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X-Ray Scanning Reduce Soluble Bioactive Molecules of Mesenchymal Stem Cells

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ABSTRACT

Background : Mesenchymal Stem Cells secrete various anti-inflammatory and regenerative SAMs-MSCs that possess immunomodulatory properties and may accelerate wound healing. As potential agent for therapeutic, SAMs-MSCs should be stable in any condition, including under X-Ray Scanning. Previous study reveal that X-Ray Scanning may induce protein damage. However, the investigation regarding the stability of SAMs-MSCs under X-Ray Scanning is limited
Objective : This study aimed to investigate the stability of SAMs-MSCs after X-Ray Scanning
Methods : Mesenchymal Stem Cell medium was filtrated using TFF method at 300 and 5 kDa filter cut off and sterilized using 0,1 um syringe. The SAMs-MSCs was underwent X-ray Scanning using public Air Port X-Ray twice. The SAMs-MSCs concentration was measured using ELISA. T-test analysis was performed for the statistical analysis with P<0,05.
Results: X-ray scanning reduce the concentration of SAMs-MSC. Previous study found that x-ray irradiation may damage protein at 6–18 keV caused by the energy deposited by photoelectrons that are generated by the interaction of X-ray photons and the protein leading photoelectron-induced damage.
Conclusion: SAMs-MSCs should not be scanned using x-ray since it was damaged through scanning. Radioprotective agent should be investigated to reduce the effect of x-ray irradiation

Keyword : MSC, Soluble molecule, TFF, X-ray radiation

INTRODUCTION

Mesenchymal Stem Cells (MSCs) are plastic-adherent cells that exhibit a fibroblast-like phenotype that were able to secrete various bioactive protein including anti-inflammatory and regenerative soluble active molecules (SAMs-MSCs) including Interleukin 10 (IL-10), Interleukin 6 (IL-6) and Transforming growth factor β (TGF-β).[1–4] Recent studies revealed that SAMs-MSC possess immunomodulatory properties that may alter the function of B cells, natural killer (NK) cells, and T cells.[5,6] Furthermore, SAMs-MSCs may accelerate wound healing by triggering fibroblast activation and promote cell proliferation[1,7]. Thus, SAMs-MSCs have promising applications as drug targets and therapeutic agents. Previous research reveals that tangential flow filtration (TFF) effectively separate and concentrate SAMs-MSCs based on molecular size[8,9].

As potential agent for therapeutic, SAMs-MSCs should be stable in any condition, including under X-Ray Scanning since it should be delivered across the world as soon as possible using air freight service. Previous study reveal that X-Ray Scanning may induce protein damage[10]. However, the

regarding the stability of SAMs-MSCs under X-Ray Scanning is limited, thus this study aimed to investigate the stability of SAMs-MSCs after X-Ray Scanning.

In MSCs case, a previous study has been revealed that oxidized cell-free DNA may serve as a mediator of MSC response to low doses of X-ray radiation through mitochondria-related genes[10]. Low-dose ionizing radiation results in upregulation of genes controlling respiratory chain, fusion/fission, and increasing the number of copies of DNA which plays a role of a stress signaling molecule[11]. A previous study in MSCs suggests that irradiation damaged the DNA may be correlated with the development of senescence in these cells[12]. Senescent cells display a host of aberrant biological characteristics, including an inability to proliferate and migrate, increased pro-inflammatory protein secretion, and genomic instability[13]. Different cells and molecules display variations in tolerance to radiation-induced damage, but the underlying mechanisms of these interpretations are not completely comprehended, and this deviation remains an essential query in radiation biology[14]. Although radiation evidence reveals that X-ray rays can increase damage to MSCs, the mechanism underlying radiation in MSCs secretomes is not known. Therefore, we aimed to determine the effect of X-ray radiation on the content of IL-6, IL-10 and TGF- β in SAMs-MSCs.

MATERIAL AND METHODS

MSCs isolation and characterization

Human primary mesenchymal stem cell at 6 passages were used for the following study. Human MSCs were maintained in complete medium containing low glucose Dulbecco's modified eagle's medium (DMEM) (Sigma, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO, USA), 1% penicillin/streptomycin (Sigma, St. Louis, MO, USA), and 1% glutamine (Sigma, St. Louis, MO, USA). For the sterilization 0,1 um syringe (Sartorius Stedim Biotech, Göttingen, Germany) filter was hired. Tangential Flow Filtration was performed using uPulse device (Formultarix, Salatiga, Indonesia) at 300 and 5 kda filter.

