels were measured before (SIL) and after (TOT-SIL) enzymatic hydrolysis by high-performance liquid chromatography (HPLC)-MS/MS in biologic samples (plasma, urine, breast cancer, and surrounding normal tissue). Fasting blood samples were taken at baseline, before the last administration, and 2 hours later. All patients were fully compliant and completed the treatment program. No toxicity was observed. SIL

and TOT-SIL were undetectable in baseline samples. Despite a high between-subject variability, repeated administration of Silyphos achieved levels of TOT-SIL of 31,121 to 7,654 ng/mL in the plasma and up to 1,375 ng/g in breast cancer tissue. SIL concentrations ranged from 10,861 to 1,818 ng/mL in plasma and up to 177 ng/g in breast cancer tissue. Median TOT-SIL concentration was higher in the tumor as compared with the adjacent normal tissue ($P = 0.018$). No significant change in either blood levels of IGF-I and nitric oxide or Ki-67 in tumors was noted. Silybin-phosphatidylcholine, taken orally, can deliver high blood concentrations of silybin, which selectively accumulates in breast tumor tissue. These findings provide the basis for a future phase II biomarker trial in breast cancer prevention. *Cancer Prev Res*; 9(1): 89–95. ©2015 AACR.

Introduction

The flavonolignan silybin is the major constituent of the seeds of milk thistle (*Silybum marianum*), a plant that has been used as hepatoprotective remedy for more than 2,000 years. Silymarin, a standardized milk thistle extract of which silybin is the main component, has been evaluated clinically in many types of liver diseases as hepatoprotective therapy (1). Recent data in rodents suggest that silymarin and silybin may be useful in the chemoprevention of several cancer types, including

breast (2, 3). Dietary Silymarin administered for 2 years decreased the incidence of mammary gland neoplasms in rats (2). Silybin also delayed the development of spontaneous mammary tumors, reduced the number and size of mammary tumor masses, and diminished lung metastasis in HER-2/neu transgenic mice (3).

Studies in healthy humans have shown that silybin when formulated with phosphatidylcholine (Silyphos; Indena SpA) improves its systemic availability compared with silymarin (4).

Several mechanisms have been proposed to explain how silybin may interfere with breast carcinogenesis (5), including the modulation of the insulin-like growth factor (IGF) system, which is a potent mitogen for mammary epithelial cells (6, 7).

Silybin is also a powerful antioxidant as a consequence of its polyphenolic structure. A case-control study has shown that serum nitric oxide (NO) levels were significantly higher in breast cancer patients compared with healthy subjects (8). NO levels may therefore be a potential marker of chemopreventive activity of antioxidants, such as silybin.

Since no clinical data of silybin in breast cancer patients have so far been obtained, we carried out a pilot presurgical study in breast cancer patients to characterize its pharmacokinetic profile and explore its pharmacodynamic effects on malignant as well as surrounding normal tissue.

¹Division of Cancer Prevention and Genetics, European Institute of Oncology, Milan, Italy. ²Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy. ³Division of Senology, European Institute of Oncology, Milan, Italy. ⁴Division of Pathology, European Institute of Oncology, Milan, Italy. ⁵School of Medicine, University of Milan, Milan, Italy. ⁶INDENA S.p.A., Milan, Italy. ⁷Division of Medical Oncology, E.O. Ospedali Galliera, Genoa, Italy. ⁸Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, United Kingdom.

A. DeCensi and B. Bonanni equally contributed to this article as senior scientists.

Corresponding Author: Matteo Lazzeroni, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy. Phone: 00390294372652; Fax: 00390294379225; E-mail: matteo.lazzeroni@ieo.it

doi: 10.1158/1940-6207.CAPR-15-0123

©2015 American Association for Cancer Research.

Materials and Methods

Patient characteristics

Twelve consecutive patients with newly diagnosed breast cancer not eligible for neoadjuvant treatment and candidate to surgical lumpectomy or mastectomy were recruited into the trial. This protocol (R621-IEO661/511) was approved by the local Institutional Review Board and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent prior to participation. Women had to be 18 years or older, have histologic confirmation of breast cancer on tru-cut biopsy, ECOG performance status of 0, and adequate normal function test.

Silybin formulation and dose

Silybin was formulated in granules to be suspended in drinkable water provided by Indena S.p.A. Each sachet contained 2.8 g of Siliphos, the soy lecithin delivery form standardized to contain $\geq 29.7\% \leq 36.3\%$ silybin by high-performance liquid chromatography (HPLC). Patients took a single sachet once daily for 4 weeks prior to surgery, in an empty stomach (30 minutes before eating, at least 2 hours after the previous meal). Supplementation was continued until the day of surgery. Date and time of last intake were recorded. The dose was chosen based on the high tolerability observed in clinical studies following repeated administration to healthy volunteers (9).

