Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 1377 - 1383
Received 20 January 2021; Accepted 08 February 2021.

A Histopathological Review of Conditioned Medium Exosomes from Wharton's Jelly Derived Mesenchymal Stem Cells Administration to Skin Collagen Deposition of Aged Wistar Rats

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ABSTRACT

Background

Prolonged and repeated exposure to UVB rays can cause photoaging, which is the formation of reactive oxygen species (ROS) that damage the DNA of skin cells. DNA damage causes destruction and inhibition of type I collagen as the largest protein in the human skin. Currently, cell-based therapies such as exosomes are known to prevent skin cell DNA damage. Wharton's Jelly conditioned media exosome (Exo-WJ) has the advantage of improving collagen deposition.

Methods

The study was a descriptive experimental study by comparing type I collagen deposition between the ageing skin of Wistar rats which were given the addition of Wharton Jelly (conditioned media exosomeExo-WJ) and control. The control group of rats (A) was given PBS 0.1 cc, the second group of rats (B) was treated with Exo-WJ concentration of 0.75 mcg, and the third group of rats (C) was treated with Exo-WJ with a concentration of 1.5 mcg. Collagen deposition was processed through Trichrome Masson staining and assessed by the pathologist through Image J digital analysis.

Result

The results of the Image J digital analysis revealed that the density of type I collagen in the control group (A) was 23.72%, in Exo-WJ 0.75 mcg group (B) was 48.41%, and in Exo-WJ 1.50 mcg group (C) was 58.5%. .

Conclusion

Giving Exo-WJ can improve collagen deposition in the skin of old Wistar rats due to UVB exposure

Keywords:

collagen, exosomes, UVB irradiation, Wharton's Jelly

Introduction

Aging is a process of decreasing the function and capacity reserve of all organs of a human being, with the skin being a reflection of the outermost organs of the body.[1] Exposure to Ultraviolet B (UVB) light with 280-320 nm wavelength, if prolonged and repeated, can cause DNA damage in the form of cross-links pyrimidine bases and leads to the formation of free radicals, namely reactive oxygen species (ROS); this condition is called photoaging.[2-3]

When photoaging occurs, collagen is damaged by glycosylation, a non-enzymatic reaction that involves the addition of reducing sugars to the collagen extracellular matrix molecules and proteins. Collagen that is repeatedly exposed to UVB irradiation will undergo degradation, inhibition of type I collagen growth, accumulation of collagen fragments and reduced structural structure in the dermis.[1,4]

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The management to prevent photoaging is by blocking damage to the DNA of skin fibroblast cells that produce type I collagen. This supports the study of cell-based therapy because of its potential for independent cell repair and differentiation.[5,6]

Exosomes are one of the intercellular communicators produced by extracellular microbubbles and can communicate with near and far cells.[7]Exosomescontain various molecules, namely: functional mRNA, microRNA, lipids and proteins, which can increase tissue regeneration. MicroRNA is a major contributor to the overall biological function of extracellular vesicles and source cells, which regulates the expression of post-transcription genes involved in stem cell differentiation pathways.[8]

Wharton's Jelly (WJ) is a gel-like tissue in the umbilical cord, rich in proteoglycan protein and contains stromal cells, such as fibroblasts, which act as pluripotent stem cells capable of developing into multiple tissues.[6,9]

The ability of the exosome depends on the original tissue that produced it. Compared to exosomes derived from bone marrow stem cells, stem cell exosomes from WJ have been shown to have better clinical applications because they have low immunogenicity, do not induce tumorigenesis, are pluripotent and non-invasive.[10]

METHODS

This study was a descriptive experimental study by comparing the deposition of type I collagen exposed to the aged skin of Wistar rats and given the addition of Wharton' Jelly conditioned media exosomes (Exo-WJ) to control.

The research was carried out in the Laboratory of Setia Budi University Surakarta and the Laboratory of Dermama Clinic Surakarta. The histopathological examination of animal skin tissue was carried out at the Laboratory of Anatomical Pathology, Faculty of Medicine, SebelasMaret University, Surakarta. The research started from November 2020 to January 2021.

This in vivo study used white male *Rattusnorvegicus*Wistar rats aged 9-10 weeks with a weight of 250-300 grams which were kept in a clean and homogeneous habitat. Male Wistar rats were obtained from the laboratory of animal experiments and had undergone an adaptation process for 7 days. Rats with obvious physical abnormalities, experiencing pain during adaptation, were excluded from the study.

