Silibinin Administration Improves Hepatic Failure Due to Extensive Liver Infiltration in a Breast Cancer Patient

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Abstract. *Background: Silibinin exerts hepatoprotective, anti-inflammatory and anti-fibrotic effects. Several pre-clinical studies have shown anti-tumoral activity of silibinin in breast cancer cell lines. Case Report: We present the case of a heavily pre-treated breast cancer patient with extensive liver infiltration. The patient presented with progressive liver failure despite several chemotherapy treatments, including paclitaxel, capecitabine and vinorelbine. After four cycles of a fourth-line chemotherapy treatment consisting of carboplatin and gemcitabine, the patient's liver blood test results deteriorated to life-threatening levels. The compassionate use of Legasil®, a new commercially available nutraceutical product containing a new silibinin formulation, was offered to the patient according to article 37 of the 2013 Declaration of Helsinki. After treatment initiation, the patient presented clinical and liver improvement, which permitted the patient to continue palliative chemotherapy. Conclusion: This is the first case report of a clinical benefit of silibinin administration in a breast cancer patient.*

Case Report

Herein, we describe the case of a 39-year-old Caucasian woman who presented with right breast cancer in November 2004. She underwent right breast radical modified mastectomy and lymphadenectomy in December 2004. The primary tumor was 6×3.9 cm, and 7 out of 20 nodes were positive. The histological diagnosis was invasive ductal carcinoma. An immunohistochemical (IHC) examination of the tumor cells was positive for estrogen receptor and negative for progesterone receptor. The tumor showed moderate membrane staining for human epidermal growth factor receptor 2 (HER-2) (2+ score). The fluorescence *in situ* hybridization (FISH) analysis was negative for HER-2 gene amplification (HER2/CEP17 ratio=1.0). The patient was staged with T3N2aM0 disease.

The patient was included in a clinical trial (GEICAM 2003-10: NCT00129935) and was randomized to receive postoperative chemotherapy with four cycles of epirubicin and docetaxel, followed by four cycles of capecitabine. The chemotherapy treatment was completed in November 2005. The patient also underwent adjuvant radiotherapy after completion of chemotherapy. The patient began adjuvant endocrine therapy with tamoxifen and completed 5 years of treatment in November 2010.

The patient showed no evidence of disease until July 2012, when she presented mild bone pain and asthenia. The laboratory analysis yielded the following values: elevated carcinoma antigen 15-3 (Ca15.3) of 254 U/ml (normal value <40), aspartate aminotransferase (AST) 229 UI/l (normal value <42), alanine aminotransferase (ALT) 60 UI/l (normal value <41) and bilirubin 1.72 mg/dl (normal value <1.0). The patient evaluation also included a bone scan, which revealed metastases in the skull, spine, ribs, right humerus, pelvic bones and both proximal femurs. A computed tomography (CT) body scan confirmed multiple lytic bone lesions and multiple hepatic metastases. The largest metastasis was 5.5 cm.

The patient started chemotherapy treatment with weekly paclitaxel in August 2012 to treat her symptomatic visceral metastases. Zoledronic acid was also administered monthly. A notable improvement in AST, ALT and bilirubin was

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achieved with the treatment. A liver biopsy was performed when the laboratory liver test results were normalized. The test results confirmed invasive ductal carcinoma that was estrogen receptor-positive, progesterone receptor-negative, and HER-2-negative. Paclitaxel treatment was discontinued in January 2013 due to grade 3 skin toxicity (nail changes) and grade 2 sensory neuropathy. There was no visceral tumor crisis and 500 mg of fulvestrant was administered from January 2013 to July 2013. Due to increased Ca15.3 levels a new chemotherapy treatment with capecitabine was administered from July 2013 to September 2013 but tumor marker progression persisted. A third-line chemotherapy treatment with vinorelbine was administered but disease progression occurred after three cycles in November 2013.

