INTRODUCTION

Osteoarthritis (OA) is one of the most common forms of arthritis that causes pain and reduces the quality of life. The risk factors for osteoarthritis are old age, obesity, history of trauma to the knee, and increased life expectancy will cause osteoarthritis every year. About 250 million people worldwide have osteoarthritis.

In osteoarthritis, there is a primary disturbance of the chondrocytes, which causes the chondrocytes to experience hypertrophy and release inflammatory mediators. These proteolytic and collagenolytic enzymes cause degradation of the matrix of proteoglycans and collagen as well as apoptosis of chondrocytes resulting in effects such as pain, impaired function, quality of life and even socio-economic so that the treatment of osteoarthritis continues to grow.

Even though there are so many therapeutic methods for OA until now none of them has consistently been able to repair joint cartilage satisfactorily.

Hyaluronic acid viscosupplement therapy used as a treatment for OA affects symptom improvement in the medium term. It lasts in the joints for several days and effects only appear after 3–6 months in humans. In addition, this therapy shows inconsistent results.

The limited number of effective treatments has prompted the development of new strategies for treating OA in the form of cell-based procedures that aim to modulate the inflammatory process and stimulate cartilage tissue regeneration. Stem cells secrete several proteins (secretome), including growth factors, chemokines, cytokines, metabolites and bioactive lipids that regulate in an autocrine or paracrine fashion modulate the inflammatory process and stimulate cartilage tissue regeneration. In the medium term. It lasts in the joints for several days and effects only appear after 3–6 months in humans.

Administration of mesenchymal stem cell derivate secretome causes lower levels of Matrix Metalloproteinase-13, Aggrecanase-2, and Interleukin-1β than hyaluronic acid in rabbit knee osteoarthritis


ABSTRACT

Background: Osteoarthritis (OA) is the most common form of arthritis, which cause pain and disability that can affect the quality of life. The inflammation in OA is chronic and characterized by the infiltration of immune cells and the secretion of cytokines. Previous OA treatment used hyaluronic acid intraarticular injection, but several studies show no satisfactory clinical outcome. This study aims to determine and compare the anti-inflammatory and anti-catabolism effects of stem cell-derived secretomes versus hyaluronic acid as the gold standard for the treatment of OA.

Methods: This research was conducted using an experimental randomized post-test-only control group design. According to veterinarians, the population in this study were local rabbits, aged 6-8 months, weighing > 1,500 grams, and healthy. Data were analyzed using SPSS version 22.0 for Windows.

Results: Twenty-two subjects were included in each treatment and control group. From the descriptive analysis, it was found that MMP-13 levels were 7.55 ± 0.23 (Treatment) (ng/mL) and 8.36 ± 1.04 (Control) (ng/mL), ADAMTS-5 levels were 0.83 ± 0.08 (Treatment) (ng/mL) and 1.14 ± 0.33 (Control) (ng/mL), and levels of IL-1β 23.0 ± 2.48 (Treatment) (ng/mL) and 26.81 ± 5.57 (Control) (ng/mL). From the inferential test, it was found that all research results were statistically significant (MMP-13= 0.81; p = 0.021; 95%CI = -1.48 – (-0.13)) (Agrekanase 2 = 0.32; p = 0.066; 95% CI = -0.53 – (-0.09)) (IL-1β = 3.76; p = 0.023; 95% CI = -7.59 – (-0.08)).

Conclusion: Administration of mesenchymal stem cell-derived secretomes resulted in significantly lower levels of MMP-13, Agrekanase 2, and IL-1β compared to administration of hyaluronic acid in rabbit knee osteoarthritis.

Keywords: Osteoarthritis, knee, secretome, MMP-13, Agrekanase-2, IL-1β.

METHODS

This research was conducted using a purely experimental research design on experimental animals using a randomized post-test-only control group design using a control group. Samples were taken from affordable populations with the following criteria: Inclusion Criteria included male rabbits with induced osteoarthritis, age 6-8 months, and weight > 1500 grams. While the exclusion criteria include: Local skin wounds, red eyes, breathing frequency >60 times/min, rectal temperature >40°C, and 6-8 months, and weight > 1500 grams. While the exclusion criteria include: Local skin wounds, red eyes, breathing frequency >60 times/min, rectal temperature >40°C, for the drop-out criteria: sick rabbits, dead rabbits.

