

INTRACAMERAL INJECTION OF LIMBAL MESENCHYMAL STEM CELLS CONDITIONED MEDIA (LMSCs-CM) IMPROVE CLINICAL OUTCOME WITH DELAYED ON Na-K ATPase CORNEAL ENDOTHELIAL PUMP RECOVERY IN PHACOEMULSIFIED RABBIT EYES

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ABSTRACT : To investigate the effect of LMSCs-CM on central corneal thickness (CCT) and Na⁺/K⁺ ATPase of endothelial cells expression in phacoemulsified rabbit eyes. This was a true experimental laboratory study on rabbits using a pre and post-test control group design. Approximately 24 rabbit eyes were exposed to phacoemulsification ultrasound, then randomly divided into 2 groups. The control group was injected using 0.2 ml of physiological saline (BSS) intracamerally, while the treatment group was injected with 0.2 LMSCs-CM intracamerally. Central corneal thickness was evaluated on the 3rd day after treatment, followed by enucleation to analyzed Na⁺/K⁺ ATPase expression using immunohistochemistry. The LMSCs-CM significantly reduced corneal edema and inflammation resulting in clinical improvement. There was no mean difference of CCT between groups ($p = 0.372$, $\alpha > 0.05$). However, CCT was higher in the control group (62.00 ± 27.41) when compared to the treatment group (50.00 ± 36.45 um). Similarly, the expression of Na⁺ / K⁺ ATPase in both groups was not significantly different ($p = 0.973$, $\alpha > 0.05$). After 3 days, LMSCs-CM exerted clinical improvements, but had a delay in functional recovery, in regards to Na⁺/K⁺ ATPase expression in the corneal endothelial cells.

Key words : Limbal mesenchymal stem cells (LMSC) conditioned media, Na⁺/K⁺ ATPase pump, endothelial cells, corneal thickness.

INTRODUCTION

Phacoemulsification is a commonly used cataract extraction surgery method with a high success rate. A study at Cicendo Eye Hospital in 2011 showed that 81% of the patients with high myopia had good vision following the surgery. Endothelial damage resulting from this procedure is influenced by various preoperative and intraoperative factors. Preoperative factors that affect corneal endothelial cell loss includes age and cataract grade (Hayashi *et al*, 1996; Budiman *et al*, 2011; Nancy and Joyce, 2012; Zavala and Jaime, 2013; Gupta *et al*, 2014). Blindness related to corneal abnormalities ranks as the 4th leading cause of blindness globally, with a prevalence of 5.1%. Studies conducted by Pirazolli *et al* found that phacoemulsification results in 16.67% loss of endothelial cells that are associated with trauma during surgery. Other studies show an endothelial cell loss of 4-15% after phacoemulsification by experienced surgeons

and 6.4% after the same procedure was done by senior residents. The main therapeutic choice for corneal endothelial damage at present is keratoplasty. However, the main problem with this method is the high postoperative cell loss when compared to penetrating keratoplasty. Another problem is the limited corneal donor available globally, meaning that new methods are needed to improve and increase the density of endothelial cell survival (Olson *et al*, 1990; O'Brien *et al*, 2004; Zavala and Jaime, 2013; Maggon *et al*, 2017).

Corneal clarity is regulated by an active metabolic pump from endothelial cells. This metabolic pump is controlled by Na⁺/K⁺ ATPase, which is located in the basolateral endothelial cell membrane. Stimulation of corneal endothelial cells from phase G1 to phase S becomes an important step in increasing the ability of endothelial cell proliferation. Methods to achieve this includes transformation of viral oncogenes, the addition

of positive growth factors such as Epidermal Growth Factor (EGF), basic Fibroblast Growth Factor (bFGF) and Nerve Growth Factor (NGF) in culture media (Nishida, 2005; Lu *et al*, 2010; Pawitan, 2014).

Recent studies have shown that the secretion factor alone, without stem cells, can repair tissue damage under various conditions. Secretome within SCCM has the capacity to be produced, cooled, packaged and transported more easily. Additionally, there is no need to match the donor with the recipient to avoid the risk of rejection. Hence, stem cell conditioned media has many promising pharmacological prospects as a regenerative drug (Pawitan, 2014). Holan *et al* (2015) stated that LMSCs have a better ability to suppress inflammation and neovascularization in ocular tissue compared to MSC bone marrow and adipose (Komaratih *et al*, 2017; Yamanaka *et al*, 2015; Garfias *et al*, 2012; Holan *et al*, 2012; Li *et al*, 2012; Tabele *et al*, 2012; Gasparroto *et al*, 2014). The purpose of this study is to investigate the effect of LMSCs-CM on central corneal thickness (CCT) and Na⁺/K⁺ ATPase of endothelial cells expression in phacoemulsified rabbit eyes.

MATERIALS AND METHODS

The main reagents to produce LMSC Conditioned medium has been produced in previous studies (Komaratih *et al*, 2017) and stored at temperatures below -80°C. 2% tetracaine hydrochloride eye drops, povidone iodine 5%, balanced salt solution (BSS), 1cc and 3cc syringes, keratome 2.2 mm, antibiotic Levofloxacin 0.5% eye drops (Neo Levo; Rohto Lab, Tokyo), visco elastic HPMC (Rohtovisc; Rohto Lab; Tokyo) 0.5% pantocain eye drops and anesthetic ingredients ketamine hydrochloride and xylazine hydrochloride. Specular microscope with the brand Nidek Co., Ltd, CEM-530 series, Nidek Co., Ltd, CV9000 series phacoemulsification machine, ultrasound Nidek Co., Ltd, US4000 series.