Treatment options were discussed with the patient and she declined chemotherapy that could cause alopecia (taxanes and anthracyclines). The laboratory results were as follows: Ca15.3, 3,523 U/ml; AST, 176 UI/l; and bilirubin, 2.42 mg/dl. The patient was treated with carboplatin (AUC 3) bi-weekly according to the patient's preferences. On January 10, 2014, adjusted gemcitabine treatment (40% of the protocol dose, 800 mg/m^2) was added to the carboplatin treatment because no analytical improvement was detected (Figure 1) and the patient's performance status was satisfactory (Eastern Cooperative Oncology Group [ECOG] score 1). Additional tests on January 24 revealed that the patient's liver function was not improving and the patient showed an increased abdominal perimeter (89 cm) due to ascites despite established diuretic treatment with furosemide 40 mg (1-1-0) and spironolactone 125 mg (0-1-0).

The patient requested additional treatment to improve her disease. We indicated that a new product containing silibinin was commercially available in Spain beginning in January 2014. The patient was informed that the product could have hepatoprotective and anticancer activity according to preclinical data but that this treatment was still experimental in humans. The patient accepted this compassionate treatment, and a signed consent form was obtained according to article 37 of the 2013 Declaration of Helsinki. A titration was started with 1 capsule of Legasil® for 3 days and an additional capsule was then added every 3 days until a 2-2-1 dosage was achieved. After initiating treatment with Legasil®, the patient showed progressive clinical improvement. The patient's abdominal perimeter decreased to 81 cm and the liver test results improved (Figure 1). The patient is currently taking Legasil® (2-2-1) and carboplatin-plus-gemcitabine with no observed toxicity. The patient has an ECOG score of 0 and the tumor marker Ca 15.3 level has decreased (Figure 2).

Discussion

The Declaration of Helsinki summarizes the ethical principles for medical research involving human subjects (1). Article 37 of the 2013 version indicates that: "In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available."

Herein, we present the first case of a breast cancer patient who received Legasil®, a product that contains silibinin plus vitamin E, to improve liver function in a compassionate use setting.

Silibinin is the primary active constituent of a crude extract (silymarin) derived from milk thistle plant (*Silybum marianum*) seeds. Silymarin contains a large fraction of flavonolignan monomers (*e.g.*, silybin, isosilybin, silychristin and silydianin) and a smaller fraction of polymeric and oxidized polyphenolic components. Silymarin has very poor bioavailability due to the poor water solubility (<0.04 mg/ml) of its flavonolignan structure (2). This property considerably limits the clinical applications and therapeutic efficiency of oral silibinin administration (3).

Pre-clinical silibinin anticancer activity has been observed in multiple types of cancers (4, 5). Our group previously published the finding that silibinin meglumine can reverse resistance to epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitors (TKIs) in PC9 cells (EGFR-mutant non-small cell lung carcinoma [NSCLC] cells) *in vitro* and *in vivo* (2, 6). The mechanism of action of silibinin in this PC9 model is based on the inhibition of epithelial-to-mesenchymal transition (EMT). Additionally, loss of responsiveness to erlotinib in EGFR-mutant NSCLC can be explained by enrichment of erlotinib-refractory aldehyde dehydrogenase (ALDH) bright cells, which exhibit stem cell-like properties. The erlotinib-refractory ALDH^{bright} cells are sensitive to the natural agent silibinin (7).

Several previous publications have suggested the presence of silibinin activity in breast cancer cell lines. Kim *et al.* recently demonstrated that silibinin induces cell death through an apoptosis-inducing factor (AIF)-dependent mechanism in MCF-7 cells and a caspase-3-dependent mechanism in MDA-MB-231 cells. The mechanism also involves reactive oxygen species (ROS) generation and Notch-1 signaling upstream of the ERK and Akt pathways (8). Ohj *et al.* suggested that silibinin suppresses 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced cell migration and matrix metallopeptidase-9 (MMP-9) expression through the MEK/ERK-dependent pathway in MCF-7 breast cancer cells (9). The combination of silibinin and cytostatic drugs was analyzed a decade ago