There were three groups of rats, namely the control group (A) with 0.1 cc of phosphate buffer saline (PBS) treatment, the second group (B) with Exo-WJ treatment with a concentration of 0.75 mcg in 0.05cc of NaCl 0.9%, and the third group (C) with Exo-WJ treatment with a concentration of 1.5 mcg in 0.1 cc of NaCl 0.9%.

Collagen deposition was processed through Trichrome Masson staining and assessed by the pathologist through Image J digital analysis.

The making of Exo-WJ

WJ tissue stem cells were obtained from the umbilical cord of a newborn. Quality control for these cells followed the criteria that the donor was a healthy donor, did not experience infection and preeclampsia, was over 18 years of age, did not experience rupture of membranes for more than 18 hours, and did not experience fever during delivery.[11]

The freshly removed umbilical cord was placed into Hank's balanced salt solution at 40 °C. The umbilical artery and vein were separated, then the WJ tissue was cut into small pieces (1-2 mm) and placed on a petri dish coated with 10μg/ml human fibronectin. DMEM media containing 10% FBS and penicillin/streptomycin was added to a dish and incubated under 5%

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CO2 level and 37 °C temperature. After 12 hours, DMEM medium was added to the dishes and changed every 3-5 days. Cells were harvested with 0.25% trypsin-EDTA and propagated. After reaching 80% of confluence, the medium was changed to the serum RPMI-free basal medium, then a 1% antibiotic-antifungal mixture was added. After 48 hours, the WJ conditioned media of stem cells were collected, filtered with a 0.22μM (Millipore) filter, and stored at -200 C before use.

The WJ conditioned media was transferred into a new tube and the Total exosome isolation of Invitrogen® reagent was added. The culture medium/reagent mixture was mixed using a vortex, or pipetted up and down until the solution is homogeneous. Samples were incubated at 2 °C - 8 °C overnight. After incubation, samples were centrifuged at a rate of 100,000 × g for 1 hour at a temperature between 2 °C - 8 °C. The supernatant was aspirated and discarded. The exosome was present in the pellet at the bottom of the tube (in most cases not visible) and was resuspended with PBS.

After the pellet had been resuspended, the exosome was ready for further analysis or purification by affinity method. The exosomes were isolated at 2 °C - 8 °C for up to 1 week, or stored for an extended period at ≤20 °C. Each 1 ml contained 1 eppendorfexosome (150mcg) diluted in 5 ml NaCl. For the treatment of experimental animals, a dose of (I) 0.75mcg and (II) 1.5 mcg for each sample in 0.05ml and 0.1ml was needed.

Study treatment

The acclimatization of the 3 groups of Wistar rats was carried out for 1 week; their dorsal hairs were shaved, then exposed using DermaPal NB UVB 311 nm phototherapy, with an output power of 3.9-6.8 mW/cm^2, Daavlin-Canada radiation, total radiation dose of rat A and B and C respectively 840mJ/cm^2 each. The irradiation was carried out 3 times a week for 4 weeks, reaching the total dose of 840mJ/cm^2.[12]

Groups A, B, and C were treated according to the provisions. The injection was done once a week on the same day for 4 weeks. Then the three groups of rats continued the irradiation schedule. After the fourth week of irradiation, the skin tissue from the back of the rats was taken with a 10 mm punch biopsy to a subcutaneous depth. The tissue was placed in a biopsy container tube containing a 10% formalin solution. The preparations were then stained with the Trichrome Masson staining method.

Collagen deposition observations were carried out using a 400x magnification microscope in 5 fields of view and the image was taken with the help of an optilab device connected to a computer. The interpretation of the results of the examination was carried out by an anatomical pathologist with repeated readings. Collagen deposition qualitative data was confirmed by image processing using Image J software to determine the collagen density.

RESULTS

The average of post-treatment collagen expression in all rats showed an improvement in the amount of collagen with different densities. The groups with the highest to lowest average collagen expression were groups C, B and A. The comparison of the mean post-treatment collagen expression can be seen in Figure 1.