The procedure in this study were reviewed and approved by the Ethics Committee of Ethical Committee of Medical Faculty Sultan Agung Islamic University Semarang in compliance with the Helsinki Declaration. The donors were fully informed regarding the purpose of study, their informed consent was obtained in written form. All protocols were performed in accordance with previous study.[15] Briefly, the umbilical cord blood from a healthy donors were stored in heparin vacutainer for mononuclear cell isolation procedures.

Mononuclear cells from umbilical cord blood were separated using Ficoll-Paque (GE Healthcare, USA) and density-gradient centrifugation at 450 xG for 30 minutes without breaking. Then, the mononuclear cell suspension layered on the top of Ficoll, centrifuged for 20 min at 840×g with low acceleration and no brake at 4 °C. The buffy coat was collected and then washed with phosphate buffer saline (PBS, Gibco, Grand Island, NY, USA) two times by centrifugation at 280×g for 8 min. Mononuclear cells were planted in complete medium contained Dulbecco's Modified Eagles Medium (Gibco, 11885084, NY, USA) with 10% fetal bovine serum (Gibco, 10270106, South American) and 100 IU/mL penicillin-streptomycin.

MSC Characterization and Validation

The MSCs differentiation capacity into osteocyte and adipocyte was performed to characterize and validate the isolated cells. Briefly 4×10^4 cells were planted six well plate using osteogenic differentiation kit medium (Stemcell Technologies, 05465, Germany) and adipogenic differentiation kit medium (Stemcell Technologies, 05412, Germany) for 21 days with 3 days changing medium interval. Alizarin Red (Sigma Aldrich, USA) and Oil Red O staining (Sigma Aldrich, USA) was performed to evaluate calcium depostion and oil droplet formation.

SAMs-MSC isolation

For SAMs-MSC isolation, the medium from HMSC on 90% confluent was collected and centrifuged at 800× g for 30 min to discard cellular debris. For the sterilization 0,1 um syringe (Sartorius Stedim Biotech, Gotingen, Germany) filter was used after centrifugation. The sterile medium was filtered using uPulse TFF Device using 300 kda and the waste was filtered using 5 kda to gain 5-300 kda SAMs-MSCs. The SAMs-MSCs was packed in 1,5 mL cryovial tube for the X-ray Scanning using public Air Port X-Ray (Ahmad Yani International Airport, Semarang, Indonesia) twice.

IL-10, TGF-β and IL-6 Concentration Analysis

The concentration of IL-10, TGF-β and IL-6 β in SAMs-MSCs pre and post X-Ray irradiation was determined with Enzyme-linked Immunoabsorbance Assay (ELISA) based on manufacture protocol at 450 nm wavelength.

Statistical Analysis

Statistical analysis was performed with T-test analysis using SPSS 22 (SPSS Inc.; Chicago; USA) with a significance value of $p<0.05$). The results of the descriptive analysis were expressed in terms of mean ± standard deviation.

RESULTS

MSC characterization and differentiation assays

MSCs isolated from the umbilical cord blood showed monolayers of fibroblast-like cells with adherence capability to the plastic surface (Figure 1A). Furthermore, differentiation capacity validation showe that isolated cells were able to differentiate into osteogenic and adipose cells after Alizarin red and Oil Red O staining (Figure 1B and 1C).

