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Flavonoids and radiation response of cancer cells: A therapeutic prospective

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Abstract

Radiation therapy has become a critical part of cancer treatment. The fact that radiation induced damage affects both cancer and normal cells limit the effectiveness of radiation therapy. Radioprotectors and radiosensitizers can increase the effectiveness of radiation therapy. Flavonoids can act both as radioprotectors and radiosensitizers. As radioprotectors, flavonoids could up-regulate the anti-oxidant enzymes and the DNA-repair processes in the cells. As radiosensitizers, flavonoids inhibited the pro-survival NFkB, Akt pathways while up-regulating the apoptotic pathways. Cancer cells have a greater demand for metals to sustain their continuous proliferation and flavonoid-metal interaction is critical for the action of flavonoids to sensitize cancer cells to radiation.

Key words : Cancer cells, Flavonoids, Radiation, Response, Therapeutic

Introduction

Radiation therapy has become an integral part of cancer treatment procedures. However, radiation therapy has its serious issue of being a non-specific treatment process as radiation can't distinguish between the cancerous and healthy cells. Damage to the nearby healthy tissues can again have both immediate effects and late effects. Immediate effects stem from disruption in the fast dividing cells which would again lead to dermatitis, mucositis, hair loss etc. while the late effects includes tissue injuries leading to fibrosis, atrophy infertility, hormonal imbalances and tumour recurrence. There are also damages to the vascular tissues surrounding the organ receiving the radiation therapy leading to telangiectasia, bleeding etc (Barnett *et al.,* 2009). A common example of normal tissue damage during radiation therapy is radiation pneumonitis caused by damage to the lung tissues when the thoracic region is subjected to radiation therapy. The damage to lung tissues is a major dose limiting factor in radiation therapy of the thoracic region (Wang *et al.,* 2012). Keeping the issue of normal tissue toxicity in mind, radiation therapy doses are being moderated to gain maximum reductions in the tumours with minimal damage

to the healthy tissues. The strict maintenance of this balance between maximizing the therapeutic potential of radio-therapy while minimizing collateral damage to the adjoining healthy cells results in improved therapeutic ratio (Barnett et al., 2009)(Figure 1).

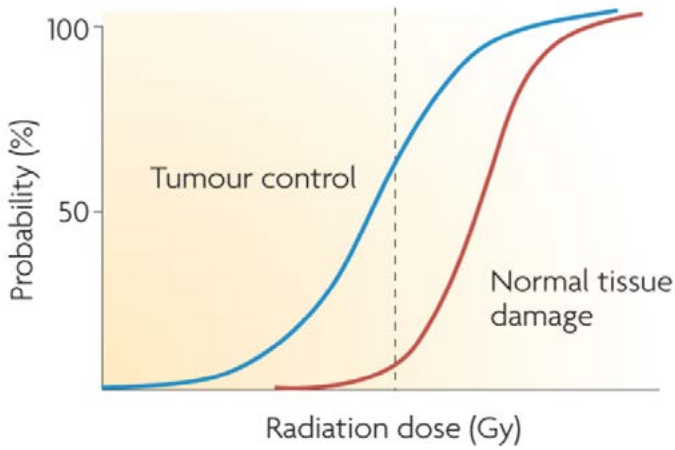

Figure 1: Dose response curve for radiotherapy showing the increasing probability of normal tissue damage along with increased tumour with an increase in radiation. The dotted line shows a theoretical dose to maximize tumour cytotoxicity with minimum damage to the normal tissues (Barnett *et al.,* 2009).

The dose response curve shows the difficulties in aiming for major tumour control with relatively fewer radiation therapies, which can be attributed to the necessity of preventing normal tissue damage.The limitation in the radiation dose that can be applied often leads to resistance of tumours in several cases, along with tumour recurrence. Both of these unfeasible events can be attributed to radiation-induced stress response in cancer cells. Repeated exposure to limited doses of radiation can promote the expression of several pro-survival factors like NFkB, Akt, MMP-2, cell cycle proteins and several pro-inflammatory cytokines, all of which contribute to increased resistance of cancer cells to radiation. The end result of these changes in gene expression in response to radiation therapy lead to lowered 2 year- and 5 year- survivality of cancer patients thus, lowering the overall efficacy of radiation therapy to treat cancers (Kim *et al.,* 2015).

The aim to improve the results of radiation therapy lead to the development of two different sets of compounds- radioprotectors and radiosensitizers. Radioprotectors protect the normal cells from radiation-induced damages thus, lowering side effects of radiation therapy while radiosensitizers sensitize the cancer cells to radiation leading to more radiation induced tumour cell death (Hazra *et al.,* 2011).

