The effects of X-rays radiation on active and passive transport of erythrocytes membrane

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Abstract

X-rays play an important role in diagnosis. But it also rendered the biological effects that damage the bilayer lipid structure and cell membrane proteins that affect the structure and permeability of the membrane, which in turn, will affect the micro-nutrient transfer through active and passive transport processes. The purpose of this study was to determine the effect of X-rays on active and passive transport of the erythrocytes membrane under in vitro method.

After obtaining approval from the Ethics Committee, erythrocyte samples were each divided into two groups, named irradiated and non-irradiated. Passive transport experiments used variation of glucose under the concentration of 20 mM, 30 mM, 40 mM, 50 mM, and 100 mM, while active transport experiments used variation of calcium under the concentration of 50 mM, 100 mM, 150 mM, and 200 mM.

The results showed that there were significant differences ($p < 0.05$) in the transport rate between the irradiated group and the non-irradiated group. The transport rate of erythrocytes in irradiated group was lower than the non-irradiated group. The effects of X-rays radiation can disrupt cell membrane function by inhibiting the active and passive transport rate on erythrocyte membrane.

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Introduction

Certain types of radiation that can be utilized in the field of dentistry are GaAs (Gallium-Arsenide) laser radiation that can be applied to pulp-capping procedures and ionizing radiation.[1] Ionizing radiation in medical field having the benefits of supporting diagnostic and radiotherapy. Aside from its benefits, ionizing radiation can also be harmful towards human body cells either directly or indirectly interacted by forming free radicals.[2]

Besides lipid bilayer, cell membrane components also comprised of membrane proteins and sugars.[3] Membrane proteins is an essential component for cell membrane, especially to perform the function of active and passive transport inside and outside the cell, signaling, and energy establishment.[3,4] Active and passive transport is very important for the survival of the cell. It is important to determine which substance is right to entering to (nutrition) and coming out from (toxins) the cell.[3]

Ionizing radiation able to cause lipid damage by increasing the lipid peroxidation and emerging lipid fragmentation, several hours after radiation exposure. The damage of the cell membrane can resulting to the cell barrier function loss that threaten the integrity of the cell itself.[5] Changes that happen in the cell structure causes disruption of cellular function.[6] The X-rays radiation able to changes the structure of erythrocytes cell membrane with the main target is the sulfhydryl bond on the membrane and will later cause disruption on the osmotic of sodium ions inside and outside of the cell.[7] Research done by Xu et al., [6] regarding the effect of gamma rays with doses variation of 10-55 Gy on the erythrocytes stated that the gamma ray radiation causes damage to erythrocytes membrane, characterized by an increased concentration of K^+ ions on the plasma that indicates membrane leakage.

X-rays radiation effect on active and

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passive transport of erythrocyte membrane still not widely studied. The purpose of this study was to analyze the X-rays radiation effects on the active and passive transport of erythrocyte membrane, by using calcium ions in active transport experiment and glucose in passive transport experiment.

Materials and methods

This study has received approval from the Ethics Committee of Hasan Sadikin Hospital Bandung. The research sample obtained from a healthy adult blood donor taken randomly in Indonesian Red Cross Bandung Branch. Heparin contained blood inserted into venoject tube 10 cc and divided into two groups, non-irradiated and 2 x 200 rad irradiated group. Samples washed with physiological saline then examined the Hb level to control the membrane integrity, then the erythrocytes dry weight determined by putting on the foil and dried in an oven at 120°C for 12 hours.

Glucose and calcium transport experiments conducted in accordance to Lineweaver-Burk plots and developed by Shahib.[8] As much as 450 µL of glucose and 450 µL of $CaCl_2$ incorporated into a separate tube, then heated at a temperature of 37°C, and after that 50 µL of erythrocytes inserted using a micropipette. Passive and active transport process was then stopped 15 seconds later by adding $HgCl_2$ solution, then centrifuged for 15 minutes on the speed of 5000 rpm. The supernatant was discarded by inverting the tube.

