

OLTIPRAZ: DNA AND RNA PROTECTION

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Summary

Present study is an attempt to investigate the radioprotective potential of *Oltipraz* against gamma radiation induced damage in testicular DNA and RNA of Swiss albino mice. For this study, the animals were divided into four (I-IV) groups. Group I had normal animals (sham control), while animals of group II were treated with *Oltipraz* alone at the dose of 100 mg/kg b.wt. for 2 consecutive days to observe any drug (*Oltipraz*) related toxicity. Group III animals were given Tween 80 (equal to the volume of the drug *Oltipraz*) and served as irradiated group while Group IV animals were given *Oltipraz* (OLT) orally for 2 consecutive days at the dose of 100 mg/kg b.wt. and served as experimental group. On the last day of drug administration, the animals of III and IV groups were exposed to 8 Gy gamma radiations and autopsied at day 6hr, 1, 3, 7, 15 and 30 days to dissect out testis for DNA and RNA estimation. The mice of irradiated group showed reduced DNA content which indicate towards radiation-induced DNA damage and apoptosis. Furthermore, RNA content was also found to significantly decrease in irradiated group mice testis as compared to sham control group that might be partly due to diminished DNA content and inhibited transcription. On the contrary, experimental group showed significantly increased DNA as well as RNA contents in mice testis. This finding provided evidence that *Oltipraz* provide protection to both DNA and RNA against radiation induced testicular damage because of its radioprotective, antioxidant and chemopreventive properties.

Keywords: *Oltipraz*, radiation, DNA, RNA, testis.

Introduction

Interaction of ionizing radiation with the biological system results in the generation of many highly reactive short-lived reactive oxygen species (ROS), mainly due to the hydrolysis of water¹, including hydroxyl radicals ($\cdot\text{OH}$), superoxide anions (O_2^-), singlet oxygen (O_2) and hydrogen peroxide (H_2O_2), which are major determinants of cellular damage. These ROS then attack cellular macromolecules like DNA, RNA, proteins, membranes etc., causing their dysfunction and damage².

A major biological effect of radiation is directly inflicted upon DNA³⁻⁴ that is why the nucleus and particularly genomic DNA of a cell is considered to be the most critical radiation-sensitive “volume”⁵ or target for irradiation-induced effects that finally leads to cell killing and cancer.

The tolerance dose to radiation in different organs varies, depending on their vital functions, ability to repair, population of dividing cells and the volume of organs irradiated. Those containing a large population of actively dividing cell (especially those in G₂ and M phases), such as haemopoietic cells, germ cells and gastrointestinal cells, are thus highly susceptible to radiation injuries as compared to bone, cartilage, muscle and nerves which are found to be more radio-resistant to such damage.

The testis which generates male germ cells is known to be one of the major radiosensitive organs in the body⁶. Testicular damage after local or whole body irradiation by external sources (eg. γ - and X-rays) has been well documented in both animals and man⁷⁻⁹. The effects of radiation are dose related resulting in temporary or permanent inhibition of spermatogenesis depending upon the cell type affected. Proliferating spermatogonia are the most radiosensitive, while differentiated spermatids/spermatozoa are the least sensitive. As well as inducing a fall in sperm output, irradiations of germ cells at lower doses, which allow the production of sperm, are clearly genotoxic in the offspring.

Nucleic acids are the main controlling centers of the cell. An ionizing radiation can cause variety of nucleic acid damaging scenarios that ultimately result into mutagenesis or cell death. DNA (blueprints of the cell), that ensure cell repair and replacement and produces a perfect copy of the original cell are considered to be the critical target molecule for the ionizing radiations. Ionizing radiations induces predominantly single strand breaks but also double strand breaks, alkali labile sites, and oxidized purines and pyrimidines¹⁰⁻¹¹. Furthermore, cells exposed to ionizing radiations can develop prolonged genetic instability that is manifested in multiple ways, including delayed reproductive death, an increased rate of point mutations, and chromosome rearrangement¹²⁻¹³.

Several drugs have been screened to provide protection against radiation induced DNA damage such as melatonin¹⁴, dithiothreitol¹⁵, substituted PROXYLs¹⁶ and Aloe vera¹⁷ etc.

Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione; RP 35972] is a synthetic, substituted 1,2-dithiole-3-thione previously used in humans as an antischistosomal agent. It posses potent antioxidant, chemotherapeutic and cancer chemopreventive properties. In animal models, *Oltipraz* was found to protect against the chemically induced carcinogens in a variety of different organs such as lung, stomach, colon, urinary bladder, liver, breast etc. *Oltipraz* also inhibits the carcinogenesis induced by polycyclic aromatic hydrocarbons and N-nitrosamines- agents that constitute some of the carcinogenic components of tobacco. Furthermore, *Oltipraz* (OLT) has

been reported to show radioprotective effects in mice via enhancing radiation (Rad)-inducible glutathione-S-transferase (GST) and microsomal epoxide hydrolase (mEH) expression in the liver¹⁸⁻¹⁹. Besides these, *Oltipraz* has also displayed antiviral, reverse transcriptase inhibiting, anti-fibrotic, anti-cirrotic and anti-aging properties in different model systems.

Materials and Methods

Animal model

Healthy young Male Swiss albino mice (*Mus musculus*, 6-8 weeks old) with an average body weight (25 ± 2 gm) were procured from IVRI Izatnagar, India. They were maintained in animal house under control condition of temperature ($25 \pm 2^\circ\text{C}$) and light (14 hrs light and 10 hrs darkness). These animals were given pelleted standard mice feed (obtained from Hindustan Lever Ltd., Delhi) and water *ad libitum*. Five to six animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland and the Indian National Science Academy (INSA), New Delhi, India.

Irradiation source

Cobalt Teletherapy Unit (ATC-C9, Canada) at Cancer Treatment Centre, Radiotherapy Department, S.M.S Medical College and Hospital, Jaipur was used for irradiation. Unanaesthetized animals were restrained in well ventilated boxes and exposed to whole-body gamma radiation at a distance (SSD) of 80 cm from the source to deliver the dose-rate of 1.59 Gy/min.

Oltipraz

Drug *Oltipraz* was obtained from Canopus Corporation, Kenilworth, Byrock, NSW, Australia. *Oltipraz* (OLT) was dissolved in 1 ml of 10% suspension of Tween 80 as a solvent immediately before use and was administered orally at the volume of 0.1 ml for two consecutive days.

Biochemical estimations

For the analysis of different biochemical parameters, mice were divided into 4 groups of 10 animals each as follows-

Group I (Sham control): Animals were administered 0.1 ml DDW (equal to the volume of Drug) once orally for two consecutive days.

Group II (Oltipraz alone): Animals were administered *Oltipraz* (OLT 100 mg/kg b.wt) once orally for two consecutive days.

Group III (Irradiated group): Animals were administered 0.1 ml of solvent (10% suspension of Tween 80) once orally for two consecutive days before 8 Gy γ -irradiation exposure.

Group IV (Experimental group): Animals were administered *Oltipraz* (OLT 100mg/kg b.wt) once orally for two consecutive days, prior exposure to 8 Gy γ -irradiation.

Testis was dissected out and the following biochemical parameters were assessed at 6 hr, day 1, day 3, day 7, day 15 and day 30 autopsy intervals-

DNA estimation assay-

DNA was quantified by the method described by Ceriotti²⁰. Testis was dissected out and homogenate was prepared in glacial distilled water (10 mg ml⁻¹). Homogenate (2ml) was taken in a centrifuge tube and 1 ml indole reagent was added. Then, 1 ml of concentrated HCl was added. Thereafter chloroform treatment was given. The water layer was separated out from the organic layer by centrifugation. The intensity of yellow colour was measured at a green filter against a blank. The volume of DNA was calculated from the graph and then the quantity of DNA in the tissue was calculated in $\mu\text{g mg}^{-1}$ tissue. The blank was 2 ml H₂O + 1ml indole + 1 ml concentrated HCl + 4 ml chloroform.

RNA estimation assay-

RNA was quantified by the method described in Ceriotti²⁰. Testis was dissected out and a homogenate was prepared in glacial distilled water (10 mg ml⁻¹). The reaction mixture contained 5 ml homogenate + 5 ml orcinol + 5ml isoamyl alcohol. The upper coloured layer was separated with the help of a Vaccumizer and the intensity was measured against red filter. The concentration of RNA from standard graph was calculated and then the amount of tissue RNA was calculated in $\mu\text{g mg}^{-1}$ tissue. The blank was 5ml H₂O + 5 ml orcinol + 5 ml isoamyl alcohol.

Statistical analysis

The results obtained in the present study were expressed as mean \pm SEM. The statistical difference between the various groups was analyzed by Student's *t*-test and the significance was observed at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ level.

