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Effect of the Botanical Compound LCS101 on Cytotoxicity of Chemotherapy

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Abstract

Many oncology patients report using botanicals while undergoing chemotherapy. There are relatively few studies on the interactions between "natural" products and chemotherapy agents, with implications regarding safety and efficacy of the conventional treatment. LCS101 is a botanical formula which has been shown to reduce the incidence of severe anemia and neutropenia, as well adverse events resulting from chemotherapy regimens for breast cancer. The formula has also been shown to increase the anti-cancer effects of doxorubicin and fluorouracil (5-FU) on breast cancer cell lines, while protecting non-tumorigenic breast cells from cell death. The present study set out to further examine the effects of LCS101 on chemotherapy, this time with gemcitabine, cisplatin, paclitaxel and etoposide. For this purpose, lung (A549), breast (MCF7), pancreatic (PANC-1) and bladder (T24) cancer cell lines were exposed to incremental concentrations of each of the four chemotherapy agents, with and without the addition of fixed dose of LCS101. A sulforhodamine B (SRB) assay was used to assess cell viability. The addition of the botanical formula was found to significantly augment the cytotoxic effects of each of the chemotherapy agents, this in all four cancer cell lines. These findings further support those of previous research on potential interactions between LCS101 with chemotherapy. Additional research is underway to examine the implications of this and other botanical formulas as an adjunct to conventional oncology treatments.

Keywords: Botanical formula; LCS101; Anti-cancer; Safety; Chemotherapy; Herb-drug interaction

Introduction

Oncology patients often seek out complementary or "alternative" medical therapies such as the use of herbal remedies, often in conjunction with their chemotherapy regimens [1,2]. Patients most often claim that they are using herbal medicine in order to relieve the side effects of conventional treatments, though many believe that these products can "cure" their cancer and "strengthen" the body's immune system [3-5]. While the use of "natural" medicine is believed to be both safe and effective [6], research has found that some of these products have potentially toxic effects, such as cyanide poisoning with amygdalin, a compound derived from apricot pits [7]; can reduce serum levels of drugs through the induction of cytochrome P450 metabolism and/or P-glycoprotein activity, as seen with *hypericum* (St. Johns wort) [8]; and can inhibit the cytotoxic effects of chemotherapy, such as that of cisplatin and carboplatin on breast cancer cell lines, as seen with the herb *Ephedra foemina* [9].

The botanical formula LCS101 was designed for the treatment of patients with breast cancer. While the formula's components were chosen according to the principles of traditional Chinese Medicine, many of them have been shown to have anti-cancer effects as well (Table 1) [10-22].

In a phase II randomized, double-blind clinical trial, patients with breast cancer who were undergoing chemotherapy (doxorubicin/cyclophosphamide, with/without paclitaxel) were less likely to develop severe anemia, leukopenia or neutropenia when treated concurrently with LCS101 (vs. placebo-treated controls) [23]. In a follow-up retrospective clinical study of 20 post-operative patients with breast cancer, the self-administration of LCS101 during adjuvant chemotherapy was associated with lower scores of severities for a number of adverse effects of the conventional treatment regimen than expected [24]. In both of these clinical trials no adverse effects were associated with the use of the botanical formula. Animal research has also found that exposure to LCS101 was not associated with either reduced body weight or changes in the animal's behavior as well as a tendency for reduction in tumor size in concurrent treatment of the formula with doxorubicin and fluorouracil (5-FU) [25].

LCS101 capsules are prepared in accordance with Good Manufacturing Practice and authorized by the Israeli Ministry of Health as safe for consumption, with no evidence of trace heavy metals, microbial contamination, pesticides or mycotoxins. The potential for negative interactions between LCS101, both as a formula and as individual components, has been addressed in part in previous research. A search of the medical literature has shown that the individual herbal components of the formula do not affect the pharmacodynamics of conventional drugs, including chemotherapy agents, or affect the cytotoxic activity of chemotherapy on cancer cell lines [26]. The interaction between LCS101 and chemotherapy has been examined on a cellular level as well. The addition of LCS101 to the chemotherapy agent's doxorubicin and 5-FU was shown to significantly increase the cytotoxic effects of these drugs on breast cancer cell lines (MCF-7, MDA-MB-231), while reducing apoptosis in non-tumorigenic human epithelial breast cells (MCF-10A) [27].