Hemolysis was done by adding 2 ml of aquadest towards the erythrocytes precipitate, mixed above the vortexer until homogeneous and allowed to stand for 10 minutes at room temperature. Then mixed with 1.5 ml Ba $(OH)_2$ and 1.5 ml of $Zn(SO4)_2$ using a vortexer until homogeneous. Once homogenous, centrifuged for 15 minutes on the speed of 5000 rpm. The filtrate was taken to determine the intra-cell glucose and calcium level. Doses variation used were 20 mM, 30 mM, 40 mM, 50 mM, and 100 mM for glucose; 50 mM, 100 mM, 150 mM, and 200 mM for calcium.

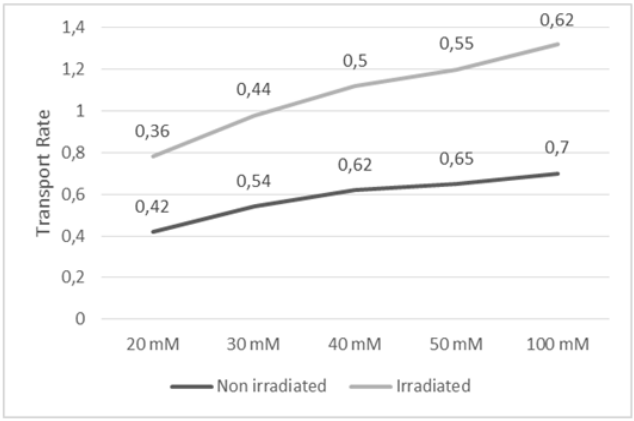
Results

The obtained data then tested with Shapiro-Wilk normality test first, and if normally distributed, we then tested with two-way ANOVA. Two-way ANOVA used to determine the effects of radiation exposure on the transport rate at various levels of glucose and calcium. Parametric test was conducted using statistical analysis program with significance level of 5%.

Table 1 showed a positive correlation ($p < 0.05$) between the increase in the glucose levels and cell transport rate, although the curve began to plateau at doses of 30, 40, and 50 mM as shown in Graph 1. This showed enzymatic resembled reaction. There was a relation between the increase of glucose levels in the incubation solution and the increase of cell transport rate, which was the rate of glucose transport in a solution of 20 mM was 0.42 mg / g dry erythrocytes/ 15 seconds and at solution of 100 mM, the transport rate was becoming 0.7 mg / g dry erythrocytes / 15 seconds.

Radiation	20 mM	30 mM	40 mM	50 mM	100 mM	Sig.
Not irradiated	0,42	0,54	0,62	0,65	0,7	0,001*
Irradiate 2 x 200 rad	0,36	0,44	0,5	0,55	0,62	
Inhibition (%)	14	19	19	15	11	
Sig.					0,000*	

Table 1. Transport rate means and inhibition percentage between non irradiated group and 2 x 200 rad irradiated group on glucose level variation. *found significant differences ($p < 0,05$).



Graph 1. The curve of glucose transport rate in irradiated erythrocytes (2 x 200 rad) towards non irradiated erythrocytes.

Transport test also conducted on 2 x 200 rad irradiated erythrocytes group. Obstacles of transport rate found on the incubation dose of 20 mM, as much as 14%, on the incubation dose of 30 mM and 40 mM increased into 19%, on the incubation dose of 50 mM averagely reduced into 15%, and on the incubation dose of 100 mM

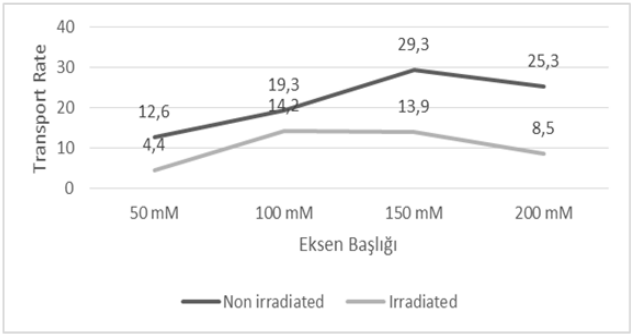
reduced more into 11%. This reflected the enzymatic reaction nature with the inhibitor.

Radiation had an influence on the glucose transport rate in erythrocyte membrane. Table 1 showed a significant difference ($p < 0.05$) in the glucose transport rate in erythrocyte membrane between non irradiated erythrocytes group and 2 x 200 rad irradiated erythrocytes group.