Results

It is evident from the table that a highly significant ($p < 0.001$) decrease in DNA content was obtained in irradiated group mice testis as compared to sham control group (Table 1). But *Oltipraz* pretreatment at the dose of 100 mg/kg b.wt. significantly inhibited this decrease in DNA content in experimental group mice as compared to their respective irradiated group.

RNA content was found to decrease in irradiated group mice with its minimum value was recorded on day 3 of post-irradiation period. *Oltipraz* (OLT) pretreatment 100mg/kg b.wt. significantly ($p < 0.01$) prevented this decline in DNA content in experimental group mice as compared to their respective irradiated group (Table 2).

Table 1. Variation in the DNA content ($\mu\text{g}/\text{mg}$ of tissue) in the testis of Swiss albino mice in different treated groups.

Treated groups	Autopsy intervals					
	6 hr	Day 1	Day 3	Day 7	Day 15	Day 30
Sham control	2.35 ± 0.15	2.38 ± 0.10	2.40 ± 0.12	2.43 ± 0.08	2.47 ± 0.16	2.49 ± 0.04
<i>Oltipraz</i> (100 mg/kg b.wt)	2.37 ± 0.16	2.40 ± 0.13	2.42 ± 0.14	2.45 ± 0.17	2.51 ± 0.05	2.57 ± 0.04
Irradiated group (8 Gy)	1.66 ± 0.20^b	1.58 ± 0.19^c	0.98 ± 0.20^c	0.76 ± 0.07^c	1.30 ± 0.05^c	1.63 ± 0.10^c
Experimental (<i>Oltipraz</i> + 8 Gy)	1.81 ± 0.23	1.96 ± 0.16^a	1.11 ± 0.09^a	1.41 ± 0.15^b	1.77 ± 0.11^c	2.17 ± 0.20^b

Significance level: $a = p < 0.05$, $b = p < 0.01$, $c = p < 0.001$

Statistical comparison : Sham control V/s *Oltipraz*
 Sham control V/s Irradiated group
 Experimental V/s Irradiated group

Table 2. Variation in the RNA content ($\mu\text{g}/\text{mg}$ of tissue) in the testis of Swiss albino mice in different treated groups.

Treated groups	Autopsy intervals					
	6 hr	Day 1	Day 3	Day 7	Day 15	Day 30
Sham control	2.45 ± 0.12	2.46 ± 0.09	2.49 ± 0.16	2.51 ± 0.16	2.54 ± 0.10	2.58 ± 0.12
<i>Oltipraz</i> (100 mg/kg b.wt)	2.46 ± 0.13	2.46 ± 0.13	2.47 ± 0.13	2.49 ± 0.09	2.51 ± 0.11	2.54 ± 0.08
Irradiated group (8 Gy)	1.49 ± 0.17^c	1.27 ± 0.13^c	0.92 ± 0.12^c	0.75 ± 0.15^c	1.10 ± 0.09^c	1.43 ± 0.11^c
Experimental (<i>Oltipraz</i> + 8 Gy)	2.08 ± 0.17^b	1.93 ± 0.21^b	1.11 ± 0.08^b	1.41 ± 0.14^b	1.75 ± 0.17^c	2.21 ± 0.23^c

Significance level: $a = p < 0.05$, $b = p < 0.01$, $c = p < 0.001$

Statistical comparison : Sham control V/s *Oltipraz*
Sham control V/s Irradiated group
Experimental V/s Irradiated group

Discussion

DNA is the first vital target molecule for radiation injury found in the biological systems. In the present study, DNA contents were found to decrease in the irradiated group mice testis as compared to sham control group. This decrease in DNA content indicates towards the DNA damage caused by irradiation. Atorino *et al.* (2001) reported that a 5 Gy irradiation dose is sufficient to induce DNA damage detectable by the Comet assay in rat germ cells²¹. DNA may be damaged due to direct hits from ionizing rays, or as secondary damage from radicals generated by radiation. In addition, oxidative stress can also cause DNA damage which includes oxidative damage to bases and the sugar-phosphates, as well as single-or double-strand breaks (SSBs or DSBs) within DNA. Moreover, the reactive aldehydes such as MDA and 4-hydroxyalkenals that are derived from the peroxidized phospholipids by ROS and free radicals can also react with DNA and causes several damage by forming exocyclic DNA adducts²². Thus, the measurement of DNA damage can be a useful indicator not only of exposure but also of risk of disease. In addition, the decrease in DNA contents could also be attributed to the inhibition of DNA synthesis. Leshner and Bauman (1968) found a reduction in the rate of DNA synthesis due to a reduction in the movement of cells from G₁ to S-phase, which was blocked by the high doses of radiation²³. Radiation induced depression in DNA synthesis is might due to decrease in the DNA polymerase activity and alteration in the pool size of DNA precursors either by a change in membrane permeability or by an alteration of other biochemical factors necessary for the synthesis of nucleosides and nucleotides. *In vitro* studies also state the similar facts that in cultured cells, ionizing radiation is able to affect DNA synthesis by blocking the initiation of replication as a result of DNA strand breaks²⁴⁻²⁵.

