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Apoptotic Radiosensitivity of Cervical Tumor Cells Enhanced by Ellagic Acid

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Abstract
Cancer is a multifactorial disease that in many cases requires multimodal treatment involving combination of conventional chemo and radiotherapy. Radiotherapy kills cancer cells and normal cells equally which imposes limitation to cancer treatment. Failure of cancer treatment in clinic by radio/chemotherapy is generally attributed to resistance of tumor.Therefore it is essential to develop strategies to increase cytotoxicity of tumor cells by radiation in combination with new tumor selective cytotoxic agents. Research has facilitated the identification of various herbal compounds capable to increase toxic effect of ionizing radiation to tumor cells and spare normal cells thereby; reducing the undesirable side effects. Ellagic acid (EA) is one such polyphenol which has shown anticancer properties *in vitro and in vivo.* Involvement of ROS is suggested in radio-cytotoxicity and apoptosis. Recent studies have shown that radiosensitivity of normal and tumor cells was governed by their susceptibility to undergo apoptotic death. Therefore, a new line of research is needed to focus on finding inducers of apoptosis in tumor cells leaving events of apoptosis in normal cells unaffected. This strategy requires the integration of radiobiological concepts and the knowledge of the molecular biological mechanisms involved in apoptosis induction. This article focuses on potential of EA as a potent radiosensitizer involving initiation of radiation induced apoptosis in cervical tumor cells.

Keywords: Ellagic Acid, Apoptotic radiosensitivity, Antioxidants, Radiosensitizer, Cervical cancer

1. Introduction

Cervical carcinoma is a leading cause of cancer related mortality in developing countries like India. Although human papillomaviruses (HPVs), a sexually transmitted virus, is a major risk factor, several molecular alterations are necessary for the progression of cervical cancer. Although surgery and radiotherapy are the commonly employed treatments for cervix cancer, local recurrence in the pelvis poses a problem. It has been known now that ionizing radiation and certain cytotoxic drugs induce oxidative stress in cells by generation of reactive oxygen species (ROS) leading to the imbalance of the cytosolic pro-oxidant and antioxidants levels resulting in cell death. ROS also leads to disruption of mitochondrial membrane potential and the release of cytochrome C in the cytosol where Apaf-1 binds to form the apoptosome complex. Most of the radio sensitizers are believed to work through up regulation of the ROS generation [1]. For many years, the cytotoxic actions of the chemotherapeutic drugs were ascribed solely to their ability to induce genotoxic death. However, evidence has accumulated to show that these agents exert their cytotoxic effects predominantly by inducing apoptosis in tumor cells [1-3]. Therefore, the important task in clinical radiobiology is to maximize the probability of eradication of all tumor cells by persuading them to undergo apoptosis and to minimize radiation effects on normal tissue keeping apoptosis cellular machinery unaffected. Thus, the search for protocols which cause minimal healthy tissue damage with sufficient damage to tumor cells would bring effective outcome in cancer patient treatment. Research should focus clinical radiobiology to find ways and means of selectively increasing the apoptosis in radio resistant tumors for treating cancer. One way to optimize radiation treatment is to selectively enhance the radiosensitivity of tumor cells with the help of various drugs having a selective action on target cells.

Many polyphenolic compounds show both pro-oxidant and antioxidant activity. Impairment of apoptosis is known to be related to cell immortality and carcinogenesis. Therefore, the induction of apoptosis in neoplastic cells is evidently vital in cancer treatment [4]. The chemotherapeutic drugs that have been observed to induce apoptosis *in vitro* and *in vivo* include cisplatinum, cyclophosphamide, pacilataxel, 5-fluorouracil, and doxorubicin [5, 6]. However, cytotoxicity of these chemotherapeutic agents is not limited to tumor cells but is also known to cause significant normal tissue toxicity [7, 8]. A class of compounds that are becoming increasingly successful in numerous *in vitro* and animal models of cancer is

phytochemicals present in diets and normally consumed as supplement. Additionally, these agents have been shown to exert anticancer and chemo preventive effects [9].The added advantage with these agents is that they are mostly non-toxic when compared with chemotherapeutic agents. EA is a naturally occurring compound in pomegranates, raspberries, blue berries and other fruits having antioxidant activity and they are shown to possess cytotoxic effects on tumor cells [2, 10, 11]. For example, EA is shown to induce apoptotic cell death within 48 hrs in cervix cancer cell lines incubated with EA. Therefore, EA offers prospects for optimizing experimental conditions to achieve greater kill of tumor cells together with gamma radiation. [2]

At the present point of time, despite popularity EA in herbal therapies, much remains to be explored on the mechanism of its anticancer effect *in vivo*.

Material and Methods

Radiation-induced apoptosis was evaluated in terms of: (a) the identification of apoptotic and necrotic cells by Annexin V, (b) *in vitro* observations of radiation-induced apoptosis, (c) up regulation of genes controlling apoptosis by Western Blot, (d) determination of caspase activity by Fluorimetry, (e) quantitative measurements of superoxides and antioxidants enzyme system by standard assays (f)clonogenic assay for cell survival, (g) FACS to quantitate apoptosis, (h)γ-H2AX foci scoring to understand the DNA damage, (i) morphological changes in the cell and the nucleus. Further research was carried out on determining the molecular pathways that are important in the expression and modulation of radiation-induced apoptosis with high priority on *in vivo* experiments. To investigate whether ROS is the mediator of apoptosis, cells were pre treated with ROS inhibitors.