Based on the research on extracellular calcium incubation dose variation showed differences in intracellular Ca^{2+} ion concentration between non irradiated group and 2 x 200 rad irradiated group ($p < 0.05$). The transport rate of Ca^{2+} in irradiated group was slower than the non-irradiated group (Graph 2). This showed that there was inhibition on transport into cells seen from extracellular incubation dose increase. Statistically, there was no significant differences ($p > 0.05$) between Ca^{2+} transport rate and incubation dose variation (Table 2).

Radiation	50 mM	100 mM	150 mM	200 mM	Sig.
Non irradiated	12,6	19,3	29,3	25,3	0,002*
Irradiate 2 x 200 rad	4,4	14,2	13,9	8,5	
Inhibition (%)	53,5	35,5	26,5	56	
Sig.				0,218	

Table 2. Transport rate means and inhibition percentage between non irradiated group and 2 x 200 rad irradiated group on calcium level variation. *found significant differences ($p < 0,05$).



Graph 2. The curve of calcium transport rate in irradiated erythrocytes (2 x 200 rad) towards non irradiated erythrocytes.

Discussion

Erythrocyte membranes often used for study because asides from its important role in physiological and metabolic functions of the body, erythrocytes also a radiosensitive cells without nucleus.[6,9] The experiment was conducted in vitro so it won't affected by stem cells. Erythrocytes don't have cell organelles so the energy source only derived from glucose that converted into ATP in glycolysis process. ATP is needed to maintain ions balance that across cell membrane and maintain the membrane proteins from oxidative damage. Examination of the effects of radiation on active and passive transport on the erythrocyte cell membrane is ideal.

Indirect effects of ionizing radiation can cause changes in cell membrane structure and components especially through ROS / RNS (Reactive Oxygen Species / Reactive Nitrogen Species). Not only cause changes in intracellular Ca^{2+} levels, but also increases lipid peroxidation, and the formation of ceramide-enriched platform and sphingolipid ceramide. These changes induce cell function change characterized into apoptosis.[5] Indrani states that the higher dose of gamma rays can prevent bacterial penetration into the collagen membrane on agar medium.[10]

Glucose transport in the membrane, included in uniport type, assisted by glucose transporter protein (GLUT1) which ensures the amount of glucose received in accordance with necessity.[11] Glucose is the energy source that is closely related to erythrocytes function, so the glucose transport is important for the erythrocytes survival.

There was inhibition on the glucose transport in the irradiated erythrocytes group (Table 1). Under dose of 2 x 200 rad, the X-rays radiation affected the glucose transport rate ($p < 0.05$). Graph 1 showed glucose transport rate of irradiated erythrocytes was always lower than non-irradiated erythrocytes. This indicated membrane damage due to X-rays radiation. Viskupicova et al.[9] stated that besides radiation, high levels of glucose could also initiated the formation of free radicals, along with the increase in lipid peroxidation that will culminated in cell membrane damage.

Study conducted by Xu et al.[6] showed that gamma rays ionizing radiation able to affected permeability, protein structure, and morphology of the membrane. This study used X-rays, as known that X-rays radiation also affected the Ca^{2+} ion transport rate in erythrocyte membrane. This was indicated by a significant difference ($p < 0.05$) in the Ca^{2+} transport rate between the irradiated group and non-irradiated group (Table 2).

Ca^{2+} ion is an intracellular messenger that has a variety of benefits to regulate cell function,

such as muscle cell contraction, neurotransmitters release, and cell motility.[3,12] X-rays radiation retarded the Ca^{2+} transport rate (Graph 2).