Radioprotectors

Radioprotectors are free radical scavengers which allow them to protect cells from free radicals that are produced in a living tissue upon exposure to radiation. Besides free radical scavenging, radioprotectors can act *via* inhibition of lipid peroxidation,

enhancement of DNA repair, stimulation of cell proliferation, immune stimulation, H-atom donation, and prevention of inflammatory reactions (Velpula *et al.,* 2013; Saaya *et al.,* 2017).

Several compounds have been tested as potential radioprotectors. These include-sulfhydryl compounds like cysteine, amifostine; antioxidants like vitamin A, C, E; angiotensin converting enzyme inhibitors like pencillamine, captopril, immunomodulators such as gamma-interferon, polysaccharides AM5, and DNA binding ligands like Hoechst-33342 (Velpula *et al.,* 2013). Till date, only amifostine has been approved for use as a radioprotector of normal tissues in cancer radiotherapy (Patyar *et al.,* 2018). Amifostine prevented xerostomia upon head and neck irradiation while in case of chemo-radiotherapy of NSCLC amifostine prevented pain and dysphagia, and also protected the normal cells from platinum induced cytotoxicity. The conversion of amifostine to its active form requires the action of alkaline phosphatase and the higher expression of alkaline phosphatase in the normal cells allows for the specific protection of the normal cells. However, amifostine has toxicity issues related to nausea, somnolence, vomiting and hypotension (Rosen *et al.,* 2015).

The safer alternatives of radioprotectors present plant extracts and plant polyphenols as natural choices. Several plants like *Withania somnifera, Curcuma longa, Eugenia jambolana, Tinospora cordifolia, Ocimum sanctum, Allium sativum, Emblica officinalis, Mentha piperita, Zingiber officinale,* etc. have shown radioprotective properties. The general method of radioprotection offered by the extracts derived from these plants consist of free radical scavenging, up-regulation of the expression of antioxidant enzymes like catalase, superoxide dismutase (SOD), glutathoine transferase, glutathione peroxidase and DNA repair enzymes and down-regulation of PKC, MAPK, cytochrome P450 which are involved in mediating radiation induced damages (Bhandari, 2013).

Flavonoids have shown radioprotective properties. Flavonoids exerted their radioprotective effects *via* free radical scavenging, protection of the immune and haematopoeitic system, anti-inflammatory mechanisms and protection of the DNA from damages (Li *et al.*, 2016). The ketone group in the flavonoid structure is important in preventing oxidative damage. Flavonoids also up-regulated the expression of anti-oxidant enzymes such as superoxide dismutase (SOD), glutathoine transferase, glutathione peroxidase and DNA repair enzymes like DNA polymerase β, while down-regulating expression of genes like PKC, MAPK, cytochrome P450, NFκB, COX-2 and LOX which are associated with promoting radiation related damages, inflammation and oxidative stress (Paul *et al.,* 2011; Jagetia *et al.,* 2012; Xu *et al.,* 2014). Flavonoids like silibin, apigenin, orientin, etc. are involved in protecting the DNA and repair of DNA in response to radiation (Satyamitra *et al.,* 2014; Li *et al.,* 2016). Almost all flavonoids show antioxidant activity with some flavonoids showing better radioprotection based on their better free radical scavenging properties. For example, genistein shows better free radical scavenging than quercetin and the combination of monoglycosylated rutin and quercetin shows better free radical scavenging and radioprotective properties as compared

to monoglycosylated rutin alone (Li *et al.,* 2016; Aizawa *et al.,* 2018). Flavonoids like apigenin, hesperidin and genistein stimulate the immune and haematopoetic system while baicalein prevents inflammatory reactions (Li *et al.,* 2016).

Radiosensitizers

Radiosensitizers have been used to get a higher cancer cell death at lower doses of radiation. Currently, the chemotherapy drugs are used as radiosensitizing drugs that are used in combination with radiation. The most common chemotherapeutic drugs used as radiosensitizers are cisplatin, 5-flourouracil and taxanes and all of them have their own toxicity issues. Cisplatin is currently used as radiosensitizer in case of NSCLC (Alcorn *et al.,* 2013; Fong, 2016). Several other compounds are currently being tested as potential radiosensitizers. These include carbogen, a mixture of 95% oxygen and 5% carbondioxide; that leads to higher oxygen concentrations in cancer cells leading to greater free radical formation upon radiation exposure and thus, higher damage to the cells; camptothecin, a topoisomerase inhibitor; patupilone, a microtubule stabiliser; and monoclonal antibodies like ertolinib (Liman *et al.,* 2015).