Specimen collection

Morning fasting blood for serum and plasma analysis was drawn at baseline, the day of surgery immediately before the last administration of Siliphos, and two hours later. Samples were stored at -80°C . Urine samples were taken at baseline and the day of surgery, before the last administration of Siliphos. Breast cancer tissue was collected by tru-cut biopsy at baseline and from surgical resections at treatment conclusion (tumor and distant surrounding noncancerous tissue), rapidly frozen in liquid nitrogen, and stored at -80°C .

Analysis of silybin

The milk thistle flavonolignans are extensively modified by phase II human enzymes. Silybin levels were first measured as free (unconjugated) silybin (SIL), and thereafter through enzymatic hydrolysis as total (free + conjugated) silybin (TOT-SIL), by HPLC-MS/MS in all types of biologic specimens (plasma, urine, breast cancer, and surrounding normal tissue). The methodology was developed by Kymos Pharma Services and proven to be accurate. The details, characterization, and validation of the method will be the subject of a future publication.

Silybin from lithium heparin plasma (0.2 mL) and urine samples (0.1 mL) was determined by HPLC-MS/MS after liquid-liquid extraction with methyl tert-butyl ether (MTBE; Scharlau). Naringenin for plasma and naproxen for urine (Sigma-Aldrich) were used as internal standards. The calibration ranges for plasma and urine were 0.5 to 500 ng/mL and 1 to 1,000 ng/mL, respectively. To determine TOT-SIL, an enzymatic hydrolysis step with β -glucuronidase (*Helixpomatia*, BBI Solutions) was added prior to run. Samples were analyzed in batches: two for plasma SIL, two for urine SIL, one for plasma TOT-SIL, and one for urine TOT-SIL. Each batch included standards and human plasma or urine controls. The concentrations of three plasma controls were 1.5, 15, and 400 ng/mL; and

the concentrations of three urine controls were 3, 30, and 800 ng/mL, respectively. The chromatographic method API4000 (A177-MS/A176-LC) was applied for plasma and API3200 (A235-MS/A237-LC) for urine.

Silybin from breast tissue (50 mg) was determined by HPLC-MS/MS after liquid-liquid extraction with MTBE. Naringenin was used as internal standard. As for plasma and urine, the enzymatic hydrolysis step was added to determinate TOT-SIL. Tissue samples were analyzed in one batch for SIL and in two batches for TOT-SIL determination. Each batch included standards (range, 2–500 ng/g) and controls (6, 24, and 400 ng/g). We used a surrogate matrix of pig muscle (Clinobs) for the preparation of standards and controls. The chromatographic method API3000 (A090-MS/A089-LC) was used.

Biomarkers

Serum concentrations of IGF-I (ng/mL) were determined by a chemiluminescent immunometric assay (Diasorin SpA) with the automatic instrument LIAISON. The sensitivity of the test was 0.8 nmol/L; intra- and interassay coefficients of variation of our in-house pooled serum control sample were 4.1% and 7.8%, respectively.

Urine concentrations of NO were determined by an ELISA assay (R&D Systems, Inc.) according to the manufacturer's instructions. The sensitivity was 0.25 $\mu\text{mol/L}$, and intra- and interassay coefficients of variation of a control sample of 30 $\mu\text{mol/L}$ were 2.5% and 4.6%, respectively. The results were normalized by urinary creatinine concentrations.

Histology

Following histologic diagnosis and IHC profiling, 5- μm -thick sections of the invasive tumors were prepared from each formalin-fixed and paraffin-embedded block of interest, i.e., those obtained upon diagnostic biopsy procedures and after surgical interventions. For invasive tumors, the histologic subtype and grade, as well as the presence of peritumoral vascular invasion, were annotated. Immunostaining was performed using anti-ER, PgR, (PharmDX) HER2 (Herceptest), and Ki-67 labeling index (LI; MIB1) antibodies, as previously reported (10–12).

Statistical methods

Correlations between biomarkers were investigated with Spearman partial correlations. We presented median values and interquartile ranges of SIL and TOT-SIL in plasma, urine, breast cancer tissue, and surrounding normal tissue. We evaluated changes in time and differences between types of tissue using nonparametric tests (Wilcoxon-rank tests).

We reported two-sided *P* values. We set the criterion for statistical significance at 5%. Data were analyzed using the SAS System Software for Windows, release 9.2. (SAS Institute).

