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ORIGINAL ARTICLE – CANCER RESEARCH

Effect of the botanical formula LCS101 on the anti-cancer effects of radiation therapy

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Abstract

Background and purpose The botanical formula LCS101 has been shown in clinical research to reduce chemotherapy-induced toxicities. In pre-clinical research, the formula demonstrated selective anti-cancer effects, in part as a result of radical oxygen species (ROS) activity of the botanical components. The present study examined the interaction between LCS101 and radiation therapy on cancer cell lines.

Methods Incremental doses of LCS101 were added to breast adenocarcinoma (MCF7), prostate (DU145), transitional cell bladder carcinoma (T24), pancreatic epithelioid carcinoma (PANC-1), and osteosarcoma (U20S) cell lines 4 h after single-dose irradiation (range 0.5–4 Gy). Cell viability was tested using sulforhodamine B (SRB) assay after 1 week, with ROS activity examined using 1 mM of the ROS scavenger sodium pyruvate (ROS scavenger), testing cell viability with an SRB assay.

Results The addition of LCS101 to MCF7 (breast) and DU-145 (prostate) cancer cell lines resulted in a dose-dependent increase in the antiproliferative effects of radiation treatment. The addition of pyruvate inhibited radiation-induced cell death in all of the cell lines treated with LCS101.

Conclusions The addition of the botanical formula LCS101 to irradiated cancer cells results in an apparent additive effect, most likely through a ROS-mediated mechanism. These findings support the use of LCS101 by patients undergoing radiation therapy, for both its clinical as well as anti-cancer effects.

Keywords Botanical formula · LCS101 · Cancer · Radiation therapy · Radical oxygen species

Introduction

Radiation therapy is a mainstay of many oncology and hemato-oncology treatment regimens, in both the curative (neo-adjuvant or adjuvant) and palliative care settings. Radiation therapy works by inducing the ionization of water molecules in cell cytoplasm, leading to the creation of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals. ROS are chemically active oxygen-containing molecules, naturally produced during mitochondrial respiration and other intracellular processes. ROS play an important role in variety of signal transduction pathways, including differentiation, proliferation and survival. The exposure of cancer cell DNA to free radicals leads to apoptosis and cell death, though some cells may recover and continue to be viable. While radiation-induced cell death is dose dependent, non-cancer cells have a greater resilience, and may recover within as little as 6 h (Hall 2000).

Many patients with cancer seek out non-conventional medicine practices, usually with the goal of alleviating symptoms and improving quality of life. One of the most popular therapies is the use of herbal products and dietary supplements, which as "natural remedies" are assumed to be both effective as well as safe (Eisenberg et al. 1993). However, some herbal products may be accompanied by adverse effects, as well as negatively interacting with conventional anti-cancer treatment. For example, the herbal product amygdalin, derived from apricot pits, was found to contain high levels of cyanide, preventing researchers from examining its efficacy as an anti-cancer agent (Moertel et al. 1982). The herb *Hypericum perforatum* (St. Johns

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wort), used to reduce depression and anxiety, induces drug metabolism via the cytochrome P450 system and increases transport P-glycoprotein activity. Both of these mechanisms can lead to significant reduction in serum levels of many drugs, including chemotherapy agents (Ron et al. 2002). Finally, *Ephedra foeminea*, a popular herbal remedy in Middle Eastern countries believed to have anti-cancer effect, was shown to significantly reduce the cytotoxic activity of cisplatin and carboplatin on breast cancer cell lines (Ben-Arye et al. 2016).

The potential for negative interactions with herbal and other dietary supplements may also have implications regarding their use by patients undergoing radiation therapy. This is most relevant with respect to the use of dietary antioxidants, which can be found in a wide range of natural products and in a number of chemical classes, including carotenoids, polyphenols, tools and triterpenes, each class with its own biological activity. The antioxidant activity of dietary supplements can vary greatly between the different classes and within each class, depending on their bioavailability, dose, and duration or route of administration (Ratnam et al. 2006). For example, vitamin C (ascorbic acid) acts as an antioxidant when taken orally, and conversely as a pro-oxidant when administered intravenously at high doses (Padayatty et al. 2004). The effect of dietary antioxidants on tumors is dose dependent, and at high doses may inhibit the growth of cancer cells without affecting the growth of normal cells (Prasad et al. 2001). At the same time, low doses of dietary antioxidants may stimulate tumor growth (Prasad and Kumar 1996), and have been shown in in vitro and in vivo models to reduce the efficacy of radiation treatment of cancer cells (Witenberg et al. 1999; Sakamoto and Sakka 1973).