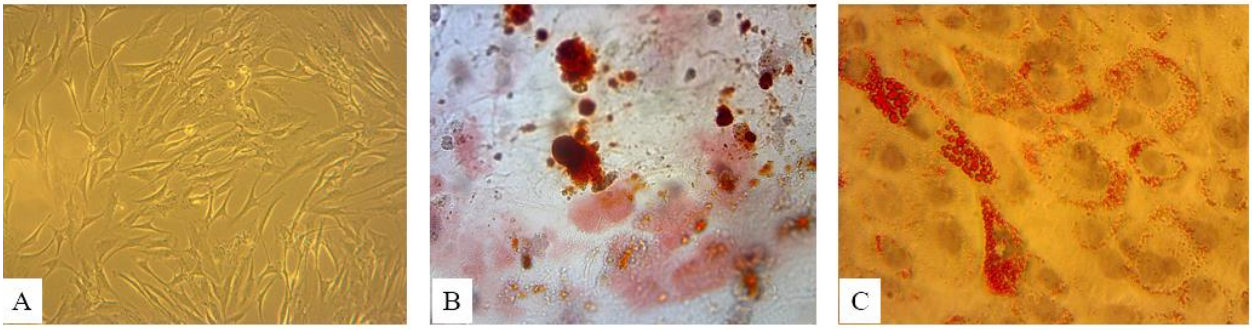

Figure 1. MSCs Validation and Differentiation Assay. Primary isolated cell showed spindle forms such as fibroblast-like cells (100x magnification) (A). The calcium deposition and oil droplet formation confirm the characteristion of MSC to differentiate into osteocyte (B) and adipocyte (C).

X-ray radiation decrease of IL-10, TGF-β and IL-6 Concentration in SAMs-MSCs

For the experiments reported here, we used TFF methods to separate SAMs-MSCs from micro vesicle and then radiated under x-ray to investigate the effect of x-ray Scanning to SAMs-MSCs. This study revealed that the concentration of IL-10, TGF-β and IL-6 post X-ray Scanning significantly reduced ($P<0,05$) (Figure 2).

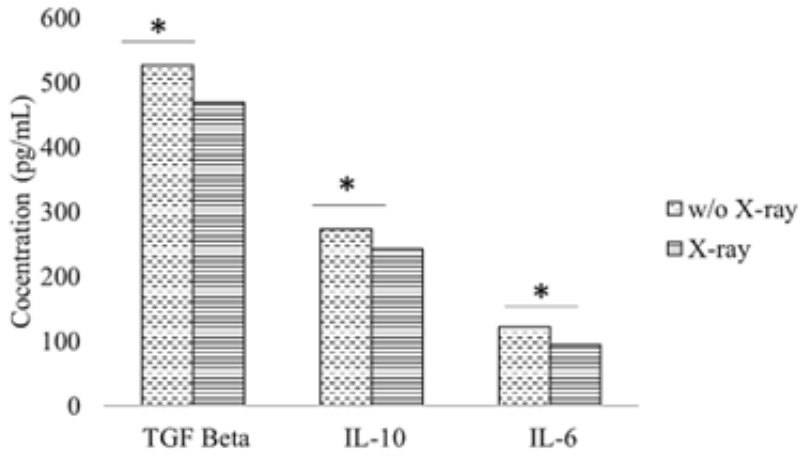

Figure 2. X-Ray Scanning significantly reduce SAMs Concentration including TGF β, IL-10 and IL-6.

DISCUSSION

Soluble active molecules of MSCs have shown to affect the niche upon injury, promoting cell proliferation, and tissue regeneration.[16,17] The proliferative potential is one of particular interest for wound healing and tissue regeneration process. Recent technologies using TFF allowed the separation of SAMs-MSCs from micro vesicle and any large protein leading to better efficacy.[18–20] As future medicine, SAMs-MSCs should be shipped using air freight for fastest route and x-ray scanning should be undergone as standard safety protocols in airport. Previous research reported that x-ray damage protein, however the effect of x-ray scanning to SAMs-MSCs concentration is not clear.

This study revealed that X-ray scanning reduce the concentration of SAMs-MSC including TGF-β, IL-10 and IL-6. Previous study found that x-ray irradiation may damage protein at 6–18 keV caused by the energy deposited by photoelectrons that are generated by the interaction of X-ray photons and the protein.[21–23] Photoelectrons are emitted along with the polarization of the X-ray beam, leading to an expectation of anisotropy in photoelectron emission and trigger photoelectron-induced damage. Released photoelectrons have sufficient energy to ionize surrounding atoms by direct collisions. For carbon, nitrogen, and oxygen this energy is of the order of 300–600 eV, this means that the photoelectron will have energy almost equal to the energy of the incoming X-ray, so it will travel rapidly through the sample, colliding with and withdrawing many electrons from other atoms until it thermalizes[22,23]. Nevertheless, it will also collide with bound electrons and remove them from atoms, directing proportionally fewer collisions than photoelectrons to thermalize[21]. Consequently, the emitted photo provokes photoelectron cascades which rapidly raises the ionization level of atoms in the protein sample.