The Ca^{2+} transport rate was slower in the irradiated group (Graph 2). The inhibition of Ca^{2+} transport rate may disturbed the concentration equilibrium of Ca^{2+} between inner and outer cell. The concentration of Ca^{2+} inside the cell must be kept as low as possible with the active transport to the cell membrane, because accumulation of Ca^{2+} inside the cell indicated pathological signs, such as sickle cell disease and thalassemia.[13,14]

In a study conducted by Karadede et al., it was found that there were no statistical significant differences in biochemical parameters (glucose, creatinine, Na +, K-, Ca2 +, albumin) in rabbits, 90 days after exposure to radiofrequency radiation of 900 MHz. Radiation is equivalent to mobile phone radiation.[15]

Generally, the hydroxyl ions produced by ionizing radiation initiates damage of membrane proteins, particularly amino acids that's vulnerable most towards radiation, and also increases the membrane permeability due to lipid bilayer damage.[16] Based on the results, X-rays radiation inhibited active and passive transport of erythrocyte membranes. The pattern of inhibition could be seen from two aspects, passive transport into cell and active transport out of cell.

Conclusions

Both glucose and calcium transport rates in irradiated erythrocytes are always below the non-irradiated erythrocyte curve, in other words radiation doses of 2x200 rad can alter the affinity of glucose and calcium. The erythrocyte membrane is an important cell barrier and has a regular transport mechanism. The erythrocyte membrane may be affected by free radicals generated by ionizing X-ray radiation. Ionizing radiation can interfere with both passive and passive transport processes simultaneously on erythrocyte membranes.

Declaration of Interest

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References

1. Mustafa EA, Kawas S Al. The effect of gallium-arsenide laser irradiation on odontogenesis. J Int Dent Med Res. 2010;3(2):52-56.
2. Desouky O, Ding N, Zhou G. ScienceDirect Targeted and non-targeted effects of ionizing radiation. J Radiat Res Appl Sci. 2015;8(2):247-254.
3. Watson H. Biological membranes. Essays Biochem. 2015;59(0):43-69.
4. Gushchin IY. Structural studies of microbial rhodopsins and other membrane proteins by means of X-ray crystallography and computer modeling. 2014. https://tel.archives-ouvertes.fr/tel-01343859.
5. Corre I, Niaudet C, Paris F. Plasma membrane signaling induced by ionizing radiation. Mutat Res Mol Mech Mutagen. 2010;704:61-67.
6. Xu D, Peng M, Zhang Z, Dong G, Zhang Y, Yu H. Study of damage to red blood cells exposed to different doses of γ -ray irradiation. Blood Transfus. 2012;10:321-330.
7. IAEA. Effects of Ionizing Radiation on Blood and Blood Components: A Survey. Vienna; 1997. http://www-pub.iaea.org/MTCD/Publications/PDF/te_934_prn.pdf.
8. Shahib M. Kinetika dan Mekanisme Transport Gula Melalui Membran Eritrosit Terutama dengan Menggunakan Pengaruh Asam Bongkrek sebagai inhibitor Enzim Sulfhidril. 1985.
9. Viskupicova J, Blaskovic D, Galiniak S, Bartosz G, Horakova L, Sadowska-bartosz I. Redox Biology Effect of high glucose concentrations on human erythrocytes in vitro. Redox Biol. 2015;5:381-387.
10. Indrani DJ, Lukitowati F, Darwis D. Effect of Gamma-ray Irradiation on Bacterial Penetration Power of Chitosan/Collagen Blend Membranes for Wound Dressing. J Int Dent Med Res. 2016;9(3):202-206.
11. Hajjawi OS. Glucose transport in human red blood cells. Am J Biomed Life Sci. 2013;1(3):44-52.
12. Bootman MD, Rietdorf K, Hardy H, et al. Calcium Signalling and Regulation of Cell Function. In: eLS. Chichester, UK: John Wiley & Sons, Ltd; 2012.
13. Clapham DE. Calcium Signaling. Cell. 2007;131(6):1047-1058.
14. Bogdanova A, Makhro A, Wang J, Lipp P, Kaestner L. Calcium in Red Blood Cells—A Perilous Balance. Int J Mol Sci. 2013;14(5):9848-9872.
15. Karadede B, Akdag MZ, Kanay Z, Bozbiyik A. The effect of 900 mhz radiofrequency (RF) radiation on some hormonal and biochemical parameters in rabbits. J Int Dent Med Res Med Res. 2009;2(3):110-115.
16. Menon SS, Uppal M, Randhawa S, et al. Radiation metabolomics: current status and future directions. Front Oncol. 2016;6:1-10.