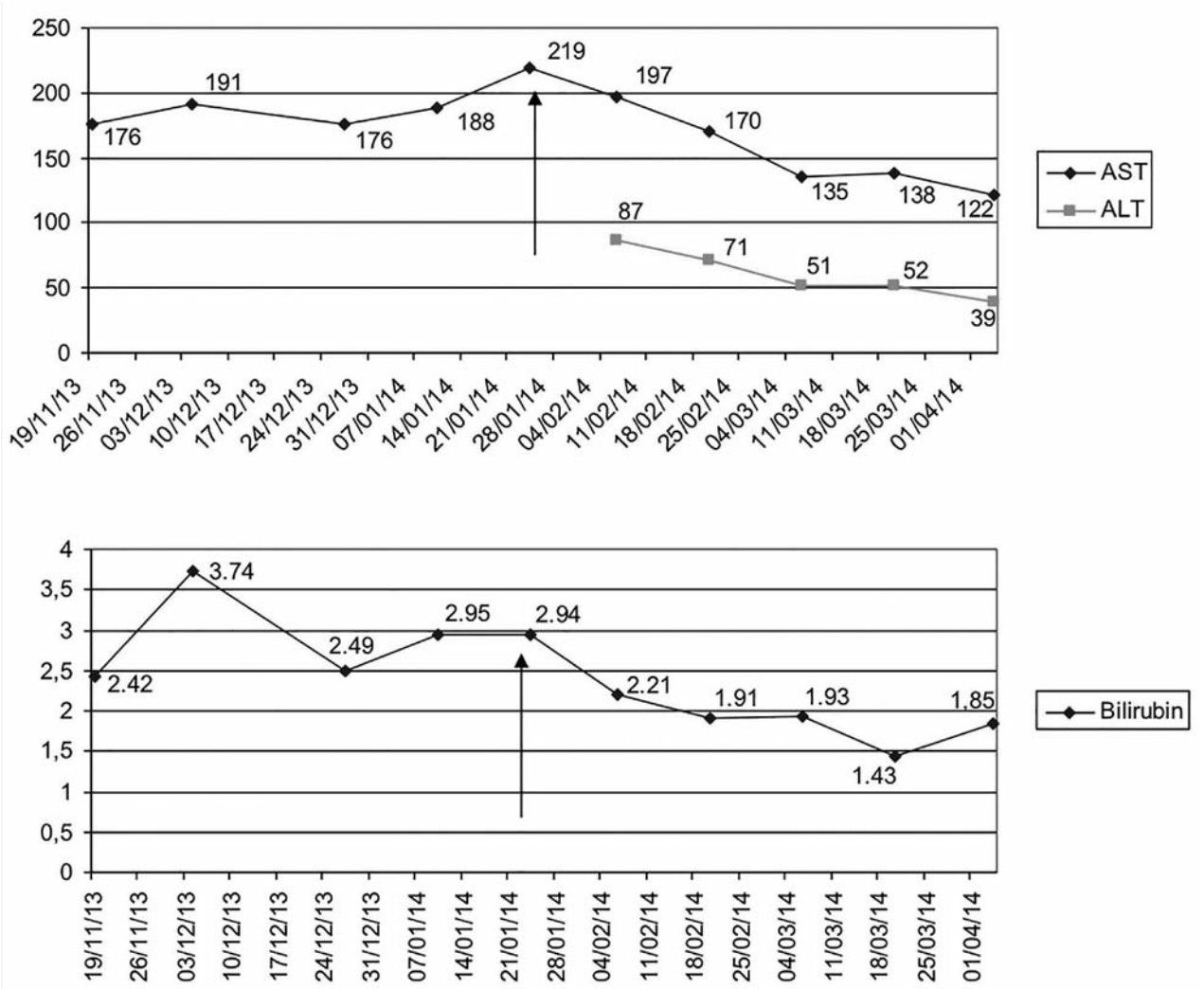

Figure 1. *The evolution of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin levels during chemotherapy treatment with carboplatin and gemcitabine. The arrow indicates the time at which Legasil® treatment was initiated.*

(10). The combination of silibinin and carboplatin showed strong apoptotic effects in MCF-7 cells. However, this effect was not observed when cisplatin was used. The combination of silibinin and doxorubicin resulted in higher rates of apoptotic death compared to each agent alone in the MCF-7 and MDA-MB468 cell lines.