Results

Twelve consecutive breast cancer patients were enrolled in this study. Table 1 describes the patient and the tumor characteristics at surgery. Women were mostly postmenopausal (58%) and moderately overweight (66% had body mass index ≥ 25). All breast cancers were hormone receptor positive (mainly luminal-B phenotype), and no one had vascular invasion or multifocality. All patients were fully compliant and completed the treatment program. No adverse events were observed.

Table 1. Main patient and tumor characteristics (at surgery)

Patients characteristics	N	%
Patients characteristics		
Age (years)		
<50	5	42%
≥50	7	58%
Menopausal status		
Premenopausal	5	42%
Postmenopausal	7	58%
Body mass index (kg/m ²)		
<25	4	33%
25–29.9	7	58%
≥30	1	8%
Tumor characteristics		
<i>In situ</i> (LCIS)	1	8%
Invasive	11	92%
Molecular phenotype		
Luminal A	5	42%
Luminal B	7	58%
TN stage		
Tis	1	8%
T1	5	42%
T2	6	50%
N0–Nx	9	75%
N1	2	17%
N3	1	8%
Vascular invasion		
Yes	2	17%
No	10	83%
Focality		
Unifocal	9	75%
Multifocal	1	8%
Multicentric	2	17%

SIL and TOT-SIL were undetectable in all compartments (plasma, urine, breast tissue) at baseline. A large degree of interpatient variability in the silybin blood levels was noted at all timepoints. The median (interquartile range, IQR) SIL and TOT-SIL concentrations after supplementation are shown in Table 2 and illustrated in Fig. 1. The median TOT-SIL plasma level was 901 ng/mL (IQR, 651–1,481) before the last administration (13–27 hours from the penultimate intake, depending on time of hospitalization) and 14,538 ng/mL (IQR, 13,147–16,828) 2 hours after the

last intake of silybin-phytosome. The median concentrations of SIL in plasma were 69 ng/mL (IQR, 13–159) and 5,847 ng/mL (IQR, 4,526–6,454), respectively (Fig. 1A).

Urine samples were also obtained at the beginning and at the end of the trial. The urine Silybin levels were higher than the plasma levels and also showed a large interpatient variability (Fig. 1B).

After 4 weeks of Siliphos administration, silybin reached the target organ and was detectable in human breast tissue. The median time interval between the last administration of silybin-phytosome and surgery was 7 hours. TOT-SIL concentration was higher in the tumor as compared with the adjacent normal tissue ($P = 0.018$). Median TOT-SIL concentration in tumors was 131 ng/mL (IQR, 35–869). Median concentration of SIL in breast cancer tissue was 33 ng/g (IQR, 4–158; Fig. 1C). Despite a high between-subject variability, plasma SIL levels showed a significant correlation with the concentration in normal tissue (Spearman coefficient = 0.690; $P = 0.027$).

The secondary endpoints consisted of an exploratory analysis of circulating and tissue biomarkers of activity. Serum and urine NO concentration, serum IGF-I level, and tissue Ki-67 LI were assessed before the agent supplementation and at the end of the trial. One patient was excluded from Ki-67 LI analysis because of the diagnosis of *in situ* disease at biopsy and an invasive breast cancer at surgery. As demonstrated in Fig. 2, there was no clear trend to a change in either the NO, IGF-I, or tissue Ki-67 LI between baseline and the end of supplementation with a notable interpatient variability (Fig. 2).

Discussion

The poor bioavailability of polyphenols is the major drawback for the development of preventive agents in the clinical setting (13). In recent years several preclinical studies concerning the effects of silybin on breast carcinogenesis (14) have been carried out. Silybin modulates imbalance in cellular homeostasis through interference with the expressions of cell-cycle regulators and proteins involved in apoptosis (15). Silybin also showed anti-inflammatory (16) as well as antimetastatic activity (17), and

Table 2. Total and free silybin levels in plasma, urine, breast cancer, and adjacent unaffected breast tissue in patients who received silybin for 28 days

	Median	Q1	Q3	Min	Max	P value
TOT-SIL (plasma) ng/mL						
Baseline	0	0	0	0	0	
Before last administration	901	651	1,481	214	4,574	
2 hours after last administration	14,538	13,147	16,828	7,654	31,121	0.0004 ^a
SIL (plasma) ng/mL						
Baseline	0	0	0	0	0	
Before last administration	69	13	159	1	523	
2 hours after last administration	5,847	4,526	6,454	1,818	10,861	0.0004 ^a
TOT-SIL (urine) ng/mL^b						
Baseline	0	0	0	0	0	
At surgery	7,131	2,015	21,095	90	26,573	
SIL (urine) ng/mL^b						
Baseline	0	0	0	0	0	
At surgery	212	71	359	7	747	
TOT-SIL (tissue) ng/g						
Tumor	131	35	869	0	1,375	
Normal breast tissue	11	0	34	0	48	0.018
SIL (tissue) ng/g						
Tumor	33	4	158	0	177	0.013
Normal breast tissue	0	0	3.79	0	19	

^a P for the difference between before last administration and 2 hours after last administration.