The descriptive analysis determined the frequency and distribution (standard deviation, mean and median, mode). Then the normality test was carried out using the Shapiro-Wilk test on MMP-13, Aggrecanase-2, and IL-1β. Then test the homogeneity of the data using Levene’s test on the research variables. For the inferential test of MMP-13, Aggrecanase-2, and IL-1β levels, in an unpaired numerical comparative study, 2 groups, with 1 measurement and normally distributed data, an independent t-test was performed using a statistical SPSS program for Windows version 22.0.

RESULTS

From the descriptive analysis, it was found that the number of samples in the treatment and control groups was 11 samples each. In the secretome group, the average MMP-13 level was 7.55 ± 0.23 ng/ml, the average Aggrekanase 2 (ADAMTS-5) was 0.83 ± 0.08 ng/ml, and the average IL-1β was 23.05 ± 2.48 μg/ml. Meanwhile, in the arthritis model group the average IL-1β was 23.05 ± 2.48 ng/ml, the average Agrekanase 2 (ADAMTS-5) was 0.83 ± 0.08 ng/ml, and the average MMP-13 level was 7.55 ± 0.23 ng/ml, the average Aggrekanase 2 (ADAMTS-5) was 0.83 ± 0.08 ng/ml, and the average IL-1β was 23.05 ± 2.48 μg/ml (Table 1).

It can be seen in the table below that there was a difference in the mean MMP-13 between the secretome and hyaluronic acid groups, with MMP-13 lower in the secretome group (7.55 ± 0.23 vs. 8.36 ± 0.23, p=0.021) (Table 2).

Based on the results of the Independent T-test for the MMP-13 variable, it was found that there was a significant difference in MMP-13 levels between the secretome and hyaluronic acid groups, with MMP-13 lower in the secretome group (7.55 ± 0.23 vs. 8.36 ± 0.23, p=0.021) (Table 2).

It can be seen in the table below that there was a significant difference in the average Aggrekanase-2 between the secretome and hyaluronic acid groups (p<0.05) (Table 2).

Based on the results of the Independent T-test for the MMP-13 variable, it was found that there was a significant difference in MMP-13 levels between the secretome and hyaluronic acid groups, with MMP-13 lower in the secretome group (0.83 ± 0.08 vs 1.14 ± 0.33, p=0.006) (Table 3). It can be seen in the table above that there was a significant difference in the mean IL-1β between the secretome and hyaluronic acid groups, with IL-1β lower in the secretome group (23.05 ± 2.48 vs. 26.81 ± 5.57, p=0.023) (Table 4).

DISCUSSION

This study showed that the sample group injected with mesenchymal stem cell-derived secretome produced lower levels of MMP-13 than the group that was given hyaluronic acid, which was statistically significant. The results of this study are supported by the results of a study by Zhang R et al., who showed that the expression of MMP-13 in the medial femoral condyle cartilage of the OA rabbit experimental animal model injected with allogeneic ADSCs was significantly lower than the rabbit group given 0.9% saline at the 6th and 10th week after induction OA. A study by van Buul et al., showed that a significant decrease in the IL-1β...
and MMP-13 genes was found in the group of mice that experienced increased MSC secretome secretion.\textsuperscript{8} Meanwhile, a study by Niada et al., stated that MMP-13 activity decreased by 61%. In the first 24 hours of giving secretome.\textsuperscript{9}

A study by Chen et al., reported that the administration of secretomes to rat samples showed apoptotic activity of cartilage cells in the administration of stem cells lower than in samples given phosphate buffer saline (PBS) with an MMP-13 ratio range of 20-40% in the administration of secretomes and administration of PBS by 40-60%.\textsuperscript{10} Meanwhile, research by Zhang et al., stated that there were significant results in reducing MMP-13 levels using samples from cartilage tissue.\textsuperscript{7}

Meanwhile, a study by Kuroda et al., which examined the effect of ADSCs in the OA model of New Zealand White rabbits, found significantly lower MMP-13 expression in the ADSCs group compared to controls (PBS plus hyaluronic acid) at the 8th and 12th weeks.\textsuperscript{11}

Meanwhile, previous studies also described a significant MMP-13 suppression in HA administration.\textsuperscript{12,13} Research by Assirelli et al., stated that secretome administration could reduce MMP-13 levels in 11 patients with knee osteoarthritis by decreasing IL-4 and inducing cartilage regeneration for 7 days.\textsuperscript{14} This study was also supported by Mancuso et al., who reported a decrease in MMP-13 levels after 10 days of secretome administration in experimental rats, followed by decreased degradation enzymes in the knee.\textsuperscript{15}

This study showed that the sample group injected intraarticularly with mesenchymal stem cell derivative secretome resulted in significantly lower levels of Aggrecanase-2 (ADAMTS-5) than the group given hyaluronic acid. In theory, the pathogenesis of OA varies widely. Niada et al., stated that ADAMTS5 is a mediator of tissue inhibitors of matrix metalloproteinases (TIMP) which play a role in the course of knee OA disease.\textsuperscript{9} In this study, it was found that there was a significant relationship with secretome administration, where there was a significant decrease in ADAMTS5 levels compared to HA administration.

