

Radioadaptation Response of Parotid Salivary Glands Acinar Cells Induced the Low Dose of X-Ray Radiation from Skull Radiography and Then Challenged The Therapy Dose of Gamma-Ray Radiation: The Measurements of Hsp70 Expression, SOD2 Activation, and MDA Concentration

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Abstract

The aim of this study was to measure the Hsp70 expression, SOD2 activity and MDA concentration on radioadaptation response of parotid saliva glands acinar cells induced the low-dose of X-ray radiation from Skull Radiography.

This research involved 24 males *Rattus Norvegicus*, wistar strains divided into 4 groups: normal control, positive control, adaptation of A (single of X-ray exposure from Skull Radiography), and an adaptation of B (chronic of X-ray exposure from Skull Radiography). The Challenge radiation exposure was performed 5 hours after adaptive radiation. The radiation was directed at the dorsal of animal's head. The animal research to be sacrificed 24 hours after challenge radiation exposure, and soon the parotid tissue was taken. Furthermore, tissue processing was performed for histopathological specimens. Hsp70 expression, SOD2 activity, and MDA concentration were measured using immunohistochemical techniques. The data were analyzed by one way ANOVA test ($\alpha = 0,05$) using SPSS software version 21.

The results showed that there were significant differences for Hsp70 expression, SOD2 activity and MDA concentration of parotid salivary glands acinar cells among 4 groups. The low-dose of X-ray radiation from Skull Radiography may induce radioadaptation response in acinar cells of parotid salivary glands.

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Introduction

The radioadaptation response was a biological response from living cells or organisms exposed the low dose radiation (LDR) as adapting dose, and cells will have greater resistance to radiation or other exposure (challenge dose) received sometime later. This response was often also called radioprotective responses, was one form of defense of cells or organisms against exposure to radiation with high dose (HDR).

Radiation from radiotherapy was one form

of HDR exposure that often gives adverse side effects on cells or tissues around the targeted radiotherapy. In the radiotherapy of head and neck cancer, hyposalivation was a common side effect, and its incidence was almost 100%.^{1,2} Meanwhile, the neck and head cancer incidence was ranked 5th, or about 2.8% of all common cancer incidence worldwide.³ Hyposalivation can lead to various disorders in the oral health, such as mouth discomfort, pain, dental caries and other mouth infections, as well as speech and swallowing.⁴ Post radiotherapy hyposalivation occurs mainly because radiation exposure causes damage to acinar cells to produce salivary fluid, especially acinar cells of the parotid salivary gland. The parotid glands are the largest salivary glands and along with the other major salivary glands account for about 90% saliva.⁵

LDR was a radiation having doses below 0.2 Gy or 200 mGy.⁶ LDR that can initiate a

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radioadaptation response was a radiation dose below 200 mGy.⁷ Medical radiography was one source of LDR exposure. Demand for medical radiography tends to increase as the level of education and welfare increases. in the community. This has an impact on the increasing trend of radiation exposure in the community or patients. Skull radiography projection was one of the most commonly performed radiographic examinations in medicine and dentistry in addition to periapical radiography and panoramic radiography. The research of Hiswara et al. found that sources to surface distance (ESD) from several radiology laboratories in Indonesia for Skull radiography was between 0.16-1.74 mGy.⁸

In this study, radioadaptation response was assessed by indicators: Heat Shock Protein (Hsp70) expression, Superoxide dismutase (SOD2) enzyme activity, and Malondialdehyde (MDA) concentrations. Hsp was a cellular or molecular defense response that God Almighty devoted to living organisms to against unfavorable injuries such as radiation exposure.⁹ The Mild or slight preconditioning stress may increase cell tolerance for subsequent dangerous stress with increasing Hsp synthesis. In the oxidative stress condition, Hsps can inhibit several pathways.¹⁰

Radiation exposure will increase the ROS formation in cells.¹¹ SOD scavenger enzymes were the major enzymes that play a role in ROS detoxification to protect cells from potential damage caused by excessive ROS formation.¹² Increased ROS in cells due to radiation exposure including LDR will stimulate increased activation of SOD especially SOD2 (manganese SOD/MnSOD). The increasing of this antioxidant enzyme activity may be to increase cell resistance when there was greater subsequent radiation.