Herbal Component	Anticancer Effects
Astragalus membranaceus	Suppression of C6 glioma cells, *in vitro* and *in vivo* [10]
Atractylodes macrocephala	Mediation of reactive oxygen species apoptosis in human leukemia cells [11]
Citrus reticulate	Induction of apoptosis in SNU-C4 human colon cancer cells [12], Induction of apoptosis in human gastric cancer cells (cas-3 pathway) [13]
Ligustrum lucidum	Induction of human glioma cell death through regulation of Akt/mTOR pathway *in vitro* and reduction of glioma tumor growth in U87MG xenograph mouse model [14]
Oldenlandia diffusa	Augmentation of oxidative burst in macrophages and inhibited tumor growth [15], Selective anti-cancer *in vitro* effects in B16-F10 mice lung cancer and Renca renal carcinoma models [16]
Paeonia lactiflora	Inhibition of bladder cancer growth in a rat model involving phosphorylation of Chk2, *in vitro* and *in vivo* [17]
Prunella vulgaris	Chemoprevention of non-small cell lung cancer (NSCLC) via promotion of apoptosis and regulation of the cell cycle [18], Suppression of PMA-induced tumor cell invasion and metastasis *via* inhibition of NF-kappaB-dependent MMP-9 expression [19]
Scutellaria barbata	Induction of oxidative stress damage with redistribution of metabolic fluxes in breast cancer cells [20], Selective cytotoxic activity on breast cancer cells [21], Augmentation of oxidative burst in macrophages and inhibited tumor growth [15, Modulation of apoptosis and cell survival in murine and human prostate cancer cells and tumor development in TRAMP mice [22]

Table 1: Anti-cancer effects of LCS101 herbal components.

The purpose of the present study was to further examine the effects of LCS101 on the cytotoxicity of four chemotherapy agents – gemcitabine, cisplatin, paclitaxel and etoposide – on four cancer cell lines - lung (A549), breast (MCF7), pancreas (PANC-1), and bladder (T24). The implications of the findings regarding the safety of using LCS101 in conjunction with conventional chemotherapy are discussed as well.

Materials and Methods

Cell lines

The following cancer cell line cultures were used in the study: American Type Culture Collection (ATCC) (Manassas, VA, USA) A549 lung carcinoma; MCF7 breast adenocarcinoma; PANC-1 pancreatic epithelioid carcinoma; and T24 bladder transitional cell carcinoma. All cell lines were propagated in RPMI1640, supplemented with 10% FBS; 2 mM L-glutamine; and 100 μg/ml Pen/Strep (Biological Industries, Beit Ha-Emek, Israel), and incubated in 37°C, 5% CO_2.

Botanical extracts and chemotherapies

The chemotherapy agent's gemcitabine, paclitaxel, etoposide and cisplatin were obtained from Sigma Aldrich Products (St. Louis, MO, USA). Standardized dried herbal extracts were purchased from Bara Herbs Ltd. (Yokneam, Israel). The formula was dissolved in PBS at a concentration of 100 mg/ml, and incubated at 60°C for 30 minutes, with occasional vortex. The solution was centrifuged at 5000 rpm for 5 minutes, and the supernatant filtered through a 0.45 μM Millex PVDF filter (Millipore Ireland Ltd., Carrigtwohill, Ireland). Solubility was estimated by cryophilization and weighting of the pellet and was estimated to be at about 50%. For convenience, the final stock concentration was designated at 100 mg/ml (w/v concentration of dried powder in PBS).

