Cancer Research Journal
2014; 2(2): 29-32
Published online March 30, 2014 (http://www.sciencepublishinggroup.com/j/crj)
doi: 10.11648/j.crj.20140202.13

Antiproliferative activity of a dietary supplement on estrogen receptor positive and negative human breast adenocarcinoma cell lines

Torricelli Piera[1], Antonelli Francesco[2], Ferorelli Pasquale[2], De Martino Angelo[2, 3], Shevchenko Anna[4], Beninati Simone[2, *]

[1]Department SPES, University of Molise, Campobasso, Italy
[2]Department of Biology, University of Tor Vergata, Rome, Italy
[3]IURS Santa Rita, Rome, Italy
[4]People's Friendship University of Russia, Moscow, Russia

Email address:
beninati@bio.uniroma2.it (B. Simone)

To cite this article:
Torricelli Piera, Antonelli Francesco, Ferorelli Pasquale, De Martino Angelo, Shevchenko Anna, Beninati Simone. Antiproliferative Activity of a Dietary Supplement on Estrogen Receptor Positive and Negative Human Breast Adenocarcinoma Cell Lines. *Cancer Research Journal*. Vol. 2, No. 2, 2014, pp. 29-32. doi: 10.11648/j.crj.20140202.13

Abstract: A commercial food supplement called Citozym (CIZ), was tested for antiproliferative activity against estrogen receptor (ER)-positive MCF-7 and (ER)-negative BT-20 human breast cancer cells, performing two independent experiments using SRB assay. A CIZ concentration of 100mg/mL, showed the most potent antiproliferative activity for cells in a potential range for further investigation on estrogen-insensitive breast cancer therapy.

Keywords: Food Supplements, (ER)+/(ER)- Breast Cancers, Cancer Cell Survival

1. Introduction

In recent years, there has been growing interest in the ability of natural compounds to serve as chemopreventive agents in the treatment of breast cancer. In most cases the progression of breast cancer occurs very slowly, spanning over a period of many years, with very distinct stages of initiation, promotion, and progression. In 2010, nearly 1.5 million people were diagnosed with breast cancer worldwide. In 2011, an estimated 230,480 new cases of invasive breast cancer were diagnosed among women in the United States, as well as an estimated 57,650 additional cases of *in situ* breast cancer according to the American Cancer Society. As a result of its long latency, even a small delay in carcinogenesis can set back the onset of disease and improve quality of life. It has been reported that less than 10% of breast cancer cases are inherited which suggests that a combination of genetics, environment, and lifestyle choices may help initiate the development of the disease [1,2]. Several migratory studies have demonstrated a link between natural dietary agents and a lower overall incidence of cancer [3]. Generally, the incidence of cancer is significantly lower in countries where people consume low-fat, plant-based diets [4]. This has led investigators to suggest that the role of the diet is an important factor in contributing to worldwide cancer incidence. The lethality of breast cancer originates from several factors including high oxidative stress of cancer cells [5] and leading to the deregulation of multiple cellular signaling pathways including those responsible for regulating cell proliferation and the initiation of apoptosis [6]. Due to its high incidence, long latency, and link to diet and lifestyle, chemoprevention through the use of natural dietary compounds may be an effective method in the management of breast cancer.

We have recently reported that CIZ, a food supplement, protected C57BL6/N mice from melanoma carcinogenesis, when chronically given before the initiation and promotion phases of melanoma cancer [7]. The aim of the present study was to investigate the antiproliferative effects of CIZ against estrogen receptor positive and negative human breast cancer cells.

In this study, we demonstrate that this dietary supplement, with antioxidant properties, could play an important role in the prevention of breast carcinogenesis.

2. Material and Methods

2.1. Human Cell Line

MCF-7 and BT-20 cell lines were obtained from the American Type Culture Collection (Rockville, MD). MCF-7 cell line represents estrogen-responsive human breast cancer cells, since express the endogenous estrogen receptor (ER)alpha, while BT-20 cell line is estrogen-insensitive. MCF-7 cells were cultured in Minimum Essential Media (MEM) with Earle Salt with 10% heat inactivated (1 h at 56 °C) fetal bovine serum (FBS), 1% of 2 mM l-glutamine, 50 IU/mL penicillin, 50 µg/mL streptomycin and 1% non-essential amino acids. Cultures were maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity [8]. BT-20 cells were maintained routinely at 37°C in 5% CO2 in RPMI 1640 supplemented with 10% heat inactivated (1 h at 56 °C) FBS, 1% of 2mM l-glutamine, 1 mM sodium pyruvate, 1% non-essential amino acids, 100 IU/mL of penicillin and 0.1 mg/mL of streptomycin.