The safety of antioxidant supplement use during radiation therapy is controversial. The most significant study to date examined a cohort of 540 patients with head and neck cancers, who were randomly assigned to receive either the antioxidant vitamin E (alpha-tocopherol), with or without beta-carotene, or a placebo preparation. Patients receiving the combined antioxidant supplementation showed a significant 38% reduction in severe adverse effects associated with radiation treatment. However, this benefit appeared to be offset by a reduction of 56% in local tumor control rates (Bairati et al. 2005). The current recommendation of the National Cancer Institute is that "until more is known about the effects of antioxidant supplements in cancer patients, these supplements should be used with caution" (National Cancer Institute: Antioxidants and Cancer Prevention 2018).

LCS101 is a botanical formula, based on the principles of Traditional Chinese Medicine (TCM) and consisting of concentrated extracts from the following herbs: *Astragalus membranaceus, Atractylodes macrocephala, Citrus reticulate, Glehnia littoralis, Ligustrum lucidum, Lycium chinense, Millettia reticulata, Oldenlandia diffusa, Ophiopogon japonicus, Paeonia lactiflora, Paeonia obovata, Poriae cocos, Prunella vulgaris*, and *Scutellaria barbata*. Extracts of these compounds are manufactured in accordance with good manufacturing practice (GMP) conditions, and imported under license (BARA Herbs Inc., Yokneam, Israel) in accordance with the regulations of the Israel Ministry of Health. All batches of the compound are analyzed and certified to be free of heavy metals, microbial contamination, pesticide residues and mycotoxins. The herbal components of LCS101 are considered to be safe for human consumption, and the research to date has found no effect on the bioavailability or efficacy of anti-cancer drugs (Mooiman et al. 2011).

In clinical studies, LCS101 treatment led to a reduced incidence of chemotherapy-induced anemia and neutropenia in patients with breast cancer undergoing anthracycline- and taxane-based therapies (Yaal-Hahoshen et al. 2011). The formula was also shown to induce immunomodulatory effects (Rachmut et al. 2013), as well as selectively inducing cancer cell death in vitro. In addition, LCS101 was shown to increase the anti-cancer effects of 5-FU and doxorubicin, while protecting the non-tumor cells from chemotherapy-induced death (Cohen et al. 2015). It was later shown that the anti-cancer activity of the formula was due, at least in part, to its ROS-related activity (Cohen et al. 2017). The present study set out to examine the interaction between LCS101 and radiation therapy on both cancer and nontumorigenic cell lines. The effects of the formula on ROS activity were examined as well.

Materials and methods

Cancer cell line cultures

The following human cancer cell line cultures were used: American Type Culture Collection (ATCC) (Manassas, VA, USA) MCF7 breast adenocarcinoma, DU 145 prostate carcinoma, T24 bladder transitional cell carcinoma, PANC-1 pancreas epithelioid carcinoma, U2OS osteosarcoma. All cell lines were propagated in RPMI1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 µg/ml Pen/Strep (Biological Industries, Beit Ha-Emek, Israel) and incubated in 37 °C, 5% CO_2.

Study compound

A dry extract powder of the formula (Bara Herbs, Yokneam, Israel) was dissolved in PBS at a concentration of 100 mg/ml, and then incubated for 30 min at 60 °C with 10-s vortex every 5 min. The solution was then centrifuged at 4300 g for 5 min, with the supernatant filtered through a 0.45-µM

Millex PVDF filter (Merck Millipore, Tullagreen, Ireland). Solubility was estimated by cryophilization and weighting of the pellet, and was estimated to be about 50%. For convenience, the final stock concentration was designated at 100 mg/ml (w/v concentration of crude powder in PBS), enabling the comparison of the individual herbal components with their variable solubilities with the formula in its entirety.

SRB viability assay

Sulforhodamine B, trichloroacetic acid, and acetic acid were from Sigma-Aldrich (St. Louis, MO, USA). 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% sulforhodamine B (w/v in 1% acetic acid). After staining, the plates were washed thrice with 1% acetic acid and 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. Survival (viability index) of cells for each treatment was calculated by dividing the treated cell values by the value of the untreated cells (untreated cell viability designated as 1).

ROS involvement in LCS101 anti-cancer effect

The cells were plated 3000 cells per well in 96-well plates and allowed to attach and grow overnight. After that, the formula was added to the final concentration of 2 mg/ml either with or without 1 mM ROS sodium pyruvate (ROS scavenger). The viability was assessed 72 h after the beginning of the treatment by SRB viability test.