There are several oral silymarin products available in Spain (*e.g.*, Legalon®, Sylarine®). Unfortunately, these products have poor bioavailability in humans (3,11). In January 2014, a product with a new formulation of silibinin (Eurosil 85®, Euromed, Mollet del Vallés, Barcelona, Spain) was launched in Spain under the commercial name of Legasil® (Rottapharm-Madaus, Barcelona, Spain). This product is available without a medical prescription because it is considered a nutritional supplement. Each Legasil® pill contains 210 mg of Eurosil85 (60% of silibinin isoforms) and 18 mg of vitamin E. This product was designed for patients suffering from liver diseases because the combination of silibinin and vitamin E exerts hepatoprotective, anti-inflammatory and antifibrotic effects (12).

The role of vitamin E in cancer is controversial (13). Previous studies indicated that its antioxidant activity could be deleterious for some cancers, as recently shown *in vivo* in human cell lung cancer cell lines (14). Conversely, it has been proposed that vitamin E could reverse multidrug resistance to paclitaxel both *in vitro* and *in vivo* (15).

A derivative of vitamin E, d-alpha-tocopheryl polyethylene glycol succinate (TPGS), has been intensively applied as a vehicle for drug delivery systems to enhance drug solubility and increase the oral bioavailability of different anticancer

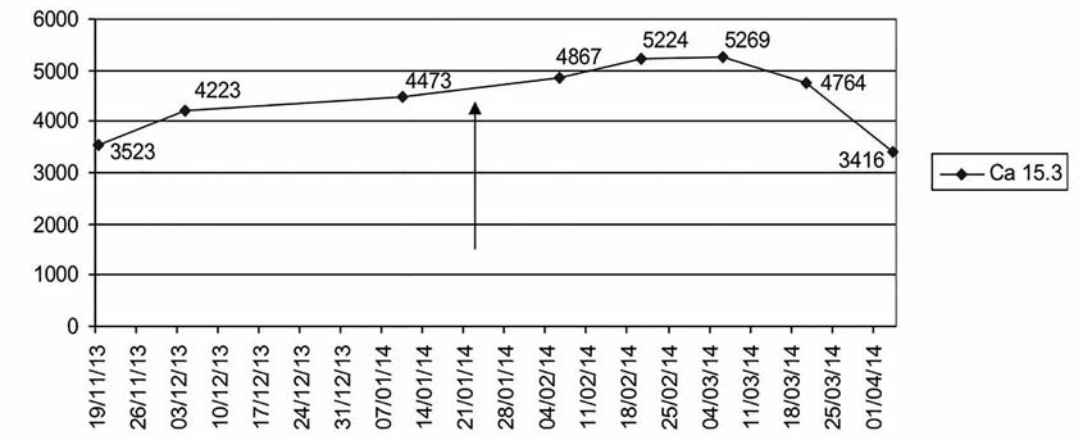

Figure 2. *The evolution of Ca 15.3 tumor marker levels during chemotherapy treatment with carboplatin and gemcitabine. The arrow indicates the time at which Legasil® was initiated.*

drugs (16). Neophytou *et al*. reported that TPGS can induce G_1/S cell cycle arrest and apoptosis in breast cancer cell lines (MCF-7 and MDA-MB-231). TPGS was also shown to induce both caspase-dependent and -independent apoptotic signaling pathways (17). Interestingly, Xu *et al*. demonstrated that silibinin-loaded lipid nanoparticles containing TPGS had inhibitory effects on the invasion and migration of MDA-MB-231 breast cancer cells through the down-regulation of MMP-9 and Drosophila embryonic protein SNAI1, commonly known as Snail (18). These pre-clinical results could explain the clinical and analytical benefits observed in our patient after Legasil® treatment was initiated.

Conclusion

The present case represents the first published experience of a patient with breast cancer showing hepatic improvement after compassionate treatment with Legasil®, a newly-available formulation of silibinin with vitamin E. This clinical experience warrants further clinical research.

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