Ionizing radiation able to cause lipid damage by increasing the lipid peroxidation several hours after radiation exposure.¹³ MDA was one of the lipid peroxidation product that initiated by ROS. In the lipid peroxidation process, increased MDA concentrations were associated with increased oxidative stress. Lipid peroxidation will be finished if there has been a balance between free radicals and antioxidant systems.¹⁴ Thus, MDA can be an indicator of the oxidative stress and can directly show free radical activity. The aim of this study was to measure the

expression of Hsp70, SOD2 activity and MDA concentration of parotid salivary gland acinar cells after the therapy dose (2 Gy) of Cobalt-60 gamma-ray radiation that previously induced by low-dose of X-ray radiation from Skull Radiography.

Materials and methods

This research was an experimental laboratory with post-test only control group design. This research has obtained the "certificate of ethics" from the research and development department of dr.Soetomo Hospital Surabaya, East Java, Indonesia with certificate number: 28/Panke.KKE/I/2017, January 20th 2017.

This experimental research using 24 of Male Rattus Norvegicus Wistars strain were divided into 4 groups: normal control (group 1), group 2 (only challenge exposure ie gamma rays radiation with a dose of 2 Gy) as positive control, group 3 (single adaptive radiation + challenge radiation), group 4 (repeat adaptive radiation (3 time with interval of 48 hours) + challenge radiation). The animal research was obtained from the Animal Reseach Units, Departement of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, East Java, Indonesia.

Before it's, the animal research was immobilized in a plastic tapered bottle without anesthesia and then fixed on a board. The adaptive radiation was performed by X-ray from skull radiography using the General X-Ray Unit (Toshiba-E 7239) at 80 kV and 15 mA, 0.16 s, and sources to surface distance (SSD) 100 cm; while the exposure of challenge radiation (2 Gy) was conducted using a Cobalt-60 teletherapy unit (XK-100 Phillip) at Radiotherapy Installation of dr.Soetomo Hospital Surabaya, East Java, Indonesia. The radiation was directed from the dorsal part of the animal's head. The Normal control group also was immobilized with similar technique and length of time.

At 24 hours after exposure to the challenge radiation, a parotid salivary gland tissue was collected immediately, and stored in a fixation solution (formalin buffer 10%) immediately too. Furthermore, the tissue was processed for the histopathology specimens using Paraffin method.

Hsp70 expression, SOD2 activity, and MDA concentration were measured using

immunohistochemical techniques (IHC). The histopathology specimens were introduced into Xylol twice each 2 minutes, then included in serial alcohol concentration ie: absolute alcohol (100%), 95%, 80% and 70% each 1 minutes, then rinsed with running water (10-15 minutes), then put into in 3% H₂O₂ solution for 30 minutes, then washed with Phosphate Buffered Saline (PBS) solution three times each 2 minutes. Then incorporated enzyme-labeled monoclonal antibodies (anti-mouse anti-Hsp70 antibody, anti-mouse anti-SOD2 antibody and anti-mouse anti-MDA antibody), Then washed with PBS solution three times each 2 minutes, washed into chromogen subtract for 5 minutes, washed with PBS three times each 2 minutes, Washed with aquadestilata, put in Mayer's Haematoxylin for six minutes, wash with running water until clean, then do dehydration, clearing and mounting.

The observation and determination were performed under the microscope with 1000x magnification at 20 fields of view then taken the average. The positive reactions of Hsp70 expression, SOD2 activity, and MDA concentration were indicated with brownish color.

The data were analyzed using One-Way ANOVA test to compare between all experimental groups. All statistical analyzes were performed with SPSS software 21 version, and for all analyzing a *P*-value <0.05 was considered statistically significant.

Results

In diagrams 1 (Figure 1) showed that groups with three times of repeat adaptive radiation provide the highest Hsp70 expression, followed by group 3, then group 2 and lowest in group 1 (normal control). The result of statistical test showed that the data of Hsp70 expression expression was not normal (*P*<0.05) and not homogeneous (*P*<0.05), so the comparative test was done by non-parametric Kruskal-Wallis test. The result of Kruskal-Wallis test was obtained *P*=0.000, meaning there was a significant difference between the 4 groups.

In diagram 2 (Figure 2): In the Group 4 showed the highest SOD2 activity followed the group 3, then group 1 and lowest in group 2. The result of the statistical test showed a significant difference among the four groups (*P*=0.000). All group pairs showed statistically significant differences (*P*<0.05), except between group 3

and group 4 (*P*=0.102) and between groups 1 and group 2 (*P*=0.691).