Combined treatment with X-ray irradiation and LCS101 formula

The cells were plated 1000 cells per well in 96-well plates. On the next day, the formula was added at rising concentration (0.25–2 mg/ml plus untreated control, each treatment performed in sextuplicates) and 4 h later the cells received single 0.5–4 Gy irradiation dose. The SRB viability assay was performed 1 week (168 h) after the beginning of the treatment. SRB assay was shown to be as effective for radiotherapy and radiotherapy/chemotherapy interaction experiments as the traditional clonogenic assay (Pauwels et al. 2003).

Statistical methods

The mean ± the standard deviation estimates were calculated from each experiment, performed in triplicates (ROS experiments) or sextuplicates (combination of formula and irradiation experiments). Each experiment was repeated at least three times (biological repeats). The data were collated and analyzed in a Microsoft Excel 2007. p value was calculated (using a Student's t test) for the combined treatment compared to irradiation alone, and for ROS experiments—compared to formula alone.

Coefficient of drug interaction (CDI) and combinational index (CI)

CDI for each point of combined treatment was calculated by the following formula: $CDI = AB/(A \times B)$, where AB is the survival rate of the combined formula and irradiation-treated group; and A or B the survival of each of the individual treatment (formula or irradiation) groups. Generally, $CDI < 1$ indicates synergism, $CDI < 0.7$ significant synergism, $CDI = 1$ additivity and $CD > 1$ antagonism. However, to compensate for deviations, natural for biological experiments, we extended the additivity settings to $0.9 \leq CDI \leq 1.1$, as established in other synergy calculation programs, such as CompuSyn. Combinational index (CI) was calculated using CompuSyn program.

Results

LCS101 induces cancer cell death through ROS

To address the question of ROS involvement in the formula effect, we used ROS scavenger pyruvate, as in our previous study on the role of ROS in the anti-cancer effect of botanicals (Cohen et al. 2017). Five human cancer cell lines were treated for 72 h with 2 mg/ml of LCS101 either alone or in the presence of 1 mM sodium pyruvate. Pyruvate effectively blocked the antiproliferative action of the formula in all tested cell lines, confirming our hypothesis on ROS involvement in the LCS101 anti-cancer effect (Fig. 1).

Combined treatment with LCS101 and X-ray irradiation

The concentrations of formula and irradiation doses were calibrated for each cell line before the presented research to set the experimental conditions in accordance with each cell line's sensitivity (not shown). Two cancer cell lines, MCF7 and DU-145, were pretreated for 4 h with rising formula concentrations and then exposed to rising doses of X-ray irradiation. A dose-dependent increase in the antiproliferative effect of irradiation was with the incremental doses of LCS101 to the irradiated cells. At low doses of the formula (0.25 mg/ml), neither anti-cancer effect was observed nor was there any change in the effect of radiation on MCF7

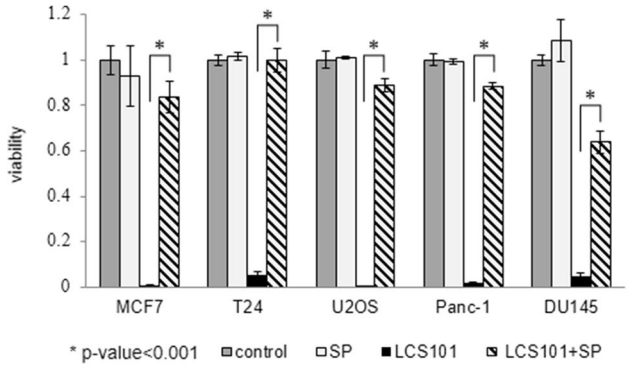

Fig. 1 ROS-mediated anti-cancer effect of LCS101. Cancer cells were treated for 72 h with 2 mg/ml of LCS101, either alone or in the presence of 1 mM sodium pyruvate (SP). Asterisks indicate *p* values of the formula + pyruvate, compared to formula alone (*$p < 0.001$)

cells. At higher doses (0.5 mg/ml), a significant increase in the effect of radiation was observed (Fig. 2).

The impact of combined treatment was estimated by two different approaches: the CompuSyn combinational index (CI) and coefficient of drug interaction (CDI). For each dose of the formula, the mean CI and mean CDI for all of the irradiation doses were calculated from several experiments. The values of CI and CDI were between 0.7 and 1.2, with the majority falling between 0.9 and 1.1 (within the range of an additive effect). These calculations indicate that LCS101 most likely has an additive effect when combined with irradiation treatment.

