Development of new formulations for mitigation of radiation injury and improving the outcome of radiotherapy

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Abstract

We have used phytochemicals, semisynthetic and synthetic molecules and rational drug design approach for prevention and treatment of radiation induced toxicity which will be useful for improving the outcome of radiotherapy and also for creating national stockpile for management of radiation emergencies in India. These novel formulations were tested in in vitro systems and their efficacy was established in preclinical animal models. The key research findings on discovery of radio-protective drugs have been transferred to private pharmaceutical company for translation to clinic. These formulations have also been studied for their safety, pharmacokinetics, detailed mechanism of action and intended route of administration. Here we present the progress on development of these new drugs and formulations and their possible health benefits to humans.

Introduction

Almost every second cancer patient receives radiotherapy in the form of external beam or internal irradiation given as brachytherapy. Despite technical improvements that allow precise deposition of dose in the tumor while progressively reducing any unwanted dose to surrounding normal tissues, many patients still suffer from side effects of normal tissue damage after radiotherapy. Acute and long term toxicities associated with radiotherapy of head & neck cancers are skin, mucosal damage and severe mucositis whereas thoracic irradiation for cancers of lung, esophagus and breast results in pneumonitis and fibrosis. There is also risk of unplanned exposures in case of nuclear emergencies or dirty bomb which emphasize the need for developing novel agents that can protect against unwanted side effects of IR. An ideal radioprotector must be non-toxic and should protect normal cells against the harmful effects of radiation without relinquishing the detrimental effects of IR on cancer cells. Several studies have been performed with objective to develop new and effective radiation countermeasures to reduce, prevent or lower the risk of normal tissue toxicity. Several drug targets such as free radicals, pro-survival transcription factors NF-kappaB and Nrf-2, cytokinereceptors, toll-like receptors, and radical scavengers are being explored.

In house research efforts:

Our research efforts are focussed on development of novel therapeutics for prevention, mitigation and treatment of radiation induced toxicity towards normal tissues and also for improving the outcome of radiotherapy. In order to develop appropriate radio-modifiers and radioprotectors, we have screened a large number of phytochemicals, synthetic compounds, semisynthetic molecules, formulations, proteins and also used in silico docking approach for rational drug design. Majority of these compounds showed either anti-oxidant or pro-oxidant action in cell free and cellular systems. The screening of these molecules during past 15 years has helped us in identification of candidate molecules for development of radio-modifier drugs. Two most important formulations developed in our lab are named as BARC Radio-modifier (BRM) and BARC Radioprotector (BRP).

BARC Radio-modifier (BRM):

BRM is a formulation based on a semisynthetic water soluble derivative of green plant pigment chlorophyll. We for the first time demonstrated the direct antioxidant activity of chlorophyllin (BRM) in terms of scavenging of different types of reactive oxygen species (ROS) using electron spin resonance (ESR) spectroscopy. It was able...
to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals as well as prevented the formation of 5,5-dimethyl-1-pyrroline-N-oxide adduct with hydroxyl radicals (DMPO-OH adduct) generated by γ-radiation in a dose-dependent manner. It also inhibited O₂⁻-dependent formation of the 2,2,6,6-tetramethylpiperidine oxide (TEMPO) radical during photosensitization of methylene blue and hydrogen peroxide induced oxidation of phenol red. BRM was also shown to act as an antioxidant and thereby prevent radiation-induced damage to biomolecules like DNA in cell free systems. Based on these cell free system findings, BRM was tested for its anti-oxidant activity in cells and animals. We found that BRM entered lymphocytes and scavenged radiation derived free radicals in a dose dependent manner in vitro as well as in the mice. It also prevented radiation induced apoptosis in the sensitive tissues including lymphocytes. It showed multiple health benefits in experimental animals including augmentation of innate as well as adaptive immune responses via upregulation of anti-apoptotic genes. It significantly protected mice against radiation induced toxicity, morbidity and mortality. In order to improve the radioprotective efficacy of BRM, we have tested several dose regimens and tried a variety of routes of administration in combination with other agents. It was found that a formulation containing BRM dose equivalent to human dose of 15 to 45 mg/kg body weight offered significant protection against whole body irradiation induced mortality in mice (Fig. 1). At these doses, it increased the abundance of hematopoietic stem cells and enhanced the production of granulocytes in the bone marrow. The hematopoietic stem and progenitor cells (HSPCs) are the progenitor cells for all blood cells. The proliferation and differentiation of HSPCs give rise to the entire hematopoietic system. HSPCs are believed to be capable of self-renewal, expanding their own population of stem cells and becoming pluripotent are capable of differentiating into any cell in the hematopoietic system. From this rare cell population, the entire mature hematopoietic system, comprising lymphocytes (B and T cells of the immune system) and myeloid cells (erythrocytes, megakaryocytes, granulocytes and macrophages) are formed.