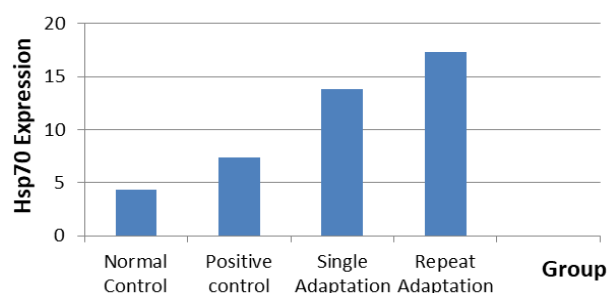


Figure 1. The Number of Hsp70 Expression on Parotid Salivary Glands Acinar Cells of Rattus Norvegicus Adapted Low-Dose Radiation from Skull Radiography, 5 Hours Later Exposed To 2 Gy Dose Challenge Radiation.

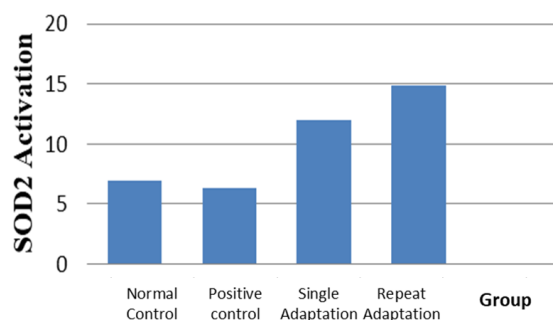


Figure 2. The Number of SOD2 Activation on Parotid Salivary Glands Acinar Cells of Rattus Norvegicus Adapted Low-Dose Radiation from Skull Radiography, 5 Hours Later Exposed To 2 Gy Dose Challenge Radiation.

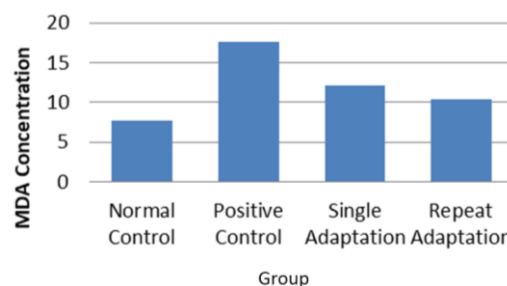


Figure 3. The Number of MDA Concentration on Parotid Salivary Glands Acinar Cells of Rattus Norvegicus Adapted Low-Dose Radiation from Skull Radiography, 5 Hours Later Exposed To 2 Gy Dose Challenge Radiation.

In diagram 3 (Figure 3): The highest MDA level was shown in group 2 and the lowest level was found in group 1 (normal control). The result of the statistical test showed a significant difference between the four groups studied (*P*=0.000). All group pairs showed statistically

significant differences ($P < 0.05$), except between groups and 3 and group 4 ($P = 0.070$).

Discussion

The results of this research once again provide evidence that LDR exposure can provide a beneficial stimulating effect. Cellular response to LDR has not always been consistent with LNT hypothesis as occurs in HDR exposure. Thus, the biological effects of HDR exposure cannot be equated with the effects of LDR exposure. In HDR exposure, no one doubts that the exposure has a damaging effect on molecules, cells or organisms in accordance with LNT hypothesis.¹⁵ In many phenomena and in many studies, LDR exposure is proven to induce a response, which is the increase of resistance to greater stress that is destructive or toxic, either the same stress or different stress. Previous research has been done on the effects of LDR on radio adaptation response from various cells or organisms both in-vitro and in-vivo. However, radiation exposure will have different effects on any cell type, that depends on many factors such as cell type, radio sensitivity, radiation dose and radiation dose rate.¹⁶ In this study the radio adaptation response was investigated on the acinar cells of parotid salivary glands. The consideration is that the parotid gland was the highest tissue in absorbing radiation compared to other tissues around it, such as a skin, bone marrow, thyroid gland.^{17,18} The results of this study become important when associated with radiation exposure as in radiotherapy head and neck areas that always cause complications, especially in the oral cavity such as hyposalivation.

