Review Article

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Potential of radiosensitizing agents in cancer chemo-radiotherapy

ABSTRACT

Potential of herbs and other plant-based formulations have been increasingly recognized in prevention and treatment of human diseases including cancer. There exist enormous prospect for screening and evaluation of herbal/plant products for developing effective radiosensitization and radioprotection relevant to nuclear research program. Investigations in our laboratory have focused on the mechanism of activity of variety of anticancer and antioxidant agents, namely, Eugenol, (EU), Ellagic acid (EA), Triphala (TPL), Tocopherol Succinate (TOS) and Arachidonic acid on normal and cancer cells with view to design effective protocols in practical radioprotection and cancer radiotherapy. This paper is mainly focused on studies on cytotoxic effects on cancer cell lines. Results have shown that these agents produced radiosensitizing action involving oxidative damage, membrane alteration and damage to nucleic acid in various human cell lines. Studies were performed employing fluorescence probes and electron spin resonance methods and gel electrophoresis protocols. It has been found that cytotoxic effect was induced by initiating membrane oxidative damage and by triggering intracellular generation of reactive oxygen species (ROS) by gamma radiation in combination with phytochemicals like TPL, EA and TOS in tumor cell line Ehrlich Ascites (EAC), Human cervical (HeLa) and breast (MCF-7) cells. Membrane damage and ROS generation was measured by DPH and DCF-FDA fluorescent probes respectively after exposure to low to moderate doses of gamma radiation. This talk will present the cytotoxic effects of phytochemicals in combination with ionizing radiation. It is emphasized that modulation of membrane peroxidative damage and intra cellular ROS may help achieve efficient killing of cancer cells which may provide a new approach to developing effective treatment of cancer.

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INTRODUCTION

Success of a strategy in improving cancer radiotherapy depends on achieving the increased tumor cytotoxicity and, at the same time, reduced adverse effects on normal tissues. This aim is not easy to achieve in clinical situation but considerable active research has been devoted to either technical advancement or by combined treatment modalities involving radiotherapy with chemotherapy and other protocols. A large number of tumors are poorly responsive or even non-responding to therapeutic drugs and radiotherapy. Increasing the doses of cytotoxic drugs and radiation fail to improve the response to these therapies and many of them display resistance to killing. An ideal strategy would be to identify anticancer agents that trigger effectively the process of cell death preferentially in tumor cells. Compounds occurring naturally in human diet are generally devoid of toxicity within certain doses. Polyphenolic compounds and flavonoids found in herbals have been reported to possess antioxidant properties, which protects normal cells from oxidative stress.[1-4] On the other hand, they also exhibit prooxidant activity, which contributes to their therapeutic actions. These compounds can both behave as prooxidant and antioxidants depending on their concentration and cytosolic redox status. Recently, it has been shown that resveratrol, a polyphenolic compound altered cell cycle progression and showed cytotoxic response to ionizing radiation.[5] It is also known that curcumin, a polyphenol, confers radiosensitizing effect in prostrate cancer cells by down regulating pro-survival factors.[6] Moreover, it is generally accepted that cancer cells are deficient in polyunsaturated fatty acids, which, in a part, is believed to contribute to observed radio and chemo-resistance. Modification of radiosensitivity of renal cancer cell line through PUFA supplementation has recently been reported.[7] The present article is aimed to give a brief review of the various natural products, which can act as potential radiosensitizer with an emphasis to present an account of ongoing research in our laboratory.

Role of ROS, Lipid Peroxidation and Apoptosis in Radiotherapy

Radiation damage to cells and tissues involve

generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) followed by alterations in lipids, DNA and proteins which eventually lead to cellular dysfunction or cell death. The consequential role of free radicals in the mechanism of cell death especially in the induction of apoptotic death has been receiving growing attention in the field of cancer radiotherapy. It needs to be noted that cancer is a result of failure of apoptosis in injured or damaged cells which is characterized by a number of features such as morphological alterations, nuclear fragmentation giving definite pattern in gel electrophoresis, loss of lipid asymmetry and imbalance of cellular signaling machinery. It has been suggested that DNA damage by ROS leads to formation of single and double stranded breaks resulting in cell cycle arrest and recruitment of DNA repair enzymes to rescue cells from the damage.[8-9] The stress-induced damage may be manifested in clonogenic cell death or may be reflected in altered signaling cascades resulting in activation of responsive genes inducing apoptosis.[10] Apart from DNA damage, another major target of radiation and ROS is believed to be the membranes of cytoplasmic organelles and plasma membrane of cells. Membrane oxidative damage is generally mediated by the degradation of phospholipids, which are the major constituents of plasma membrane. It is observed that membrane lipids are easily peroxidised by ROS produced by ionizing radiation, causing structural and functional impairment.[11-14] Oxidative damage leads to an alterations in the both lipid bilayer fluidity and permeability properties. In the context of cellular response to radiation, the contributions of radiation oxidative damage to membranes as well as to DNA damage via ROS appears rather complex and these pathways of initiators of cytotoxicity seem to be intimately linked in the development of radiation induced deleterious cellular effects. Since cancer cells are known to be the consequence of resistance to apoptosis, it is logical to search for agents that can trigger and modulate the oxidative stress in the cells including the radiation induced damage. Evidently, alterations in the lipid membrane due to peroxidative damage process may form a potential initiator of radiosensitizing effect in combination with radiation and drugs acting through modulation of membrane associated events involved in the mechanism of induction of apoptosis.