Other recent studies show that that OH-radical is the greatest active type of ROS liable for most damage to biomolecules overexposure to ionizing radiation[10,12,24,25]. In line with its destructive effect at increased concentrations, H2O2 plays a critical signaling and regulatory role, resulting in the activation of the protective systems of cells and stimulating their survival under oxidative stress, in case induced by ionizing radiation. H2O2 intervenes in diverse responses as a secondary precursor[10,24]. Recent facts on the role of H2O2 in the intra- and intercellular signaling in mammalian cells, including its impact on cellular growth, death, and aging. However, the limitation of this study is that we did not examine ROS or other DNA damage markers that can prove this theory.

CONCLUSION

This study demonstrated that SAMs-MSCs should not be scanned using x-ray since it was damaged through scanning. Radioprotective agent should be investigated to reduce the effect of x-ray irradiation.

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AUTHORS' CONTRIBUTIONS

All authors made influential contributions to the reported manuscript, be it in its conception, study design, implementation, data acquisition, analysis, and interpretation, or in any of these areas; participate in compiling, revising, or critically reviewing articles; give final approval for the version to be published; has approved the journal whose articles have been submitted; and agree to be responsible for all aspects of the work.

COMPETING INTERESTS

The authors declare that there was no conflict of interest.

REFERENCES

1. Putra A, Alif I, Hamra N, et al. MSC-released TGF-β regulate α-SMA expression of myofibroblast during wound healing. *J Stem Cells Regen Med*. 2020;16(2):73. doi:10.46582/JSRM.1602011
2. Nakajima M, Nito C, Sowa K, et al. Mesenchymal Stem Cells Overexpressing Interleukin-10 Promote Neuroprotection in Experimental Acute Ischemic Stroke. *Mol Ther - Methods Clin Dev*. Published online 2017. doi:10.1016/j.omtm.2017.06.005
3. Gu Y, He M, Zhou X, et al. Endogenous IL-6 of mesenchymal stem cell improves behavioral outcome of hypoxic-ischemic brain damage neonatal rats by supressing apoptosis in astrocyte. *Sci Reports 2016 61*. 2016;6(1):1-16. doi:10.1038/srep18587
4. Masyithah Darlan D, Munir D, Karmila Jusuf N, Putra A, Ikhsan R, Alif I. In vitro regulation of IL-6 and TGF-ß by mesenchymal stem cells in systemic lupus erythematosus patients. *Med Glas (Zenica)*. 2020;17(2):408-413. doi:10.17392/1186-20
5. Lotfinia M, Lak S, Ghahhari NM, et al. Hypoxia pre-conditioned embryonic mesenchymal stem cell secretome reduces IL-10 production by peripheral blood mononuclear cells. *Iran Biomed J*. 2017;21(1):24-31. doi:10.18869/acadpub.ibj.21.1.24
6. Nugraha A, Putra A. Tumor necrosis factor-α-activated mesenchymal stem cells accelerate wound healing through vascular endothelial growth factor regulation in rats. *Universa Med*. 2018;37(2):135. doi:10.18051/univmed.2018.v37.135-142
7. Darlan DM, Munir D, Putra A, Jusuf NK. MSCs-released TGFβ1 generate CD4+CD25+Foxp3+ in T-reg cells of human SLE PBMC. *J Formos Med Assoc*. 2021;120(1):602-608. doi:10.1016/J.JFMA.2020.06.028
8. Putra A, Ridwan FB, Putridewi AI, et al. The Role of TNF-α Induced MSCs on Suppressive Inflammation.pdf. 2018;6(10):1779-1783.
9. Putra A, Widyatmoko A, Ibrahim S, et al. Case series of the first three severe COVID-19 patients treated with the secretome of hypoxia-mesenchymal stem cells in Indonesia. *F1000Research 2021 10228*. 2021;10:228. doi:10.12688/f1000research.51191.3
10. Kostyuk S V., Proskurnina E V., Konkova MS, et al. Effect of Low-Dose Ionizing Radiation on the Expression of Mitochondria-Related Genes in Human Mesenchymal Stem Cells. *Int J Mol Sci 2022, Vol 23, Page 261*. 2021;23(1):261. doi:10.3390/IJMS23010261
11. Wölfle U, Esser PR, Simon-Haarhaus B, Martin SF, Lademann J, Schempp CM. UVB-induced DNA damage, generation of reactive oxygen species, and inflammation are effectively attenuated by the flavonoid luteolin in vitro and in vivo. *Free Radic Biol Med*. 2011;50(9):1081-1093. doi:10.1016/j.freeradbiomed.2011.01.027
12. Bylicky MA, Mueller GP, Day RM. Radiation resistance of normal human astrocytes: the role of non-homologous end joining DNA repair activity. *J Radiat Res*. 2019;60(1):37-50. doi:10.1093/JRR/RRY084
13. Taub AF. Cosmeceuticals Using Growth Factors and Stem Cells. 2021;5:47-62. doi:10.1159/000491845

