Abstract

Intestinal epithelial cells and stem cells are highly sensitive to ionizing radiation as they are continuously proliferating. These cells are damaged during radiotherapy of gastro-intestinal (GI) cancers resulting in severe side effects including diarrhoea, loss of appetite and weight loss. Here we describe a novel strategy to mitigate radiation induced GI syndrome using a pro-oxidant molecule 1,4-naphthoquinone (NQ). Administration of NQ to mice rescued them against whole body irradiation (8Gy) induced mortality by perturbing cellular redox leading to activation of pro-survival transcription factor Nrf-2 in intestinal epithelial cells.

Introduction

Enhanced use of radiation in various aspects of human life may lead to situations like planned or unplanned exposure to radiations. Planned exposure is the result of the use of radiation in diagnosis and treatment in radiation therapy. In spite of the fact that radiotherapy is an effective tool for cancer treatment, the normal tissues adjacent to the tumor are exposed to radiation which limits the therapeutic efficacy. High sensitivity of the intestine tract due to high proliferation rate is one of the factors contributing to the adverse side effects of cancer radio therapy. Ionizing radiation induced mortality is largely dependent on the dose of radiation received by organism. Acute effects after Whole body irradiation of 8Gy are known as gastro-intestinal syndrome that causes death within 7 to 10 days. Gastro-intestine (GI) tract is lined by numerous luminal protrusions villi and submucosal invaginations called crypts of Lieberkuhn constituting the absorptive surface of small intestine called as jejunum. GI syndrome is characterized by loss of absorptive surface of small intestine due to denudation of villi and apoptosis in crypts.

Although different radiation countermeasures are currently under development but none of them have been approved by food and drug administration for human use. Previously reported countermeasures against GI syndrome like fibroblast growth factor, TLR 5 agonist CBLB502, S1P and anti-ceramide antibody offered protection by reducing apoptosis in intestinal crypts (1,2). Radiation exposure is followed by a marked increase in oxidative stress induced cellular damage, thus targeting a molecule which serves as a master regulator of antioxidant and cytoprotective gene expression seems a logical approach. Nuclear factor erythroid 2- related factor 2 (Nrf-2) is a redox sensitive transcription factor which upon activation translocates into the nucleus and binds to antioxidant response element (ARE) and induces the expression of phase II detoxifying enzymes, anti-oxidant enzymes and stress responsive proteins. 1,4-naphthoquinone (NQ), a pro-oxidant, is a strong electrophile with high...
affinity for cellular nucleophiles like thiols of cysteine group present in proteins contributing to its biological activity. Hence we postulated to explore the potential of NQ to protect against radiation induced gastrointestinal syndrome and also delineate the underlying molecular mechanism.

Results

**NQ protected against radiation induced cell death in INT 407 (human intestinal epithelial cells)**

NQ treatment prior to radiation dose of 6Gy significantly protected INT 407 cells against radiation induced loss of clonogenicity. Radiation induced apoptotic death is marked by an increased caspase activity. NQ pre-treatment significantly inhibited radiation induced caspase-3 activity.

**NQ modulated cellular redox status by increasing cellular ROS and depleting GSH/GSSG**

Perturbation in cellular redox by NQ treatment was marked by a transient increase in basal ROS levels compare to control as measured by redox sensitive fluorescent dye (Fig. 1A) and a transient depletion in GSH/GSSG ratio (Fig. 1B).

**Fig. 1A**: A transient increase in basal ROS levels compare to control as measured by redox sensitive fluorescent dye

**Fig. 1B**: A transient depletion in GSH/GSSG ratio

**NQ induced activation and upregulation of Nrf2 and its dependent gene in INT 407**

NQ treatment showed an increase in nuclear translocation of Nrf2 in a time dependent manner (Fig. 2A). NQ treatment significantly enhanced the mRNA expression of Nrf2 and its dependent gene hemeoxygenase 1(HO1) in a time dependent manner (Fig. 2B).

**Fig. 2A**: An increase in nuclear translocation of Nrf2 in a time dependent manner
Inhibitors of Nrf2 and its upstream kinases Erk abrogated NQ mediated protection

ATRA (Nrf2 inhibitor) and PD98059 (Erk inhibitor) significantly reverted the NQ mediated protection against radiation induced loss of clonogenicity in INT 407 cells (Fig. 3) suggesting the pivotal role of Nrf2 pathway as underlying mechanism.

NQ administration protected mice against WBI induced mortality

Administration of NQ (2mg/kg bw 4 doses) prior to irradiation protected mice against WBI 8Gy induced mortality. NQ treated mice showed significantly higher survival after WBI (Fig. 4)

NQ protected against WBI induced gastrointestinal injury:

Normal villous architecture, epithelial alignment and crypts were observed in NQ administered group when compared to shortened and oedematous villi with epithelial irregularities in radiation treated group (Fig 5.).
Discussion

Earlier findings from our laboratory showed the potential of NQ to protect against hematopoietic system (3). Our current findings highlight the efficacy of NQ to protect gastro-intestinal tract from radiation induced toxicity. INT 407 cells served as a model for in vitro studies. Clonogenicity is defined as the potential of single cell to form a colony determining its proliferating efficiency. NQ significantly protected against radiation induced loss of clonogenicity. Radiation induced cell death is mainly due to induction of apoptosis which is marked by higher caspases activity. NQ protected against radiation induced increase in caspase activity. Balanced cellular redox status is required for normal functioning of cell. However transient modulation in cellular redox is associated with the induction of redox sensitive cytoprotective transcription factors like Nrf2. NQ being a pro-oxidant modulated cellular redox by transiently enhancing the basal ROS levels and depleting the abundant redox couple GSH/GSSH ratio (Fig. 1A and B). Further NQ showed an increase Nrf2 nuclear translocation and an increase in mRNA expression levels of Nrf2 and its dependent cytoprotective gene (Fig. 2A and B). Inhibitors of Nrf2 and its upstream kinases Erk, which is responsible for Nrf2 phosphorylation thus activation, reverted the effect of NQ on radiation induced cell death (Fig 3) implicating the pivotal role of Nrf2 pathway in NQ functioning as radioprotector. Further in vivo studies suggested the potential of NQ to act as radioprotector.

As discussed previously, doses from 8Gy and above induces mortality due to induction of GI syndrome. NQ significantly rescued mice from WBI 8Gy induced mortality (Fig. 4). NQ treated mice showed normal epithelial lining indicating intact absorptive surface of intestine when compared to denudated and oedematous villi with irregularities in epithelial lining in WBI group (Fig 5). Our findings highlight the efficacy of NQ to ameliorate radiation induced gastro-intestinal syndrome by modulating cellular redox and activating Nrf2 pathway.

References