^bCreatinine normalization.

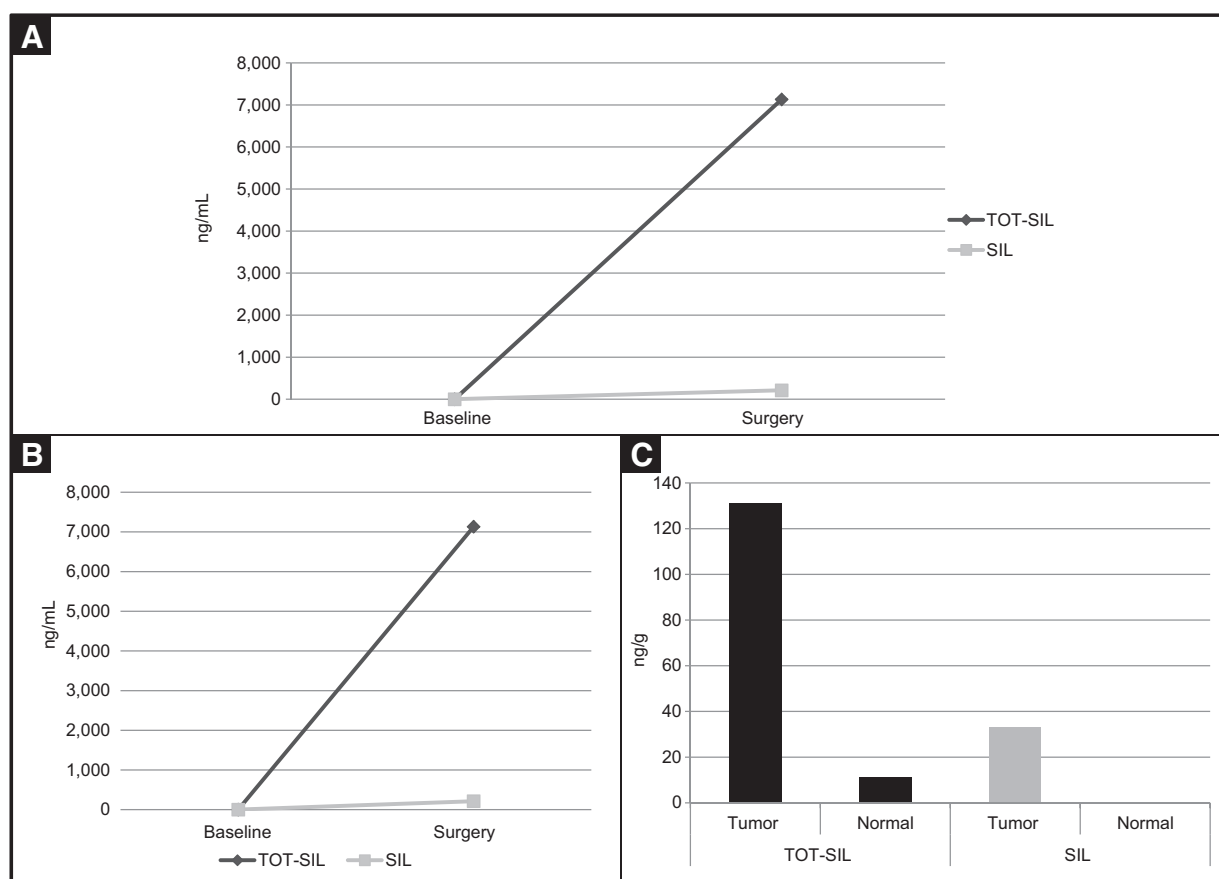


Figure 1. Median silybin level in plasma (A), urine (B), and tumor/normal breast tissue (C) before and after Siliphos supplementation.

synergistic effects with doxorubicin and cisplatin in both estrogen-dependent and independent human breast carcinoma cells (18). However, quantitative data on human breast tissue levels of silybin are absent, thus hampering the understanding of the biologic activities of this compound in breast cancer prevention and treatment.

The most interesting result of the present study is that oral silybin-phosphatidylcholine selectively accumulates in breast tumor tissue. In addition, the absence of relevant adverse events at 2.8 g daily dose for 4 weeks is consistent with a previous study in human volunteers (4) and with a clinical trial in patients with localized prostate cancer in which 13 g silybin was administered orally daily for 14 to 31 days (19).