According to their growth profiles, the optimal plating density of the cell lines was determined to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at 492 nm and cell number when analyzed by SRB assay. Citozym (CIZ) was obtained from CITOZEATEC, S.r.l. (Peschiera Borromeo, Milano, Italy). The main components of CIZ are as follows (units/100 g): 500 mg of vitamin C, 56 mg of vitamin B5, 56 µg of vitamin D, 3.3 mg of vitamin B9, 222 mg of pyruvic acid, 120 mg of citric acid, 250 mg of tartaric acid, 14 g of dextrose, 11 g of fructose, 7.5 g of mannitol, 7 g of maltose, 5.5 g of sorbitol, 2.5 g of lactulose, 3.2 g of inositol and 2 g of xylitol.

2.2. Cytotoxicity Assay

For the cytotoxicity assay, cells were washed with phosphate buffer saline (PBS) (Oxoid Ltd., UK) free of magnesium and calcium. The PBS was decanted and cells detached with 0.025% trypsin-EDTA (Sigma-Aldrich) and PBS was added to a volume of 50 mL. The cell pellet, obtained by centrifugation (1000×g, 5 min) was resuspended in 10 mL of medium to make a single cell suspension. Viable cells density were counted by trypan blue exclusion in a haemocytometer and then diluted with medium to give the previously-determined optimal plating density for MCF-7 and BT-20. 100 µL/well of these cell suspensions were seeded in 96-well microtiter plates and incubated at 37 °C to allow for cell attachment. After 24 h the cells were treated with two final concentration of CIZ (CIZa: 50mg/mL and CIZb: 100mg/mL). Two cytostatic drugs, Vincristine sulphate (Sigma-Aldrich, Lot No. 34H0447) and dithranol (Hillcross Pharmaceuticals) were used as positive controls (data not shown). The suitable concentration of CIZ was considered to be that which gave more than 80% survival at exposure time 72 h. The plates were incubated for selected exposure times of 24, 48,72 and 96 h. At the end of each exposure time the medium was removed and the wells were then washed with medium, and 200 µL of fresh medium were then added. The plates were incubated at 37 °C for a recovery period of 2 days and cell growth was then analyzed using the SRB assay. Three replicate plates were used to determine the cytotoxicity of each CIZ dilution

2.3. Sulphorhodamine B (SRB) Assay

The antiproliferative SRB assay was performed to assess growth inhibition by a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the dye SRB [9]. In brief, cells were fixed by layering 100 µL of ice-cold 40% trichloroacetic acid (TCA, Aldrich Chemical) on top of the growth medium. Cells were incubated at 4 °C for 1 h, after which plates were washed five times with cold water, the excess water drained off and the plates left to dry in air. SRB stain (50 µL in 1% acetic acid) (Sigma- Aldrich) was added to each well and left in contact with the cells for 30 min, after which they were washed with 50 mL 1% acetic acid, rinsed four times until only dye adhering to the cells was left. The plates were dried and 100 µL of 10 mM Tris base pH 10.5 (Sigma-Aldrich) were added to each well to solubilize the dye. The plates were shaken gently for 20 min on a shaker and the absorbance (OD) of each well was read on a SLT 340 ATTC plate reader (SLT Lab instrument, Australia) at 492 nm. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells).

2.4. Statistical Analysis

Student's two-tailed test was employed to assess the statistical significance of difference between the untreated controls and CIZ-treated samples. All treatment experiments were repeated at least three times to generate statistically relevant data.

3. Results

3.1. Cytotoxic Activity

The results of cytotoxicity evaluation of the two CIZ dilutions, at several exposure times is shown in Figure 1. Data show that both dilutions CIZa and CIZb (w/v) exhibited low cytotoxic activity. CIZa dilution showed a negligible cytotoxic activity (Figure 1A). CIZb dilution (Figure 1B) being the more cytotoxic against BT-20 cell line where the percentage survival of cancer cell for this concentration at exposure time 24, 48, 72 and 96 h were 93.0%, 90.6%, 75.0% and 70.3%, respectively. On the contrary the percentage survival of MCF-7 cancer cell at exposure time 24, 48, 72 and 96 h were 98.0%, 95.6%, 90.4% and 80.8% respectively. Therefore, by controlling the cytotoxicity of CIZ, we were able to discriminate between the anti-proliferation as a block of cell growth and cell death for toxic effect. The knowledge of this fact, can play a more important role for the potential application in chemotherapy.