Natural Products as Potential Radiosensitizers

A large number of natural compounds have shown potential of cytotoxic effects in a variety of pathological situations including cancer either alone or together with radiation. The compounds exhibiting radiosensitizing effects are believed to work at different levels of cellular phases and one of the suggested mechanisms of treating cells with the particular radiosensitizer before irradiation probably consists in synchronizing them in sensitive cell cycle phase. Moreover, presence of compound during irradiation amplifies their effects by multi-factorial mechanisms including toxic reactions of free radicals. Those compounds that are given after radiation show the effect by inhibiting repair of the radiation induced lethal and sub-lethal damage apart from down regulation of numerous pro-survival factors.

Studies on Effects of Ellagic Acid on Cancer Cells

The various dietary modulators and herbal compounds are biological modifiers, which work as radiosensitization by multiple mechanisms. Studies in our laboratory on the combined effects of radiation and ellagic acid (EA) both *in vitro* and *in vivo* on normal and tumor cells have shown a generation of increasing ROS as a function of radiation dose especially after 3 Gy in human cervical cell line, HeLa. EA was found to be prooxidant *in vitro* at a concentrations ~ 100μM in HeLa cells. Moreover, generation of ROS was found to increase with the increasing concentration of EA. Treatment of cells with EA and radiation showed enhanced ROS generation. Tumor transplanted mice subjected to radiation and EA treatment showed a loss in cellular viability after 1 hr, which increased significantly after 24 hr. These results indicated the up regulation of oxidative stress induced cytotoxicity of tumor cells (unpublished results, Bhosle and Mishra)

Studies on Effects of Tocopherol Succinate on Cancer Cells

Experiments involving tocopherol succinate (TOS), an esterified analogue of Vitamin E, together with gamma radiation showed enhanced induction of apoptosis in MCF-7 breast cancer cell line as measured by fluorescent probes, Annexin V and MC 540. Combined treatment also showed substantial decrease in cellular viability. The combined treatment was found to cause increased destabilization of lysosomes and changes in the plasma membrane fluidity indicating involvement of oxidative stress and membrane destabilization contributing to observed enhanced apoptosis (unpublished results, Kumar and Mishra).

Studies on Effects of Triphala on Cancer Cell

The use of herbals and dietary modulators in combination with radiation have enhanced tumor killing but protected normal cells against radiation. The use of certain phytochemicals as radioprotectors have been reported in literature.[15] Results from our laboratory have shown that Ayurvedic formulation, Triphala in combination with gamma radiation lead to radiosensitization of tumor cells, MCF-7 breast cancer cell line. It was further found that triphala spared normal cells, such as, mouse hepatocytes and spleen cells at concentrations that were toxic to MCF-7 [Cancer Lett. 2005, Sandhya T. et al].

Studies on Eugenol Effects on Liposomes

Results from our laboratory have shown that irradiated phospholipids liposomal as model membranes exhibit significantly enhanced bilayer rigidity due to free radical mediated reaction of lipoxy radicals.[16] It has been demonstrated modulation of physico-chemical properties of model as well as cellular membranes by inclusion of antioxidants like eugenol caused inhibition of membrane oxidative damage.[17,18]

Studies on Arachidonic Acid Effects on Cancer Cells

Cancer cell membranes are known to be deficient in polyunsaturated fatty acids, which renders them radio-resistant. The modulation of plasma membrane by PUFA's can lead to oxidative stress, increased lipid peroxidation and decrease in the cells ability to proliferate. Our results have suggested that enriching the cell membrane of tumor cells with arachidonic acid lead to decreased cellular ability to proliferate as assayed by MTT method. Results have shown that enriching these membranes with PUFA resulted in sensitivity to radiation. Moreover, combined treatment showed increased production of ROS, and decreased viability of cells [unpublished results, Girdhani and Mishra].

Challenges and Future Directions

Treatment of various solid tumors such as renal cell carcinoma (RCC), Prostrate cancer, Head and Neck cancers and Breast cancers is a challenge. Approximately, one third of RCC presented clinically are in metastatic stage at the time of diagnosis and is usually followed by poor prognosis with only 0% to 18% of stage IV patients surviving the 5-year period [19,20]. Prostrate cancer, which is of second largest incidence, amongst the male populations, is only modestly responsive or non-responsive to radiotherapy or chemotherapy. Postoperative radiotherapy can be effective in achieving local control in these tumors. The limitation of this approach in prolonging survival appears to be caused by the intrinsic radioresiatance of these tumor cells. The success of radiotherapy, therefore, depends on increasing the sensitivity of the malignant cells to radiation induced cell kill coupled with a reduction in metastasis phenotypes of these cells. Various dietary modulators and phytochemicals can work as excellent adjuvants to radiation therapy in a variety of cancers. These phytochemicals work at increasing oxidative damage or by synchronizing the cells to a radiosensitive phase of cell cycle thus causing enhanced killing. The future perspectives lie in identifying more such compounds and elucidating the mechanism through which they act for developing effective protocol for cancer radiotherapy.

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