Treatments and viability assay

Sulforodamine B, trichloroacetic acid, acetic acid was from Sigma Aldrich (St. Louis, MO, USA). The cells were plated 3000/w over 96 well plates and allowed to attach and grow overnight. After that treatments were added in triplicates and the cells were propagated for an additional 72 h. SRB viability test was performed in the following way: the cells were fixed for 1 h with 10% trichloroacetic acid (v/v in RPMI1640), washed trice with DDW, dried and stained for 1 h with 0.057% sulforodamine B (w/v in 1% acetic acid). After staining, the plates were washed three times with 1% acetic acid and then dried, and 200 μl 10 mM Tris was added to each well to solubilize SRB. The absorbance was measured at 570 nm using ELISA reader. Each experiment was repeated at least three times.

Statistical methods

The mean ± the standard deviation estimates were calculated for each experiment and performed in triplicate. The data were collated and analyzed in a Microsoft Excel 2007. P-value was calculated for combined treatment compared to chemotherapy alone, using a Student's t-test.

Results

Initially, each of the concentrations of drugs and botanical formula were calibrated individually for each cell line, in accordance with the sensitivity of each cell line. For each of the cancer cell lines, the concentrations of the LCS101 and chemotherapy agents were selected according to their individual dose-response curves, on the slope between 0 (no response) and 100% (full response) plateaus. Each line

was exposed to incremental concentrations of gemcitabine (Gem), cisplatin (cis-PL), paclitaxel (taxol) and etoposide (VP-16), with a fixed single dose of LCS101 for each cell line. The outcomes of the herb-drug interaction for each cell line are shown in Figure 1. The addition of LCS101 to conventional chemotherapy agents was shown to augment the effects of the conventional treatment in all of the four tested cancer cell lines.

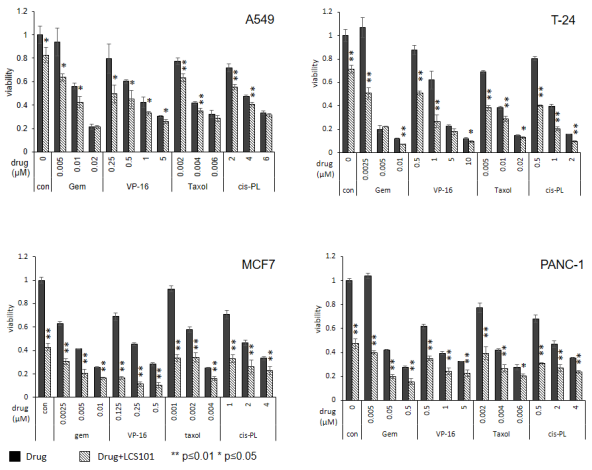

Figure 1: Effects of LCS101 on chemotherapy-induced apoptosis: Cancer cell cultures were treated for 72 h with incremental concentrations of the chemotherapy agent's gemcitabine (Gem), etoposide (VP-16), taxol (Taxol) or cisplatin (cis-PL), either alone or in combination with single fixed concentration of LCS101 (1 mg/ml for T-24, Panc-1 and A549, 0.75 mg/ml for MCF7). Cancer cell viability was examined via SRB staining. Asterisks indicate p-values of combined treatment vs. treatment with chemotherapy alone (**- p<0.01, *- p<0.05).

Discussion and Conclusion

The use of herbal medicine by oncology patients during active cancer care is widespread. Yet most patients do not disclose this practice to their oncologists, either because they fear an antagonistic response or because they are not asked [28]. The unmonitored use of herbal medicine in the oncology setting can have significant implications, as well as benefit, regarding patient safety and treatment outcomes, and emphasizes the importance of an open and non-judgmental patient-oncologist dialogue [29]. At the same time, there is need for research to identify herb-drug interactions which can compromise patient safety and affect conventional treatment outcomes. This includes examining herb-drug interactions which, in addition to impacting the pharmacodynamics of conventional drugs, can also reduce the cytotoxic effects of a number of chemotherapy agents.