In our study, the presence of metabolic conjugates of silybin has been shown indirectly. Silybin metabolites were calculated as silybin after enzymatic hydrolysis. Raised levels of the parent molecule after enzymatic hydrolysis suggested the presence of metabolites. A previous study in colorectal cancer patients demonstrated that silybin undergoes multiple conjugation reaction in humans (20). Hoh and colleagues identified conjugate species, such as silybin-mono-glucuronide and silybin-mono-sulfate. These two metabolites have at least two intact phenol moieties. On the assumption that the antioxidant activity of silybin is a function of its polyphenolic chemical structure, the presence of these two conjugate species suggests that significant amounts of circulating

silybin metabolites may maintain the same antioxidant potency of the parent molecule (20).

The plasma levels of silybin described in our study can be compared with those reported previously in healthy volunteers and cancer patients. In a study on 9 healthy subjects, a form of silybin-phosphatidylcholine (Silipide) at 720 mg given daily for 8 consecutive days provided a mean peak plasma level of 180 ng/mL (0.18 μ g/mL) reached 1 hour after administration of the last dose. Repeated administration of the same supplement given at the dose of 1,440 mg daily for 7 days in colorectal cancer patients achieved levels of SIL of 145 to 1,930 ng/mL (0.3 to 4.0 μ mol/L) 1 to 4 hours after the last Silipide administration and was dependent on Silipide dose. In our study with 2.8 g/day Siliphos (~900 mg of silybin) for 4 weeks, the median plasma concentration for SIL was 5,847 ng/mL 2 hours after the last administration and 69 ng/mL before the last dose. Median plasma concentrations for TOT-SIL were 14,538 ng/mL and 901 ng/mL, respectively, indicating that the vast majority of circulating silybin during the 24 hours was present as metabolites.

Levels of silybin in human breast cancer and normal breast tissue have not been described previously. In our study, the breast tissue levels of silybin were highly variable, which may, at least to some extent, be the consequence of the variability in the time period (2–12 hours) between the last dose and surgery. Median TOT-SIL concentration was higher in the tumor as compared with