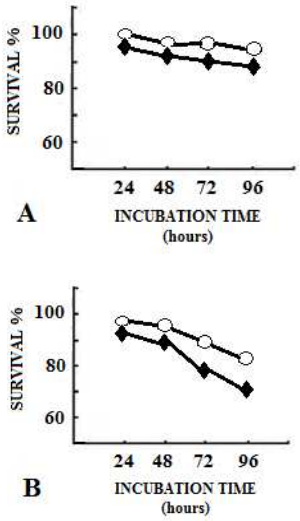

Figure 1. *Survival assay on (**A**) CIZa- (w/v) and (**B**) CIZb (w/v)-treated BT-20 (◆) and MCF-7 (○) breast cancer cells at several incubation times. Data represent the mean of three determinations with a S.D. lower than 10%.*

3.2. Antiproliferative Activity

Incubation of (ER)-positive MCF-7 and (ER)-negative BT-20 human breast cancer cells with CIZb (Figure 2A), showed, a significative antiproliferative activity during the time, against BT-20 cell line (9.4%, 17.0%, 30.0% and 51.0% at 24, 48, 72 and 96 hours of incubation, respectively), but it showed a lower antiproliferative activity against MCF-7 cancer cell line (3.8%, 5.6%, 13.0% and 20.6% at 24, 48, 72 and 96 hours of incubation, respectively). CIZb treatment confirmed that this concentration was the more effective against both the breast cell lines, compared with the CIZa-treatment (Figure 2B)

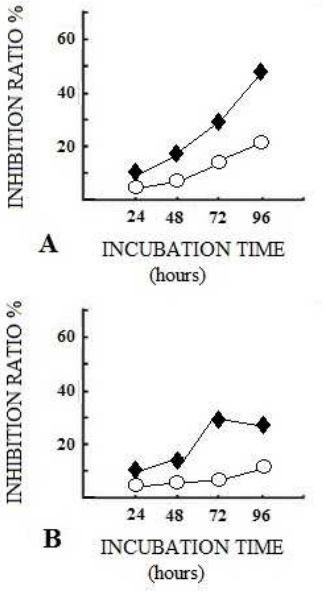

Figure 2. *SRB assay on (**A**) CIZb- and (**B**) CIZa-treated BT-20 (◆) and MCF-7 (○) breast cancer cells at several incubation times. Data were normalized for cytotoxicity and represent the mean of three determinations with a S.D. lower than 10%.*

4. Discussion

Many breast cancers have on their cell surface receptors for estrogen, progesterone, or both. This feature shows the histological examination carried out on the material taken during a biopsy or surgery and allows to define the tumor as "estrogen and / or progesterone positive" [10]. This means that the female sex hormones stimulate its growth, and you can choose a treatment that act at this level. If the tumor does not present these receptors, and is therefore called "negative" for these two factors, hormone therapy is not indicated. There is strong epidemiological, experimental and clinical evidence that the etiology of breast cancer is closely related to long-term exposure of breast epithelium to sex steroid hormones [11]. Estrogens can enhance the development of breast cancer by stimulating cell proliferation rate and thereby increasing the number of errors occurring during DNA replication, as well as by causing DNA damage via their genotoxic metabolites produced during oxidation reactions. Anti-oxidant supplements, including tamoxifen, raloxifene and anastrozole, have been tested with promising results in the block of the binding between estrogens and receptors on cancer cells, thus preventing the recurrence of the tumor [12, 13]. Once the two types of breast cancer have been defined, it is appropriate to think of other therapies that may take advantage of the differences between cancers. In case of hormone receptor negative breast tumors is however preferable the chemotherapy and radiotherapy, even when they are not visceral metastases. Furthermore, radiotherapy and chemotherapy are used as a precautionary and postoperative treatment of estrogen and / or progesterone negative breast cancer, to reduce the size of the tumor and in advanced disease. Therefore, in this case, the therapeutic treatment is more drastic compared to that for the hormone receptor-positive tumors [14]. The results obtained in our work clearly indicated that incubation of estrogen receptor (ER)-positive MCF-7 and ER-negative BT-20 human breast cancer cells with CYZ at a final concentration of 100mg/mL (CIZb), was preferentially active against BT-20 cancer cells. On the basis of previous observations about therapies for this type of tumor, it is of interest that a dietary supplement showed a higher antiproliferative activity against the (ER)-negative breast cancer cell line. Although it was not demonstrated any effect on the progression of the cancer, the results presented may suggest that CIZ can have the potential of a possible positive contribution as an adjunct in improving the quality of life of patients with (ER)-negative breast cancer. In conclusion, CIZ deserves further epidemiological investigations and by the time our preliminary results can therefore not be extrapolated directly to human. However, It is apparent from these remarks that dietary food supplements continue to show an important role as adjuvant in the therapy of many common pathologies in man.

