Effect of chebulinic acid on Bcl-2 and p53 protein expression in A431 human skin cancer cells in vitro

U. Vidhya Rekha1,*, M. Anita1, S. Bhuminathan1, G. Jayamathi1, K. Sadhana1, J. Bhuveneswarri1, V. Ramya2, Preethe Paddmanabhan3, Selvaraj Jayaraman4, D. Priscilla4

ABSTRACT

Aim: The present study was designed to evaluate the effect of chebulinic acid on Bcl-2 family protein and p53 expression in A431 human skin cancer cells in vitro. Cell Culture and Treatment: The skin cancer cell line, A431 was purchased from the National Centre for Cell Sciences, Pune, India, and cultured in Dulbecco’s Modified Eagle’s Medium supplemented with fetal bovine serum, penicillin G, and streptomycin. The cells were placed in 5% CO2 incubator and the further experiments were started after the confluence stage was attained. The cells were incubated at 37°C with the various doses of chebulinic acid (10, 25, 50, and 100 μg/ml) in a CO2 incubator to find out the inhibitory concentration of IC50. Results: A431 skin cancer cell line showed a significant increase in the levels of Bcl-2, p-Bcl2, and Bcl-xL whereas decreased in the levels of p53 at the doses of 50 and 100 μg. However, an effective dose of chebulinic acid (100 μg/ml) altered the levels bring back to normalcy. Conclusion: The anticancer potential of the chebulinic acid is mediated through the controlling of apoptotic signaling molecules such as Bcl-2, Bcl-xL, and tumor suppressor proteins p53. Hence, this study concludes that chebulinic acid can be used as one of the potential therapeutic herbal drugs for the treatment of skin cancer. Further studies on the molecular mechanisms of the action of chebulinic acid are warranted to ascertain its potential.

KEY WORDS: B-cell lymphoma 2, B-cell lymphoma-extra-large, Chebulinic acid, Skin cancer, Tumor protein p53

INTRODUCTION

Cancer has become one of the major sources of death in the world. It is estimated that there are nearly 2–2.5 million cancer cases at any given point of time. Over 7 lakh new cases and 3 lakh deaths occur annually due to cancer.1 Skin cancer is one of the major types of malignancies in the world and the morbidity rate of skin cancer is rising day by day, and it has become a major disease that is detrimental to human health.2 Skin cancer can be an uncommon change in the skin.3 The second consists of basal cell and squamous cell carcinomas are the most common malignancy.4 It is one of the most common cancer in the world (1.04 million cases). Globally, about 1 in 6 deaths is due to cancer.5 Skin cancers are named for the type of cells that become malignant (cancer).6 There are three types of skin cancer present, i.e., basal cell carcinoma, squamous cell carcinoma, and melanoma.7 The skin is composed of three layers: Epidermal, dermal, and subcutaneous, of which epidermis is adversely affected by abiotic factors.8 UV radiation is one of the major causative factors for the development of skin cancer and 90% of skin cancer are caused by UV exposure.9 Skin pigmentation is caused by the assembly of melanin granules which is synthesized in melanocytes and shifted to keratinocytes.10 Skin cancer can take place during any stage of life, in which skin cells start to grow in an uncontrolled way and get accumulated due to damage to their DNA and start to grow in an uncontrolled way; various types of skin disorders seem to be produced by cells from the immune (defense) system of the body. These cells are either multiplying too rapidly or behaving in an abnormal manner.11 At present, the treatments of skin cancer include surgery, treatment, radiation, and chemotherapy.
radiotherapy, and chemotherapy, or combinations of those treatments, but the cure rate is displeasing. There has been great interest in discovering natural herbal compounds to be used in treating skin cancer.[12] Apoptosis is an essential phenomenon to maintain the cellular balance between cell differentiation and cell death, and the apoptotic pathway, thus, performs a critical role in the removal of genetically damaged cells from the body.[13]

Triphala is one of the most accepted herbal preparations in the world used to treat variety of diseases.[14] It is commonly used in the Indian ayurvedic medicine and Triphala is a combination of the dried fruit powder of three different plants namely Terminalia chebula, Terminalia belerica and Emblica officinalis. Chebulagic acid and chebulinic acid are known to be present in triphala as major constituents.[15] These compounds are reported to have anticancer and antiproliferative properties.[16,17] Chebulinic acid is an ellagitannin widely present in fruit up to 30%.[18] A lot of studies report that chebulinic acid has shown multiple therapeutic effects including obstacle of cancer cell progression in human leukemia K562 cells,[19] colon adenocarcinoma HT-29 cell lines, regressing the contractile responses of cardiovascular muscles.[20] and accumulating experimental evidence and has also suggested that triphala is a promising herbal formulation for cancer therapy. It has been reported that triphala not only shows chemo, radio, and oxidant-protective activities, which indicates that triphala has the potential to prevent oncogenesis[21] and antifungal, antibacterial activities, etc. Most anticancer drugs are based on a differential killing of cancer cells.[22] However, no available information on effects of chebulinic acid on apoptotic signaling molecules in human skin cancer cell lines. In the present study, we investigated to chebulinic acid on apoptotic signaling molecules in A431 human skin cancer cells in vitro.