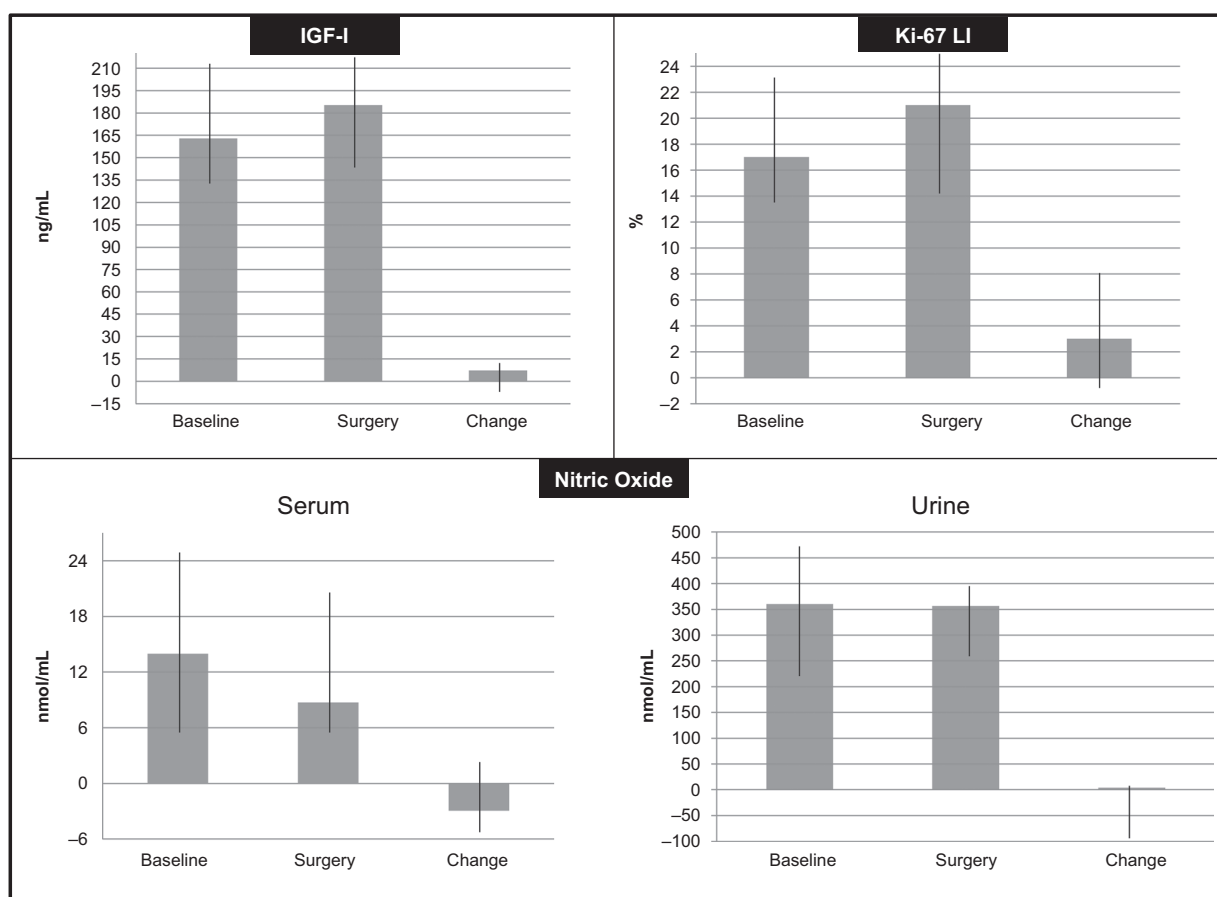


Figure 2. Median concentration and median change of serum IGF-I, tissue Ki-67 LI, and NO in serum/urine before, after Siliphos supplementation.

the adjacent normal tissue. Possible explanations of this different retention effect might be related to variances between physiologic and tumor vascularization, nonspecific interactions with the tumor microenvironment, and a lack of lymphatic drainage in tumors.

If we consider the hepatoprotective effect of silybin, a tissue concentration ranging from 290 to 482 ng/g (0.6–1 $\mu\text{mol/L}$) is indicative of a biologic activity (20). In our study, the highest concentration in breast cancer tissue was 1,375 ng/g (corresponding to $\sim 2.8 \mu\text{mol/L}$) for TOT-SIL and 177 ng/g for SIL, a level that is likely to exert potential pharmacologic effects also in the light of the active concentrations reported in cultured DU-145 prostate cancer cells, where treatment with 2 $\mu\text{mol/L}$ silybin resulted in a 54% growth inhibition (21). However, to date, none of the *in vitro* studies has investigated the bioactivity of silybin metabolites yet.

Moreover, we explored the tissue and blood marker changes with respect to IGF pathway, oxidative stress, and cell proliferation. The results presented here suggest that the concentrations of SIL and TOT-SIL achieved after consumption of Siliphos 2.8 g daily for 4 weeks do not positively influence circulating levels of IGF-I (Fig. 2). Similarly, no modulation was reported in a previous study on colon cancer patients in which silybin was administered for 7 days, although the authors postulated that their 1-week treatment might have been too short to achieve a long-lasting

effect on the IGF axis (20). In our pilot trial, Siliphos was administered for a longer period and at higher doses compared to that study, but seemed to be insufficient to affect circulating IGF-I. The same assumption might be applicable for tumor Ki-67 LI. The slight increase between biopsy and surgery is in line with prior experience, indicating the need for a control group in these biomarker trials (22).

Given the exploratory nature of the secondary endpoints, we cannot draw definitive conclusions about the most appropriate dose and treatment duration for efficacy. Recently silybin has shown to inhibit the phosphorylated signal transducer and activator of transcription protein-3 (pSTAT3; ref. 23), a marker of poor prognosis for many cancers, including breast (24). In a preclinical model of human gastric cancer, Wang and colleagues reported that the mechanism of action of silybin was related to a specific suppression of pSTAT3 and downregulation of STAT3 target genes, including Mcl-1, Bcl-xL, and survivin (25). A future clinical trial might explore silybin activity as a pSTAT3 inhibitor in breast cancer patients.

We finally studied NO concentration in serum and urine. NO plays a dual role in specialized tissues and cells, possessing either antioxidant or pro-oxidant properties (26). NO is an essential physiologic signaling molecule, mediating various cell functions in the nervous, immune, and cardiovascular systems but inducing

cytotoxic and mutagenic effects when overexpressed (26). A case-control study evaluating the diagnostic and prognostic values of the angiogenic serum factors, including NO in breast cancer patients, showed a significant correlation between tumor microvessel density (associated with decreased overall survival in patients with breast cancer) and serum NO compared with healthy controls (27). In our study, breast cancer patients showed a modest reduction in serum NO levels after Siliphos supplementation, providing the rationale for further investigations on its potential beneficial effects in cancer prevention.