Acknowledgements

The authors wish to thank Dr. Paolo Bonomo and Dr. John Phersuk for technical assistance in the experiments with breast cancer cells. We are grateful to A.I.A.S Italy, for financial support for studying the possibility of alternative therapies for cancer patients.

Conflict of Interest

The authors have no potential conflicts of interest.

References

[1] Carter B. S., Beaty T. H., Steinberg G. D., Childs B. & Walsh P. C. Mendelian inheritance of familial prostate cancer. Proc. Natl. Acad. Sci. U.S.A. 1992; 89: 3367–3371.

[2] Muraca L, Leung D, Clark A, Beduz MA, Goodwin P. Breast cancer survivors: taking charge of lifestyle choices after treatment. Eur J Oncol Nurs. 2011;15(3):250-253.

[3] Meiyanto E, Hermawan A, Anindyajati. Natural products for cancer-targeted therapy: citrus flavonoids as potent chemopreventive agents.Asian Pac J Cancer Prev. 2012;13(2):427-436.

[4] Arcangeli S., Pinzi V., Arcangeli G. Epidemiology of prostate cancer and treatment remarks. World J. Radiol. 2012;4:241–246.

[5] Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy.Nat Rev Drug Discov. 2013;12(12):931-947.

[6] Vera-Ramirez L, Sanchez-Rovira P, Ramirez-Tortosa MC, Ramirez-Tortosa CL, Granados-Principal S, Lorente JA, Quiles JL.Free radicals in breast carcinogenesis, breast cancer progression and cancer stem cells. Biological bases to develop oxidative-based therapies.Crit Rev Oncol Hematol. 2011;80(3):347-368.

[7] Antonelli F & Beninati S. Enhanced survival of B16-F10 melanoma tumour-bearing C57BL6/N mice treated with a mixture of antioxidants in: Recent Res Devel in Life Sci. 2011;5:51-60 Research Signpost, Trivandrum India

[8] Keawpradub N, Amooquaye E.E, Burke PJ, Houghton PJ. Cytotoxic activity of indole alkaloids from Alstonia macrophylla. Planta Medica 1999;65: 311–315.

[9] Skehan P, Storeng R, Scudiero D, Monks A, McMahon I, Vistica D, Waren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer drug screening. Journal of National Cancer Institute. 1990: 82:1107–1112.

[10] Lipsett MB. Prospects in endocrinology for chemotherapy. Cancer Res. 1969;29(12):2408-2411.

[11] Jensen EV, and Jacobson H. I. Basic Guides to the Mechanism of Estrogen Action. Recent Progr. Hormone Res.1962;18: 387-408.

[12] Cuzick J. IBIS II: a breast cancer prevention trial in postmenopausal women using the aromatase inhibitor anastrozole. Expert Rev Anticancer Ther. 2008;8(9):1377-1385.

[13] Klepin HD, Geiger AM, Bandos H, Costantino JP, Rapp SR, Sink KM, Lawrence JA, Atkinson HH, Espeland MA. Cognitive Factors Associated with Adherence to Oral Antiestrogen Therapy: Results from the Cognition in the Study of Tamoxifen and Raloxifene (Co-STAR) Study.Cancer Prev Res (Phila). 2014;7(1):161-168.

[14] Osborne CK, McGuire WL.Therapy for cancer of the breast. Current status of steroid hormone receptors. West J Med. 1979;130(5):401-407.