14. Estaphan S, Abdel-Malek R, Rashed L, Mohamed EA. Cimetidine a promising radio-protective agent through modulating Bax/Bcl2 ratio: An in vivo study in male rats. *J Cell Physiol*. 2020;235(11):8495-8506. doi:10.1002/jcp.29692
15. Chen MX, Ouyang GF. Differentiation potential of umbilical cord blood-derived mesenchymal stem cells. *Chinese J Tissue Eng Res*. Published online 2013. doi:10.3969/j.issn.2095-4344.2013.32.020
16. Shin S, Lee J, Kwon Y, et al. Comparative proteomic analysis of the mesenchymal stem cells secretome from adipose, bone marrow, placenta and wharton's jelly. *Int J Mol Sci*. Published online 2021. doi:10.3390/ijms22020845
17. Mizukami A, Thomé CH, Ferreira GA, et al. Proteomic identification and time-course monitoring of secreted proteins during expansion of human mesenchymal stem/stromal in stirred-tank bioreactor. *Front Bioeng Biotechnol*. Published online 2019. doi:10.3389/fbioe.2019.00154
18. Busatto S, Vilanilam G, Ticer T, et al. Tangential flow filtration for highly efficient concentration of extracellular vesicles from large volumes of fluid. *Cells*. Published online 2018. doi:10.3390/cells7120273
19. Dehghani M, Lucas K, Flax J, McGrath J, Gaborski T. Tangential Flow Microfluidics for the Capture and Release of Nanoparticles and Extracellular Vesicles on Conventional and Ultrathin Membranes. *Adv Mater Technol*. Published online 2019. doi:10.1002/admt.201900539
20. Pires IS, Palmer AF. Selective protein purification via tangential flow filtration – Exploiting protein-protein complexes to enable size-based separations. *J Memb Sci*. Published online 2021. doi:10.1016/j.memsci.2020.118712
21. Nass K. Radiation damage in protein crystallography at X-ray free-electron lasers. *Acta Crystallogr Sect D Struct Biol*. Published online 2019. doi:10.1107/S2059798319000317
22. Sanishvili R, Yoder DW, Pothineni SB, et al. Radiation damage in protein crystals is reduced with a micron-sized X-ray beam. *Proc Natl Acad Sci U S A*. Published online 2011. doi:10.1073/pnas.1017701108
23. Close DM, Bernhard WA. Comprehensive model for X-ray-induced damage in protein crystallography. *J Synchrotron Radiat*. Published online 2019. doi:10.1107/S1600577519005083
24. Gudkov S V., Guryev EL, Gapeyev AB, et al. Unmodified hydrated C60 fullerene molecules exhibit antioxidant properties, prevent damage to DNA and proteins induced by reactive oxygen species and protect mice against injuries caused by radiation-induced oxidative stress. *Nanomedicine Nanotechnology, Biol Med*. 2019;15(1):37-46. doi:10.1016/J.NANO.2018.09.001
25. Wang J. X-ray radiation-induced addition of oxygen atoms to protein residues. *Protein Sci*. 2016;25(8):1407-1419. doi:10.1002/PRO.2934