Abou-Sief *et al.* (2003) reported a highly significant decrease in both DNA and RNA content on exposure to 6 Gy of γ -irradiation²⁶. This decrease in DNA and RNA content is mainly attributable to the generation of reactive oxygen species (ROS) such as O₂^{•-}, H[•], HO₂[•], [•]OH and H₂O₂ within the organism. Although these ROS are being produced continuously during normal respiration as well as through other biological pathways but exposure to ionizing radiations raises the generation of ROS in biological systems and are regarded as the major causative factor responsible for molecular damage. It is well known that the molecule most often reported to be damaged by ionizing radiation is DNA²⁷. ROS can induce damage to both nuclear and mitochondrial DNA. The nature of damage depends mainly on the dose and duration of the radiation exposure. Any damage inflicted on DNA molecule further results in the diminished RNA contents and suppressed protein expression within the organism.

Recently, Sharma and Sisodia (2009) observed a significant decrease in DNA and RNA contents in the testis of Swiss albino mice on exposure to 5 Gy of gamma-radiation. They correlated this decrease in DNA and RNA contents with the acute germ cell death resulting from the whole body exposure to 5 Gy gamma irradiations. The prolonged interphase or delayed onset of DNA

synthesis after irradiation further contributes to this decreased DNA content which in turn account for the suppressed RNA transcription within the testis²⁸.

Mechanism of radioprotection by Oltipraz-

Molecular and biochemical studies suggest that *Oltipraz*, a synthetic dithiolethione affords cellular protection by inducing the expression of a battery of Phase II detoxification enzymes. *Oltipraz* produced significant elevations in the detoxification potential of the host²⁹. This finding provided the first evidence that *Oltipraz* may be effective in increasing cellular protection. The protective effect of OLT has been attributable to its ability to induce Phase 2 detoxifying enzymes such as microsomal epoxide hydrolase (mEH), and glutathione-S-transferase (GST) as well as the inhibition of certain cytochrome P-450s (eg. P-4501A2 and3A4)^{19,30-31}.

The chemopreventive agent, *Oltipraz* also stimulates the repair of damaged DNA caused by the free radicals generated by irradiation. *Oltipraz* increases the nucleotide excision repair (NER) which represents the major pathway of elimination of chemical carcinogen DNA adducts. In vitro studies carried out by O'Dwyer et al. (1997) showed that pretreatment with OLT resulted in either decreased accumulation or enhanced removal of platinum DNA adducts in colon carcinoma HT29 cells³² and the mechanism is based on the fact that OLT may act by directly quenching the ability of radicals to bind DNA as well as by inducing enzyme-mediated inactivation but the actual way by which OLT may influence the rate of repair is still unclear. One possibility is that OLT treatment results in the up-regulation of NER genes as seen in case of detoxication genes³³. In addition, *Oltipraz* have the potential to alter intracellular redox characteristics via transcription factor activation, which further aids in providing immediate protection against DNA adduct formation.

Another possibility that helps in repairing damaged DNA involves the ability of *Oltipraz* to elevate GSTs and GSH levels within the host tissues. This view is based on the observations of Kensler et al. (1985) that the ability of *Oltipraz* to inhibit DNA damage via GST induction is suggested by the inverse correlation between the amount of toxicant bound to DNA following treatment with carcinogen plus *Oltipraz* and the level of GST activity within the liver ($r = 0.95$)³⁴.

Furthermore, induction of MnSOD by *Oltipraz* which contributes to its chemopreventive properties also helps in preventing the formation of DNA adducts and thereby, reducing the DNA damage³⁵ (Helzlsouer and Kensler, 1993). It is based on the fact that as MnSOD levels increases, reactive oxygen species are decreased, therefore reducing the likelihood of DNA mutations or DNA adducts.

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