Up to now there were no data showing that silybin was able to penetrate human breast tissue. Repeated administration of Siliphos achieved levels of silybin in breast cancer tissue similar to those known to be pharmacologically active in other models. In light of breast cancer chemopreventive activity of silybin in rodents, the silybin levels achieved in human breast cancer tissue after consumption of safe Siliphos doses support its further exploration as a potential chemopreventive agent for breast cancer. A randomized window of opportunity trial is justified.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Lazzeroni, H. Johansson, D. Serrano, M. Cazzaniga, P. Caldarella, G. Pruneri, A. Riva, G. Petrangolini, P. Morazzoni, A. DeCensi, B. Bonanni

Development of methodology: M. Lazzeroni, H. Johansson, P. Caldarella, G. Pruneri, B. Bonanni

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Lazzeroni, A. Guerrieri-Gonzaga, H. Johansson, D. Serrano, M. Cazzaniga, A. Puccio, S. Mora, G. Pagani, G. Pruneri, G. Petrangolini

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Lazzeroni, S. Gandini, H. Johansson, P. Caldarella, G. Pruneri, A. Riva, G. Petrangolini, P. Morazzoni, A. DeCensi, B. Bonanni

Writing, review, and/or revision of the manuscript: M. Lazzeroni, A. Guerrieri-Gonzaga, H. Johansson, M. Cazzaniga, P. Caldarella, G. Pruneri, A. Riva, G. Petrangolini, P. Morazzoni, A. DeCensi, B. Bonanni

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Lazzeroni, A. Guerrieri-Gonzaga, V. Aristarco, S. Mora, A. Riva

Study supervision: M. Lazzeroni, P. Caldarella, A. Riva, B. Bonanni

Acknowledgments

The authors thank Margherita Omesso for language editing of the article, Indena S.p.A. for the support in the analytical evaluation and for kindly providing silybin-phosphatidylcholine at no cost, and Kymos Pharma Services for providing the analytical procedures for the quantification of silybin.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 5, 2015; revised October 6, 2015; accepted October 22, 2015; published OnlineFirst November 2, 2015.

References

- Loguerco C, Festi D. Silybin and the liver: from basic research to clinical practice. *World J Gastroenterol* 2011;17:2288-301.
- Anonymous. Toxicology and carcinogenesis studies of milk thistle extract (CAS No. 84604-20-6) in F344/N rats and B6C3F1 mice (Feed Studies). *Natl Toxicol Program Tech Rep Ser* 2011;1-177.
- Provinciali M, Papalini F, Orlando F, Pierpaoli S, Donnini A, Morazzoni P, et al. Effect of the silybin-phosphatidylcholine complex (IdB 1016) on the development of mammary tumors in HER-2/neu transgenic mice. *Cancer Res* 2007;67:2022-9.
- Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E. Pharmacokinetic studies on IdB 1016, a silybin- phosphatidylcholine complex, in healthy human subjects. *Eur J Drug Metab Pharmacokinet* 1990;15:333-8.
- Deep G, Agarwal R. Antimetastatic efficacy of silibinin: molecular mechanisms and therapeutic potential against cancer. *Cancer Metastasis Rev* 2010; 29:447-63.
- Hawsawi Y, El-Gendy R, Twelves C, Speirs V, Beattie J. Insulin-like growth factor - oestradiol crosstalk and mammary gland tumourigenesis. *Biochim Biophys Acta* 2013;1836:345-53.
- Wang HJ, Tashiro S, Onodera S, Ikejima T. Inhibition of insulin-like growth factor 1 receptor signaling enhanced silibinin-induced activation of death receptor and mitochondrial apoptotic pathways in human breast cancer MCF-7 cells. *J Pharmacol Sci* 2008;107:260-9.
- Gupta RK, Patel AK, Kumari R, Chugh S, Shrivastav C, Mehra S, et al. Interactions between oxidative stress, lipid profile and antioxidants in breast cancer: a case control study. *Asian Pac J Cancer Prev* 2012;13: 6295-8.
- Flaig TW, Gustafson DL, Su LJ, Zirrollo JA, Crighton F, Harrison GS, et al. A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. *Invest New Drugs* 2007;25:139-46.
- Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst* 2007;99: 167-70.
- Viale G, Regan MM, Maiorano E, Mastropasqua MG, Dell'Orto P, Rasmussen BB, et al. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer. *BIG 1-98. J Clin Oncol* 2007;25:3846-52.
- Ellis MJ, Tao Y, Luo J, A'Hern R, Evans DB, Bhatnagar AS, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst* 2008; 100:1380-8.
- Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat Prod Rep* 2009;26:1001-43.
- Ramasamy K, Agarwal R. Multitargeted therapy of cancer by silymarin. *Cancer Lett* 2008;269:352-62.
- Kim TH, Woo JS, Kim YK, Kim KH. Silibinin induces cell death through reactive oxygen species-dependent downregulation of notch-1/ERK/Akt signaling in human breast cancer cells. *J Pharmacol Exp Ther* 2014;349: 268-78.
- Kim S, Kim SH, Hur SM, Lee SK, Kim WW, Kim JS, et al. Silibinin prevents TPA-induced MMP-9 expression by down-regulation of COX-2 in human breast cancer cells. *J Ethnopharmacol* 2009;126:252-7.
- Dastpeyman M, Motamed N, Azadmanesh K, Mostafavi E, Kia V, Jahanian-Najafabadi A, et al. Inhibition of silibinin on migration and adhesion capacity of human highly metastatic breast cancer cell line, MDA-MB-231, by evaluation of beta1-integrin and downstream molecules, Cdc42, Raf-1 and D4GDI. *Med Oncol* 2012;29:2512-8.
- Tyagi AK, Agarwal C, Chan DC, Agarwal R. Synergistic anti-cancer effects of silibinin with conventional cytotoxic agents doxorubicin, cisplatin and carboplatin against human breast carcinoma MCF-7 and MDA-MB468 cells. *Oncol Rep* 2004;11:493-9.
- Flaig TW, Glode M, Gustafson D, van BA, Tao Y, Wilson S, et al. A study of high-dose oral silybin-phytosome followed by prostatectomy in patients with localized prostate cancer. *Prostate* 2010;70:848-55.
- Hoh C, Boockch D, Marczylo T, Singh R, Berry DP, Dennison AR, et al. Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences. *Clin Cancer Res* 2006;12:2944-50.
- Zi X, Zhang J, Agarwal R, Pollak M. Silibinin up-regulates insulin-like growth factor-binding protein 3 expression and inhibits proliferation