Various plant extracts have also shown radiosensitizing properties, such as *Panax ginseng, Azadirachta indica, Tinospora cordifolia, Trametes versicolor, Withnia somnifera, Erythroxylum tuberosum* (Hazra *et al.,* 2012; Macedo *et al.,* 2016). Several flavonoids have been reported to have radiosensitizing properties and they include genistein, quercetin, flavopiridol, myricetin, apigenin and vicenin-2 (Garg *et al.,* 2005; Malik *et al.,* 2016; Prasad *et al.,* 2016; Baruah *et al.,* 2018). Flavonoids cause radiosensitizing effects on the cancer cells through various mechanisms. Radiation up-regulates PI3K/Akt, ERK, NFkB pathways which then set in motion several resistance mechanisms like up-regulation of anti-apoptotic proteins and DNA repair proteins (Abotaleb *et al.,* 2018). Flavonoids also inhibit the PI3K/Akt,ERK and NFκB pathway and promote apoptosis. In combination with radiation, flavonoids cause a greater reduction in cancer cell colony forming ability and also cause cell cycle arrest. Flavonoids exhibit a pro-oxidant effect leading to greater DNA damage. Flavonoids also cause down-regulation of the DNA repair pathways. Flavonoids promote apoptotic proteins and down-regulate the anti-apoptotic proteins. All of these factors contribute to the sensitization of cancer cells to radiation (Baruah *et al.,* 2018; Baruah *et al.,* 2019).

Flavonoids and metals and free radicals

The investigators group have checked the ability of three different flavonoids as radiosensitizers and also conducted test to check for their ability to ensure higher survival of embryonic HEK293T cells when exposed to radiation. The srudy included the flavonoids vicenin-2, naringenin and quercetin for their ability to sensitize lung cancer cells to radiation and all of them showed significant radiosensitizing ability. The specificity for flavonoid action could be attributed to the ability of flavonoids to undergo oxidation with metals resulting in the production of free radicals along with H_2O_2 which

leads to increase in flavonoid-induced apoptosis of cancer cells (Eghbaliferiz *et al.,* 2016; Uivarosi *et al.,* 2017). This complements the free radicals that are produced by radiation alone resulting in an excess of free radicals that the cancer cells have to put up with. An increased amount of free radicals was observed in the cancer cells when the cells were treated with vicenin-2 (Baruah *et al.,* 2019) and when the cancer cells were treated with the other two flavonoids (unpublished results). Cancer cells have higher levels of Cu ions due to increased requirement of Cu by the proteins involved in cell proliferation and angiogenesis (Wang *et al.,* 2010). The high Cu levels in turn allows higher levels of free radical and H_2O_2 production in the presence of flavonoids and thereby contributing to increased rate of apoptosis in the cancer cells (Eghbaliferiz *et al.,* 2016; Uivarosi *et al.,* 2017). At lower concentrations of flavonoid when the production of free radicals and H_2O_2 was less, flavonoids reported a cytoprotective activity on the cancer cells, which was observed in case of vicenin-2 (Sak, 2014; Baruah *et al.,* 2018), further highlighting the importance of free radical production by flavonoids to carry out their cytotoxic activities on cancer cells. The chelation of Cu by flavonoids also affects the Cu-dependent pro-cancerous processes and this area has now turned into a new focus point for researchers (Wang *et al.,* 2010). Normal cells have significantly lowered levels of metals as compared to cancer cells (Wang *et al.,* 2010) which could be taken as a primary indicator of an explanation regarding our observation of how the flavonoids we tested could offer radioprotection to the embryonic cells while being radiosensitive to the cancer cells. The flavonoids don't produce excessive free radicals in the embryonic cells that could affect the health of the cells.

Another aspect of the flavonoid-metal interaction has been the increase of lipophilicity of flavonoids when they are complexed with metals. Increased lipophilicity allows flavonoids to bind to the hydrophobic sites in the proteins, leading to clustering of the proteins to the complex, thereby affecting their functions. Flavonoids have also been reported to affect the membrane structure and the formation of lipid rafts which greatly affects the functioning of proteins like Akt and NFkB (Tarahovsky *et al.,* 2014). In our studies we have observed the lowering of the levels of activated Akt, which could be attributed to the disruption of lipid rafts. The lowering of activated Akt led to the lowering of the downstream anti-apoptotic proteins (Baruah *et al.,* 2019). However, we have studied on only a single cancer cell line and as such, the activities of the flavonoids need to be tested on more cancer models before establishing them as radiosentitisers and moving into clinical trials. The elucidation of the molecular mechanisms of the radioprotective effect of these flavonoids also remain fertile ground for future research.

Conclusion

Flavonoids can act both as radioprotectors and radiosensitizers and thus, have immense scope in being considered as regular clinical part of radiotherapy, thereby improving the effectiveness of radiation therapy without compromising the safety of the healthy cells and tissues adjacent to a tumour. The interaction of flavonoids and metals in both cancer cells and healthy cells, both with and without radiation, needs to be further

elucidated along with the molecular mechanism that these interactions might give rise to events on *in vivo* models.

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