This study is similar to Niada et al., who concluded that giving secretome can reduce ADAMTS5, which has an anti-inflammatory effect.\textsuperscript{8} The study of Zhu et al., reported that secretome injected into experimental rats showed a significant reduction in ADAMTS5 levels (in vitro) and an increase in type II collagen synthesis activity in knee joint cartilage cells.\textsuperscript{16}

Van Buul reported that administering mesenchymal stem cells had a therapeutic effect on knee OA patients with significant changes in the amount of ADAMTS5 and ADAMTS4 levels (p <0.001) after 2 days of secretome administration.\textsuperscript{8}

Research by Yan et al., found that administration of ADSC secretome to mice with knee osteoarthritis decreased ADAMTS-5 at 2 weeks post-injection.\textsuperscript{17} Likewise, a study by Jia et al., examined OA rats, then given a secretome, with evaluation using histopathological examination and examination of ADAMTS-5 levels, it was concluded that there was a significant decrease in ADAMTS-5 with a decrease in macrophage infiltration in the knee joints of experimental rats.\textsuperscript{18}

The next result of this study was lower IL-1β levels in the group of rabbits that were injected with mesenchymal stem cell derivative secretome compared to the group that was given hyaluronic acid, which was statistically significant.

An in vitro study by van Buul et al., who used samples of synovial explants from the human knee, supported the results of this study.\textsuperscript{8} Synovial explants exposed to MSC-conditioned media showed lower IL-1β gene expression than controls.\textsuperscript{8} The results obtained from this study are thought to be a result of the ability of MSCs to generate a regenerative intra-articular microenvironment by increasing the recruitment, activation, and differentiation of endogenous stem cells in repairing cartilage.

The limitation of this study was used only one examination time, so it could not provide a justification basis for the difference in MMP-13 levels at different examination times. Different time points will also provide benefits in determining the effect of general secretome administration in this study, i.e., curative or preventive impact on the progressivity of OA. This study's period of experimental studies was also not long enough to evaluate the long-term stability of cartilage. Hence, this study did not use a control group to examine the effects of secretome on healthy joints (the group of rabbits that were not OA-induced but injected secretome). Studying the effects of secretomes on healthy joints will add to

\textsuperscript{901} Bali Medical Journal 2023; 12(1): 899-903 | doi: 10.15562/bmj.v12i1.4169
the study's strength. This study has also not examined the regenerative aspects of post-administration of mesenchymal stem cells secretomes, such as cartilage thickness, chondrocyte cell count and experimental animal behavior, supporting the justification of secretory administration in a clinical setting.

Our suggestion regarding this study, further studies are needed on the role of mesenchymal stem cell secretomes in the progression of OA using more varied administration points and doses so that scientific evidence can be justified to support phase 1 clinical trials. Hence, further studies are needed on cartilage regeneration after using mesenchymal stem cell secretomes, which include clinical effects, such as cartilage thickness, chondrocyte cell count, and behavior in experimental animals before proceeding to the stage of clinical trials in patients.

Based on the above, this study resulted in a novelty in the form of secretome administration can reduce levels of MMP-13, aggrecanase-2, and IL-1β more than administration of hyaluronic acid in rabbit osteoarthritis by reducing pro-inflammatory mediators and proteolytic enzymes.

CONCLUSION
Administration of mesenchymal stem cell derivative secretome resulted in lower levels of MMP-13, Aggrecanase-2, and IL-1β compared to administration of hyaluronic acid in rabbit knee osteoarthritis.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

ETHICAL CLEARANCE
Ethics approval has been obtained from the Ethics Committee, Faculty of Medicine, Universitas Udayana, Prof. dr. I.G.N.G Ngoerah Hospital, Bali, with number 62/UN14.2.2.VII.14/LT/2022 prior to the study being conducted.

FUNDING
None.

AUTHOR'S CONTRIBUTION
All authors equally contribute to the study from the conceptual framework, data acquisition, and data analysis, until reporting the study results through publication.

REFERENCES

ORIGINAL ARTICLE


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