- of androgen-independent prostate cancer cells. *Cancer Res* 2000;60:5617–20.
22. Gandini S, Guerrieri-Gonzaga A, Pruneri G, Serrano D, Cazzaniga M, Lazzeroni M, et al. Association of molecular subtypes with Ki-67 changes in untreated breast cancer patients undergoing pre-surgical trials. *Ann Oncol* 2014;25:618–23.
 23. Bosch-Barrera J, Menendez JA. Silibinin and STAT3: A natural way of targeting transcription factors for cancer therapy. *Cancer Treat Rev* 2015;41:540–6.
 24. Chen Y, Wang J, Wang X, Liu X, Li H, Lv Q, et al. STAT3, a Poor Survival Predictor, Is Associated with Lymph Node Metastasis from Breast Cancer. *J Breast Cancer* 2013;16:40–9.
 25. Wang YX, Cai H, Jiang G, Zhou TB, Wu H. Silibinin inhibits proliferation, induces apoptosis and causes cell cycle arrest in human gastric cancer MGC803 cells via STAT3 pathway inhibition. *Asian Pac J Cancer Prev* 2014;15:6791–8.
 26. Bakan E, Taysi S, Polat MF, Dalga S, Umudum Z, Bakan N, et al. Nitric oxide levels and lipid peroxidation in plasma of patients with gastric cancer. *Jpn J Clin Oncol* 2002;32:162–6.
 27. Hewala TI, bd El-Moneim NA, Ebied SA, Sheta MI, Soliman K, bu-Elenean A. Diagnostic and prognostic value of serum nitric oxide, tumor necrosis factor-alpha, basic fibroblast growth factor and copper as angiogenic markers in premenopausal breast cancer patients: a case-control study. *Br J Biomed Sci* 2010;67:167–76.

Cancer Prevention Research

A Presurgical Study of Oral Silybin-Phosphatidylcholine in Patients with Early Breast Cancer

Matteo Lazzeroni, Aliana Guerrieri-Gonzaga, Sara Gandini, et al.

Cancer Prev Res 2016;9:89-95. Published OnlineFirst November 2, 2015.

Updated version Access the most recent version of this article at:
doi:[10.1158/1940-6207.CAPR-15-0123](https://doi.org/10.1158/1940-6207.CAPR-15-0123)

Cited articles This article cites 26 articles, 5 of which you can access for free at:
<http://cancerpreventionresearch.aacrjournals.org/content/9/1/89.full#ref-list-1>

Citing articles This article has been cited by 1 HighWire-hosted articles. Access the articles at:
<http://cancerpreventionresearch.aacrjournals.org/content/9/1/89.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerpreventionresearch.aacrjournals.org/content/9/1/89>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.