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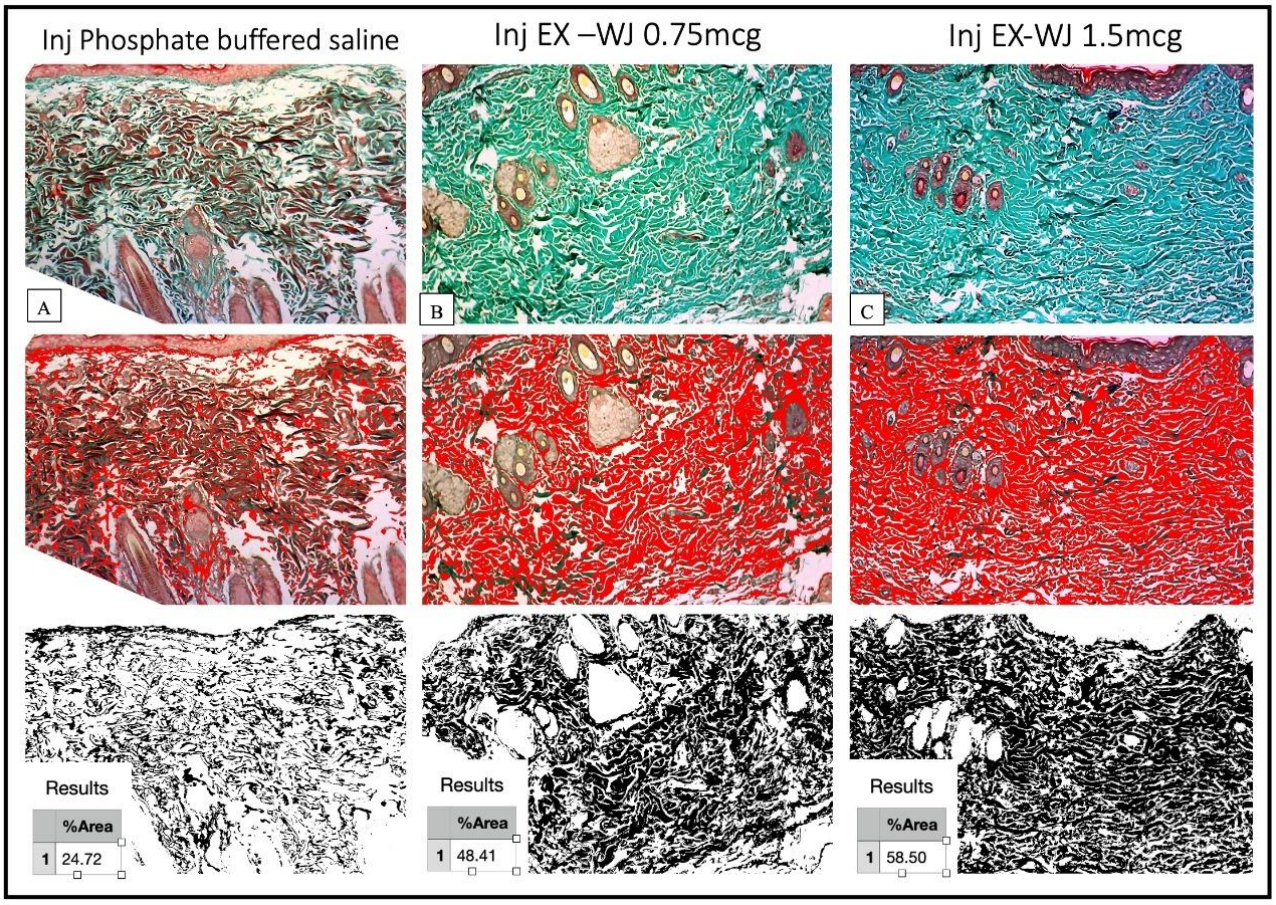

Figure 1. Image J analysis on the sampling of the three groups showed the deposition of collagen in group A was 23.72% (PBS control), group B was 48.41% (Exo-WJ 0.75mcg), and group C was 58.5% (Exo- WJ 1.50mcg)

Exposure to ultraviolet rays has a significant impact on the body. UV rays will damage the heterocyclic DNA bases in skin cells which are recipients of UVB photons. On exposure to a single dose of UVB rays, erythema symptoms will appear which will decrease within 24 hours. On repeated exposure there will be a cumulative effect and erythema develops. Symptoms of erythema after UVB exposure will appear within three to five hours later and peak in 12-24 hours later, then decrease in 72 hours. Before the occurrence of erythema there will be vasodilation of blood vessels. On histopathological examination with a 1-μm strip of skin irradiated with a single dose of UVB, damage to keratinocyte cells has been found 30 minutes after exposure, and most clearly seen 24 hours later. After 72 hours the damaged keratinocyte cells turn into parakeratosis and endothelial cell enlargement occurs. [13]