Discussion

The use of herbal medicine is very popular among patients with cancer, and many of these products have the potential to reduce the symptom load related to conventional oncology treatments. Clinical research has shown that LCS101 has the potential to reduce the prevalence of hematological toxicities (anemia, leukopenia/neutropenia), as well as potentially reducing the symptom load in female patients diagnosed with breast cancer (Yaal-Hahoshen et al. 2011; Samuels et al. 2013). However, the potential for negative interactions with conventional treatments, including radiation therapy, has prevented the inclusion of herbal and other dietary supplements in standard cancer care. The findings of the present study demonstrated the safety of the botanical formula LCS101, which did not interfere with the effects of radiation when administered at lower concentrations, and exhibited an additive effect at higher concentrations.

To gain a better understanding of the mechanism of action of the combined LCS101-radiation treatment, the ROS scavenger pyruvate was added to the exposed cancer cell lines. On their own, 7 of the 11 botanical LCS101 components have been shown in earlier research to exhibit anti-cancer activity (Cohen et al. 2015), with 5 of these exhibiting ROS activity (Cohen et al. 2017). Ionizing radiation at high doses induces oxidative stress and cell death, while low-dose irradiation activates cellular

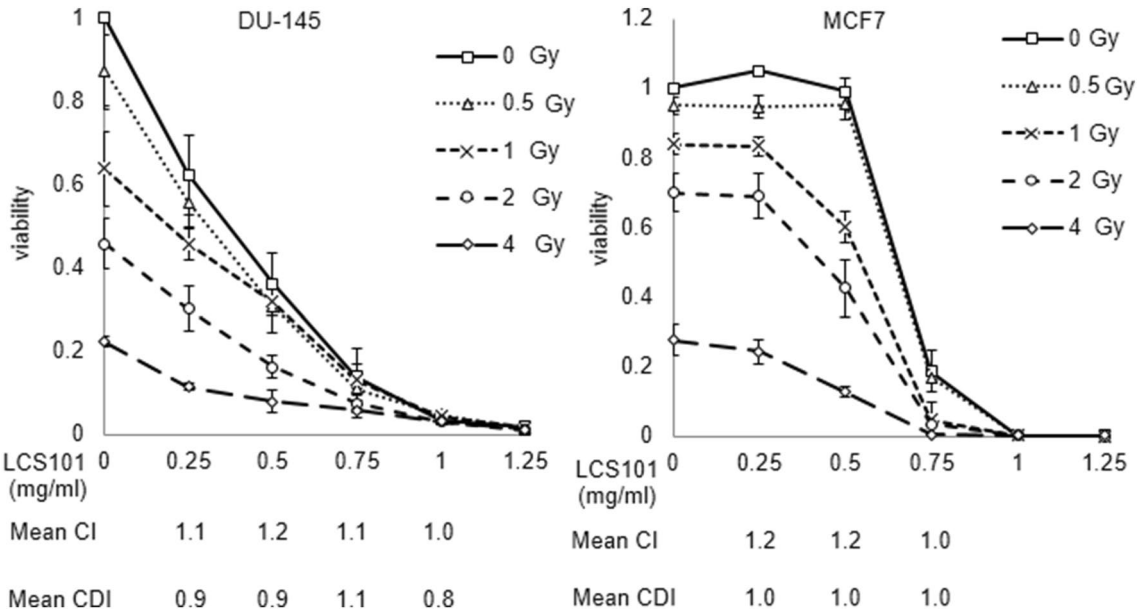

Fig. 2 The combined treatment with LCS101 and X-ray irradiation. Cancer cells were pretreated for 4 h with incremental concentrations of the formula, and then exposed to the graded X-ray doses (Gray; Gy). Asterisks indicate *p* values of the combined treatment compared to irradiation alone (**$p < 0.001$)

antioxidative mechanisms and thus protects the cells from further oxidative damage (Kataoka 2013). As seen in previous research, the ROS scavenger pyruvate inhibited the effects of LCS101 on the irradiated cells, indicating that a ROS-mediated mechanism was responsible for this effect. The findings of this study are encouraging, though further research—both pre-clinical and clinical—is needed to further examine the use of LCS101 as an adjunct to conventional oncology treatment regimens, including radiation therapy.

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Compliance with ethical standards

Conflict of interest Dr. Yair Maimon is a shareholder of LifeBiotics Ltd. No conflict of interest exists for any of the other authors.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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