In this study, the radio adaptation response was assessed by Hsp70 expression indicator, SOD2 activity, and MDA concentration. Hsp70 was a cytoprotective protein expressed as an adaptation or defense mechanism of various organisms; from bacteria to mammals to help to survive and can adapt to various stresses from the surrounding environment.^{10,19}

Radiation was one source of external stress that has a destructive biological effect through oxidative stress conditions due to the formation of free radicals. Hsp70 was a protein that can inhibit oxidative stress in several levels. Hsp70 acts as a chaperone protein, assist a folding of the protein, prevents protein aggregation, Hsp70 can also improve functionalization of

proteins or other enzymes to work optimally. Hsp70 can also inhibit proteins or other molecules that lead to cell damage. The results of this research, the group given chronic adaptive radiation exposure obtained the highest expression of Hsp70, and the lowest was obtained in the control group. This proves that LDR as adaptive radiation exposure before HDR exposure can increase Hsp70 expression. LDR is the signal for an induced transcription factor of heat shock factor (HSF-1). In the nucleus, HSF-1 will bind the heat shock element (HSE) resulting in phosphorylation. The binding activity of HSF-1 with DNA will enhance the transcription, synthesis, and functionalization of Hsp70, so that when subsequent radiation exposures, the cells have been adapted to enhance the transcription and synthesis of Hsp70.

SOD enzyme especially SOD2 or Mn-SOD was one of cytoprotector enzymes that play a role to protect the cell in oxidative stress condition. The SOD enzyme catalyzes superoxide radicals (form after exposure to ionizing radiation) into hydrogen peroxide and oxygen. Our results found that low dose X-ray radiation from skull radiography as adaptive radiation was able to induce the radio adaptation response of parotid salivary glands acinar cells that given 5 hours before the challenge exposure of HDR. There was an increase of SOD2 activity in the group receiving adaptive radiation exposure compared to the control group and the group without adaptive radiation exposure. From the comparative statistical analysis show a significant difference. Miura (2004) in his article review suggests that endogenous antioxidants will increase as a result of low-dose radiation exposure. Feinendeigen in his article suggests that antioxidant defenses such as SOD play a role in the mechanism of adaptive response.⁷ Jin et al. Research found that SOD2 activity increased in cells (Human skin keratinocytes) given LDR exposure (10 cGy) and then exposed to radiation 5 Gy (with an increase of approximately 36%) compared to groups exposed only to radiation 5 Gy. Furthermore, they say that MnSOD was a major antioxidant in mitochondria mammalian cell that has been known to be involved and mediated in the radioadaptation response.²⁰

Increased ROS formation due to adaptive radiation exposure will induce an increase in cellular antioxidant activity such as SOD2. In

addition, Hsp70 which was also induced transcription and synthesis will help improve functionalization of proteins or other enzymes including SOD2. Thus, when there was subsequent radiation exposure, the induction of this enzyme was already adapted, thereby reducing the damage of the more severe cells. This was evidenced in the group given adaptive radiation exposure, its MDA concentration after exposure to the challenge radiation, there was found to be lower than the group without adaptive radiation. This indicates the role of the SOD2 enzyme (and possibly other antioxidant enzymes, such as catalase (not measured in this study) to inhibit oxidative damage. As is well known, MDA is one of the end products of the lipid peroxidation process due to increased ROS formation. MDA was a molecule that often measured to show free radical activity indirectly.

The presence of SOD2 was important because DNA damage and other molecules in cells due to radiation are mostly (70%) caused by free radicals or through indirect effects. SOD2 was located only within the mitochondria (SOD1 in the cytoplasm and SOD3 in extracellular space), this indicates that the mitochondria are the key subcellular organelles involved in the adaptation response.^{16,21} SOD2 is located only within the mitochondria (SOD1 is present in the cytoplasm and SOD3 is present in extracellular space). This indicates that mitochondria were subcellular organelles that have an important role in the adaptation response.²²

The adaptation used in this study was X-ray from the Skull Radiography projection. This projection was one of the extraoral radiographic examinations that many do in the practice of medicine and dentistry. Generally, radiation exposure derived from diagnostic radiography may be categorized in LDR. The results of this study were consistent with previous studies using radiation from diagnostic radiography as an adaptation. Redpath et al. in their in-vitro study found that LDR exposure from diagnostic X rays may protect against neoplastic transformation.²³ Phan et al. conducted a study using an adaptation of CT-Scan 1x/week for 10 weeks, they received fewer DNA damage (DSBs) than the group that was not given adaptation after both groups were given HDR.²⁴ Pramojanee et al. in their study found a decrease in ROS production at 4 h after LDR exposure from dental radiography of 1.5 my dosage.²⁵

Conclusions

Low-dose X-ray radiation from skull radiography can induce radio adaptation response of parotid salivary glands acinar cells through indicators: Increased Hsp70 expression, increased MnSOD activity and decreased MDA concentration. The LDR exposure given repeatedly provides a better radioadaptation response compared to that given only single exposure.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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