MATERIALS AND METHODS

Procurement of Cell Line and Treatment

The skin cancer cell line, A431 was purchased from the National Centre for Cell Sciences, Pune, India, and cultured in Dulbecco’s Modified Eagle’s Medium supplemented with fetal bovine serum, penicillin G, and streptomycin. The cells were placed in 5% CO₂ incubator and the further experiments were started after the confluency stage was attained. A431 cells were incubated at 37°C with the various doses of chebulinic acid (50 and 100 μg/ml) in a CO₂ incubator to find out the optimal dose of chebulinic acid.

Protein Expression Analysis by Western Blotting Method

Western blot analysis after the 24 h treatment period, the cells were lysed in RIPA buffer containing ×1 protease inhibitor cocktail, and protein concentrations were determined. Cell lysate (50 μg) was electrophoresed in 12% sodium dodecyl sulfate polyacrylamide gel and then transferred into PVDF membranes. The membranes were incubated with primary antibodies against Bcl-2, p-Bcl-2, Bcl-xL, and p53 (1:2000) in Tris-buffered saline. After washing, the membranes were incubated with HRP conjugated antimouse IgG (1:5000) and goat-anti-rabbit IgG (1:5000). Protein bands were detected using chemiluminescence system (ECL Kit) and quantified in ChemiDoc XRS Imaging System, Bio-Rad (USA).

Statistical Analysis

One-way analysis of variance and Duncan’s multiple range test were used to assess the significance of individual variations between the control and treatment groups using a computer-based software (GraphPad Prism version 5). In Duncan’s test, the significance was considered at the level of P < 0.05.

RESULTS

Effects of Chebulinic Acid on Bcl-2 and p-Bcl-2 Protein Expression in A-431 Cells

Compared to control, both Bcl-2 [Figures 1a and b] proteins were in A-431 cell were significantly (P< 0.05) raised in untreated cancer cells. Treatment with chebulinic acid notably reduced in the Bcl-2 and p-Bcl-2 protein levels.

Effects of Chebulinic Acid on Bcl-xl and p53 in Cancer-induced A-431 Cells

Compared to control, Bcl-xl [Figure 2a] protein expression was found to be elevated in untreated cancer cells whereas tumor suppressor protein p53 [Figure 2b] was significantly (P< 0.05) reduced in cancer-induced group. Treatment with chebulinic acid significantly reverted the protein levels.

DISCUSSION

There are no highly effective drugs to treat most cancers. There is a common call for new drugs that are mostly effective, possess low toxicity, and have a minor environment impact and the novel natural products offer opportunities for innovation in drug discovery, and there is a natural product plays a major role in cancer prevention and treatment. A substantial number of antitumor agents currently used in the clinic are of natural origin.[23] The present study elucidates the biological effect of chebulinic acid, a major component of T. chebula on apoptotic signaling molecules. Skin cancer can be prevented by controlling or eliminating these causative agents. It can be effectively removed by obstruct blood supply to the tumor (antiangiogenesis), which curbs tumor growth and enhances patient survival. Most cancer cells develop ways to evade
apoptosis or exhibit defective apoptosis mechanisms, thus allowing uncontrollable cell development. Proteins of the Bcl-2 family mediate mitochondrial permeability and it is key regulators of the intrinsic apoptotic pathways. Bcl-2 proteins contain sections of amino acid sequence resemblance, known as Bcl-2 homology (BH) domains. The family consists of the antiapoptotic Bcl-2 group (such as Bcl-2, Bcl-xL, and Mcl-1), the proapoptotic Bax group (Bax, Bak, and Bok), and the proapoptotic BH3 domain-only group (including Bad, Bid, Noxa, and Puma). In the present study, the effect of chebulinic acid focuses on increased levels of B-cell lymphoma 2 (Bcl-2), phosphorylation of Bcl2 (p-Bcl2), and B-cell lymphoma-extra-large (Bcl-xL), and decreased in the levels of tumor protein p53 of A431 human skin cancer cells. Chebulinic acid treatment significantly altered these apoptotic signaling molecules to reach normal range. In the current study, treatment with chebulinic acid (50 and 100 µg/ml) significantly decreased the levels of Bcl-2, p-Bcl2, and Bcl-xL and increased the level of tumor protein p53. These levels of Bcl-2, p-Bcl2, Bcl-xL, and tumor protein p53 on cancer-induced cell lines produced distorted and poorly outlined the treatment with chebulinic acid bring back these abnormal changes to near normal may be through its anticancer property.

**CONCLUSION**

The present study thus explores the anticancer/cytotoxic potential of the chebulinic acid in A431 skin cancer cell lines. The anticancer potentials of the
chebulinic acid are mediated through the controlling of apoptotic signaling molecules such as Bcl-2, Bcl-xL, and tumor suppressor proteins p53. Hence, this study concludes that chebulinic acid can be used as one of the potential therapeutic herbal compounds for the treatment of skin cancer. Further studies on the molecular mechanisms of action of chebulinic acid are warranted to ascertain its potential.

REFERENCES