Wharton's Jelly is a gel-like tissue in the umbilical cord, rich in proteoglycan protein and contains stromal cells, such as fibroblasts, which act as pluripotent stem cells capable of developing into multiple tissues.[9]Mesenchymal stem cells are populations of progenitor cells from the mesoderm lineage and have been shown to be an important mediator in the inflammatory environment.[14] Some of their effects include: limiting inflammation by releasing cytokines; aiding healing by expressing growth factors; altering the immune response by secreting immunomodulatory proteins; increasing the response of endogenous repair cells, and in some tissues (such as bone) playing a role in the maturation of functional cells. This mechanism

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is not independent and therefore it is known that the therapeutic effect of MSC depends on the pathophysiology of the disease.[15]

This study used 3 groups of rats, namely the control group using PBS solution and two groups with treatment usingdifferentExo-WJ concentrations. PBS is an isotonic buffer solution that is used in a variety of biological applications such as: washing cells, transporting tissue, and dissolving, because it has acidity, osmolarity and ion concentration similar to human body fluids.[16] This PBS solution was used as a placebo with the hope of not having an impact on the skin of the Wistar rats in this study.

The determination of the exosome dose of the conditioned media of Wharton 'Jelly's mesenchymal stem cells was based on various studies previously used in humans. According to Willis et al., Exosomes can provide therapy for various diseases where they provide a wound healing effect on human skin at doses of 100mcg and 160mcg.[17] This study determined the basic dose used between the two doses, namely 150 mcg which was then diluted according to the weight requirements of the Wistar rats (250-300 grams). The two doses specified were 0.75mcg (in a 0.05 ml dosage) and 1.50 mcg (in a 0.1 ml dosage).

The results of the Image J digital analysis revealed that the density of type I collagen in the control group (A) was 23.72%, in the Exo-WJ 0.75 mcg group (B) was 48.41%, and in the Exo-WJ 1.50 mcg group (C) was 58.5%. This showed that the deposition of type I collagen in the control group is weaker and in the treatment groups there was an increase of more than 2 times. There was a clear increase in the groups given with exosome from the conditioned media of WJ stem cells with increasing doses.

The results showed that giving Exo-WJ 0.75mcg and Exo-WJ 1.5mcg improves collagen type I deposition in the skin of male Wistar rats aged due to exposure to UVB rays compared to PBS control. This study is in accordance with the research conducted by Diana on Exo-WJ which has been shown to be effective in increasing migration and aging fibroblasts.[18] According to Bongso and Fong, the ability of exosomes depends on the original tissue producing them.[11] According to Kim et al. in his research, it was revealed that the administration of exosomes from mesenchymal cord stem cells can be well absorbed in the skin tissue and has been shown to increase collagen and elastin synthesis in skin tissue in a relatively short time (3 days).[19] This is in line with this study which has also shown an increase in the density of type I collagen in the administration of WJ exosomes which also come from the umbilical cord.

Mesenchymal stem cell exosomes have been recognized as a good therapy in tissue repair and suppression of inflammation as well as anticancer therapy. The current study has shown that exosomes produce a therapeutic effect largely through transfer of miRNA and protein, which triggers various signaling pathways in the receiving cells.[20] Kim et al. demonstrated that umbilical cord exosomes contain a high number of growth factors (especially EGF and bFGF) which are associated with skin rejuvenation that stimulates fibroblast proliferation and migration as well as collagen and elastin synthesis.[19] Much literature suggests that exosomes provide benefits at several important stages in wound repair and skin regeneration, such as cell migration and proliferation, angiogenesis and deposition collagen, particularly with AKT / ERK signaling activation. [21,22]

The effectiveness of the Exo-WJ can be reviewed in studies with a longer observation distance. Research by Fang et al showed that mesenchymal stem cell exosomes from the umbilical cord tested on mice showed a clinical improvement in the skin of mice at day 25 compared to day 14. This suggests that exosome administration has a long-term improvement in collagen deposition in the skin if an observation is made with a longer time.[23]

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CONCLUSION

Based on the results of the study and discussion, it was found that a significant difference was found between the PBS control group and the groups given with exosome media conditioned by Wharton's Jelly's 0.75 mcg and 1.5 mcg mesenchymal stem cells. Giving Exo-WJ has been proven to improve collagen deposition in the aged skin of the Wistar rats due to UVB exposure. Further research is needed to determine the effectiveness of giving Exo-WJ by using a larger sample.

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