The findings of the present study show that the botanical formula LCS101 does not reduce the cytotoxic effects of the chemotherapy agent's cisplatin, paclitaxel (taxol), gemcitabine or etoposide, this in four cancer cell lines – lung, breast, pancreatic and bladder. Instead, the addition of LCS101 led to increased death of cancer cells to the chemotherapy agents. These findings support those of a previous study, in which the addition of LCS101 to doxorubicin and fluorouracil (5-FU) led to an increased cytotoxic effect on breast cancer cell lines (MCF-7, MDA-MB-231), while "protecting" non-tumorigenic human epithelial breast cells (MCF-10A) from any harmful effects. These effects were shown using 3 different methods: XTT viability assay, cell-cycle analysis and Western blot [27]. These findings are encouraging, though further research – both pre-clinical and clinical - is still needed to further examine the use of LCS101 or other botanical compounds as an adjunct to the conventional chemotherapy regimen.

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Authors' contributions

All of the authors participated in the conception and design of the research and preparation of the study article.

Competing Interests

Dr. Yair Maimon is a shareholder of LifeBiotics Ltd.

References

1. Vapiwala N, Mick R, Hampshire MK, Metz JM, DeNittis AS (2006) Patient initiation of complementary and alternative medical therapies (CAM) following cancer diagnosis. Cancer J 12: 467-474.
2. Yates JS, Musteian KM, Morrow GR, Gillies LJ, Padmanaban D, et al. (2005) Prevalence of complementary and alternative medicine use in cancer patients during treatment. Support Care Cancer 13: 806-811.
3. Roberts CS, Baker F, Hann D, Runfola J, Witt C, et al. (2005) Patient–physician communication regarding use of complementary therapies during cancer treatment. J Psychosoc Oncol 23: 35-60.
4. Boon HS, Olatunde F, Zick SM (2007) Trends in complementary/alternative medicine use by breast cancer survivors: Comparing survey data from 1998 and 2005. BMC Women Health 7: 4.
5. Matthews AK, Sellergren SA, Huo D, List M, Fleming G (2007) Complementary and alternative medicine use among breast cancer survivors. J Altern Complement Med 3: 555-562.
6. Saibul N, Shariff ZM, Rahmat A, Sulaiman S, Yaw YH (2012) Use of complementary and alternative medicine among breast cancer survivors. Asian Pac J Cancer Prev 13: 4081-4086.
7. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, et al. (1993) Unconventional medicine in the United States. Prevalence, costs, and patterns of use. N Engl J Med 328: 246-252.
8. Moertel CG, Fleming TR, Rubin J, Kvols LK, Sarna G, et al. (1982) A clinical trial of amygdalin (Laetrile) in the treatment of human cancer. N Engl J Med 306: 201-206.
9. Mathijssen RH, Verweij J, De Bruijn P, Loos WJ, Sparreboom A (2002) Effects of St. John's Wort on irinotecan metabolism. J Nat Cancer Inst 94: 1247-1249.
10. Ben-Arye E, Mahajna J, Aly R, Ali-Shtayeh MS, Bentur Y, et al. (2016) Exploring an herbal "wonder cure" for cancer: a multidisciplinary approach. J Cancer Res Clin Oncol 142: 1499-1508.
11. Sun JY, Yang H, Miao S, Li JP, Wang SW, et al. (2009) Suppressive effects of swainsonine on C6 glioma cell in vitro and in vivo. Phytomedicine 16: 1070-1074.
12. Huang HL, Chen CC, Yeh CY, Huang RL (2005) Reactive oxygen species mediation of baizhu-induced apoptosis in human leukemia cells. J Ethnopharmacol 97: 21-29.
13. Kang SA, Park HJ, Kim MJ, Lee SY, Han SW, et al. (2005) Citri Reticulatae Viride Pericarpium extract induced apoptosis in SNU-C4, human colon cancer cells. J Ethnopharmacol 97: 231-235.