BRM also increased serum levels of granulocyte colony stimulation factor (G-CSF) concurrent with higher frequency of neutrophils in peripheral blood. Neutrophils are the first line of defense in the immune system and they protect the body against most of the infections. Ionizing radiation induced depletion of neutrophils increases the susceptibility to infections. The depletion of hematopoietic stem cells following exposure to radiation suppresses hematopoiesis resulting in long term immune suppression. Percent granulocytes are a biological marker for efficacy of BRM as a radioprotector in vivo. The increase in granulocytes in response to BRM treatment and increased abundance of hematopoietic stem cells would boost the immune system and thus improve the survival following exposure to ionizing radiation. We indeed found that BRM significantly protected mice against whole body irradiation induced mortality. The safety and toxicity profile of BRM was evaluated in detail and it was found that an acute dose of 5000 mg/kg body weight was well tolerated in rodents. Further, it did not induce any toxicity in mice when a repeated daily dose of 1000 mg/kg body weight was administered for 28 consecutive days.

Our recent studies revealed that BRM significantly prevented radiation induced toxicity in the lungs, bone marrow and gastro-intestinal tract. However, BRM did not offer protection to lymphoma cells, lung cancer cells or breast cancer cells against radiation. On the contrary, it increased the radiosensitivity of these cancer cells by inducing mitotic catastrophe and delaying DNA repair (Fig. 2). BRM also sensitized the human breast cancer cells to ionizing radiation in vivo (Fig. 3A and B).

Based on these findings in the pre-clinical settings, it is proposed that BRM would find application as an adjuvant during cancer radiotherapy. A USA patent application entitled ‘A method of adjuvant treatment with chlorophyllin containing therapeutic preparation including for radioprotection of normal tissues during radiation therapy and kit therefor’ has been filed.
In order to translate these findings to clinic, the technology for development of tablets and oral admissible formulations, it has been incubated with M/S Innovative Drug Research Solutions, Bangalore. The BRM tablets may be administered alone or in combination with pharmaceutically acceptable carriers, vehicles or diluents, in either single or multiple doses. Thus, for purposes of oral administration, tablets containing various excipients have been prepared (Fig. 4). The composition suitable for oral administration will provide immediate, delayed, extended or controlled release of BRM. BRM exhibited dissolution profile such that almost 80% of the active pharmaceutical ingredient is released in less than 25 minutes.

These formulations will be useful for prevention and treatment of radiation induced toxicity in people exposed to radiation under planned exposure settings. It can also be useful for increasing the radiosensitivity of...
breast cancer stem cells and lung cancers. Another novel therapeutic use of BRM based formulations will be for increasing hematopoietic recovery in lymphopenic patients.

BARC Radioprotector (BRP):
The lectin-based formulation developed in our laboratory will be useful for mitigation of radiation induced toxicity in people who may accidentally receive whole body or partial body radiation exposure at high (supra lethal) doses of ionizing radiation. We found that a single intravenous injection of lectin to mice offered protection against 10-12 Gy  \( \gamma \) - radiation-induced mortality and male sterility and also abrogated 12 Gy induced decrease in testosterone levels in the serum of male mice (Fig. 5A & B).

We have also discovered the anti-tumor, immune-suppressive and anti-inflammatory molecules from medicinal plants (Terminalia arjuna, Plumbago zeylanica, Mirabilis jalapa, Sisandra chinensis, Curcuma longa and Withania somnifera), tea / coffee and synthetic sources and tested them in preclinical experimental models.

Conclusions
Our preclinical studies on BRM and BRP have shown very promising efficacy as radio-modifier and radioprotective agents. Clinical trials in human subjects will be conducted for using these candidates as drugs for improving the outcome of radiotherapy / chemotherapy. The initial trials will be carried out in collaboration with a pharmaceutical company, clinical research organization and Tata Memorial Centre, Mumbai.

References:
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