Results and Discussion

For *in vitro* studies, HeLa cells treated with EA and gamma radiation showed increased p53 protein expression and superoxide levels and decreased antioxidant enzymes. It was also found that EA and radiation enhance caspase-3 activity via oxidative stress, increased levels of phospholipase C intracellular calcium cause a drop in mitochondrial potential. EAC transplanted Swiss mice treated with radiation and EA both shows decrease in cell count as compared to the untreated animals and animals treated with radiation or EA alone. Clonogenic assay indicated that combined treatment of EA (10 µM) and IR significantly reduced the capacity of HeLa cells to form colonies compared with individual treatments. FACS results suggest that EA treatment induced apoptosis more potently in the G1-S phase than G2-Mphase of the cell cycle. Therefore it can be concluded that EA assisted in radiosensitizing the cells and lead them to undergo apoptosis. It is noteworthy that in presence of EA during irradiation HeLa cells were sensitized and hence more number of foci was observed in the combined treatment. of HeLa cells treated with EA for 48 h resulted in so obvious cell shrinkage, cellular detachment, and loss of the originally confluent monolayer. DAPI staining revealed the occurrence of nuclear condensation, DNA fragmentation, and perinuclear apoptotic bodies in HeLa cultures treated with EA, but not in control cultures [12, 13].

Cancer cells are said to have high levels of ROS and therefore are more susceptible to oxidative stress. This is one of the reason that pushes the cell towards apoptosis. These results therefore suggest that EA augments the cytotoxicity of radiation induced oxidative stress in HeLa as well as in EAC cells. It also illustrates that EA and radiation-induced apoptosis in cervix cancer cell line are mediated through increased ROS, activation of PLC, drop in MMP, and increased intracellular calcium levels [2, 12, 13].

Extensive work has been documented in the literature to suggest that ionizing radiation cause tumor cell apoptosis which is mediated by Reactive oxygen species. Therefore herbal chemotherapeutics can be used which can augment or enhance effect of radiation dose on the cancer cells and simultaneously protect the normal cells. It has been of utmost importance that oxidative damage should be increased in tumor cells as radiation is reported to fail in the later stages of cancer treatment due to radio resistance. Our work concentrated to investigate the effect of EA on gamma radiation treated HeLa cell line. We observed induction of apoptosis in HeLa cells which was mediated through increased ROS, Increased calcium levels, activation of PLC and drop in the mitochondrial potential. These results might present a base for prominent reduction of cancer cell using EA as an adjunct to radiotherapy and an opportunity to lower the toxic radiation doses to improve the quality of life.

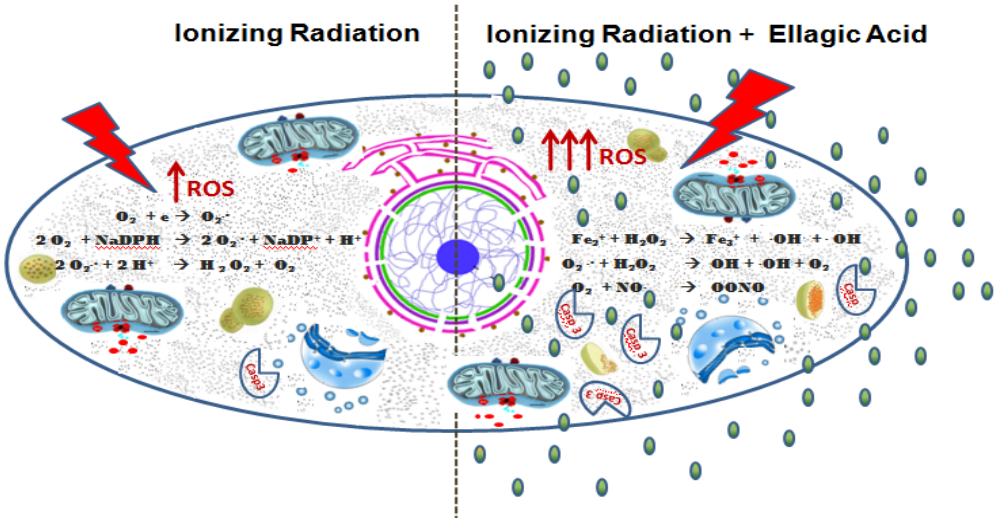

Fig: 1 Proposed Model for treatment of cancer cell with radiation alone and in combination with Ellagic acid.

When the cells are treated with radiation alone there is an increase in the ROS levels. But use of chemotherapeutic dietary compound like Ellagic acid (green) increases ROS by many folds, damages DNA, drops the mitochondrial potential by release of cytochrome C (red), increases caspase-3 expression pushing the cells apoptosis.

Conclusion

Plant based dietary compounds have been progressively acknowledged for being valuable in the inhibition and treatment of cancer. Hence there exists a huge possibility for screening and valuation of natural products in the development an effective radiosensitizer and radioprotector that would be significant for cancer patients undergoing therapy. This study describes the effect of the flavonoid EA, as a radiosensitizer when administered alone or in combination with gamma radiation, on the growth of human cervical cancer HeLa cells in vitro. EA enhanced tumor radio-toxicity in cervical cancer lines by induction of apoptosis. Hence these results may provide insights in developing therapeutic regimes using EA for patients undergoing radiotherapy for resistant tumors.

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