14. Kim MJ, Park HJ, Hong MS, Park HJ, Kim MS, et al. (2005) Citrus Reticulata blanco induces apoptosis in human gastric cancer cells SNU-668. Nutr Cancer 51: 78-82.
15. Jeong JC, Kim JW, Kwon CH, Kim TH, Kim YK (2011) Fructus ligustri lucidi extracts induce human glioma cell death through regulation of Akt/mTOR pathway in vitro and reduce glioma tumor growth in U87MG xenograft mouse model. Phytother Res 25: 429-434.
16. Wong BY, Lau BH, Jia TY, Wan CP (1996) Oldenlandia diffusa and Scutellaria barbata augment macrophage oxidative burst and inhibit tumor growth. Cancer Biother Radiopharm 11: 51-56.
17. Gupta S, Zhang D, Yi J, Shao J (2004) Anticancer activities of Oldenlandia diffusa. J Herb Pharmacother 4: 21-33.
18. Ou TT, Wu CH, Hsu JD, Chyau CC, Lee HJ, et al. (2011) Paeonia lactiflora Pall inhibits bladder cancer growth involving phosphorylation of Chk2 in vitro and in vivo. J Ethnopharmacol 135: 162-172.
19. Feng L, Jia X, Zhu M, Chen Y, Shi F (2010) Chemoprevention by Prunella vulgaris L. extract of non-small cell lung cancer via promoting apoptosis and regulating the cell cycle. Asian Pac J Cancer Prev 11: 1355-1358.
20. Choi JH, Han EH, Hwang YP, Choi JM, Choi CY, et al. (2010) Suppression of PMA-induced tumor cell invasion and metastasis by aqueous extract isolated from Prunella vulgaris via the inhibition of NF-kappaB-dependent MMP-9 expression. Food Chem Toxicol 48: 564-571.
21. Klawitter J, Klawitter J, Gurshtein J, Corby K, Fong S, et al. (2011) Bezielle (BZL101)-induced oxidative stress damage followed by redistribution of metabolic fluxes in breast cancer cells: A combined proteomic and metabolomic study. Int J Cancer 129: 2945-2957.
22. Fong S, Shoemaker M, Cadaoas J, Lo A, Liao W, et al. (2008) Molecular mechanisms underlying selective cytotoxic activity of BZL101, an extract of Scutellaria barbata, towards breast cancer cells. Cancer Biol Ther 7: 577-586.
23. Wong BY, Nguyen DL, Lin T, Wong HH, Cavalcante A, et al. (2009) Chinese medicinal herb Scutellaria barbata modulates apoptosis and cell survival in murine and human prostate cancer cells and tumor development in TRAMP mice. Eur J Cancer Prev 18: 331-341.
24. Yaal-Hahoshen N, Maimon Y, Siegelmann-Danieli N, Lev-Ari S, Ron IG, et al. (2011) A prospective, controlled study of the botanical compound mixture LCS101 for chemotherapy-induced hematological complications in breast cancer. Oncologist 16: 1197-1202.
25. Samuels N, Maimon Y, Zisk-Rony RY (2013) Effect of the Botanical Compound LCS101 on chemotherapy-induced symptoms in patients with breast cancer: A case series report. Integr Med Insights 8: 1-8.
26. Rachmut IH, Samuels N, Melnick SJ, Ramachandran C, Sharabi Y, et al. (2013) Immunomodulatory effects of the botanical compound LCS101: implications for cancer treatment. Onco Targets Ther 6: 437-445.
27. Mooiman KD, Goey AK, Meijerman I, Beijnen JH, Schellens JH (2012). Letter to the editor regarding "A prospective, controlled study of the botanical compound mixture LCS101 for chemotherapy-induced hematological complications in breast cancer" by Yaal-Hahoshen et al. Oncologist 16: 1197-1202. Oncologist 17: 740-741.
28. Cohen Z, Maimon Y, Yoeli-Lerner M, Yang P, Samuels N, et al. (2015) Selective anticancer effects and protection from chemotherapy by the botanical compound LCS101: Implications for cancer treatment. Int J Oncol 46: 308-316.
29. Samuels N, Ben-Arye E, Maimon Y, Berger R (2017) Unmonitored use of herbal medicine by patients with breast cancer: reframing expectations. J Cancer Res Clin Oncol 143: 2267-2273.