This is experimental pre and post study in eyes of New Zealand white rabbit conducted in Stem Cell research and development center, Universitas Airlangga. All experiments were conformed to the local ethics review board, faculty of veterinary medicine Universitas Airlangga. The inclusion criteria of the experimental unit of this study were: New Zealand white rabbit adults older than 12-18 months with a body weight of 2.5-3.5 kg. The exclusion criteria for the experimental unit of this study included: animals declared by veterinarians that were proven to have unhealthy eye conditions as well as diseases or have the potential to transmit disease during the study. Criteria for dropping out test rabbits are sickness, death, the occurrence of complications such as

corneal perforation, vitreous prolapse, infection, bleeding during and after surgery.

Limbal Mesenchymal Stem Cells-CM production

Isolation of LMSCs and production of LMSCs-CM were previously described by Komaratih *et al* (2019).

Phacoemulsification and Intracameral Injection of LMSCs-CM

Approximately 24 eyes were used in this study. Central corneal thickness was measured as the pre-test data. The eyes then underwent phacoemulsification, with a single operator using the general technique as follows: phacoemulsification ultrasound exposure power was 70% burst panel mode with a duration of 5 minutes 10 seconds power on and 10 seconds power off (Total exposure time 2.5 minutes), parameter including aspiration flow rate 25 ml/s and the height of the bottle is 90 cm. The incision was constructed according to the phacotip (2.2 mm). The bevel-up phacotip position is placed in the anterior chamber without touching the endothelium and lens capsule. The corneal endothelial cells were damaged according to the procedure developed by Nemet *et al* (2000) using the NIDEK CV 9000 phacoemulsification machine. The eyes were then randomly divided into a control group, received intracameral BSS injection and treatment group, received intracameral 0.2mL LMSCs-CM injection.

On the 3rd day post-surgery, the CCT was analyzed using ultrasound pachymetry followed with enucleation of the eye. The cornea of the eye was then taken and a histopathological examination of IHC staining with Na⁺ / K⁺ ATPase α 1 antibody was carried out.

Statistical analysis

The collected data was analyzed using paired t-test with SPSS 17.0 software and $\alpha = 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Clinical evaluation

Ocular anterior segment examination, biometric measurements of axial length and specular microscopy of corneal endothelial cell density (ECD) was carried out before the procedure using a handheld slit lamp. The conditions for functioning eyes were within normal limits and no significant differences between groups was observed, as shown in Tables 1 and 2.

In the above data, the average length of the eyeball axis in the treatment group was 15.83 mm and in the control group it was 15.41 mm. From the data, the normal distribution was obtained in both groups (p treatment =

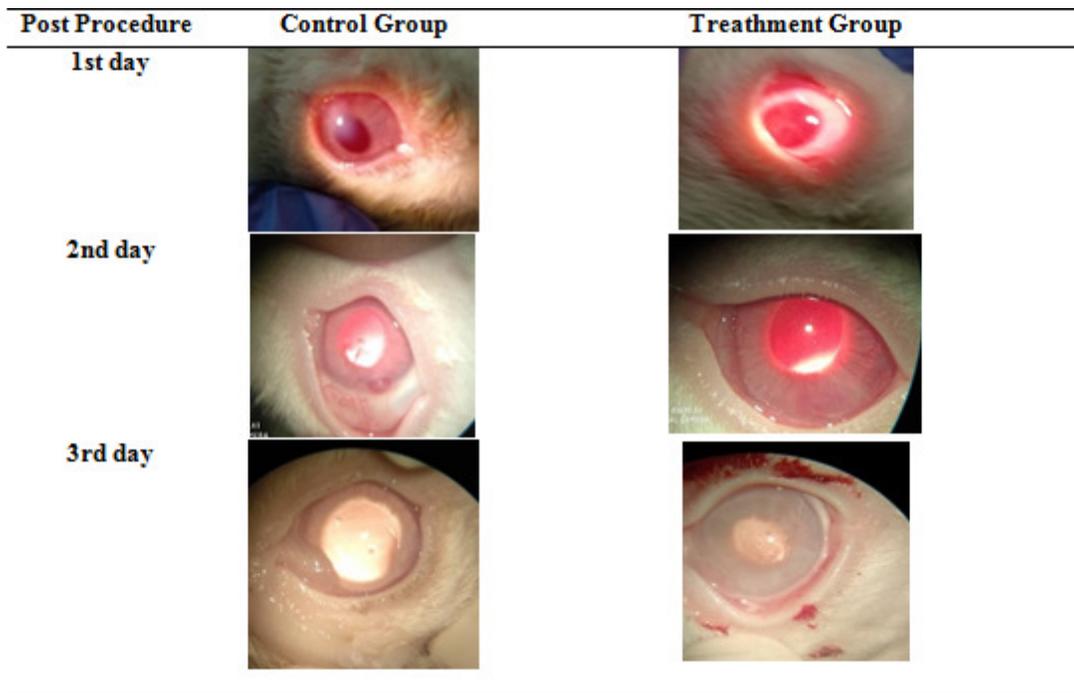


Fig. 1 : Anterior Segment Evaluation on days 1, 2, and 3 post phacoemulsification.

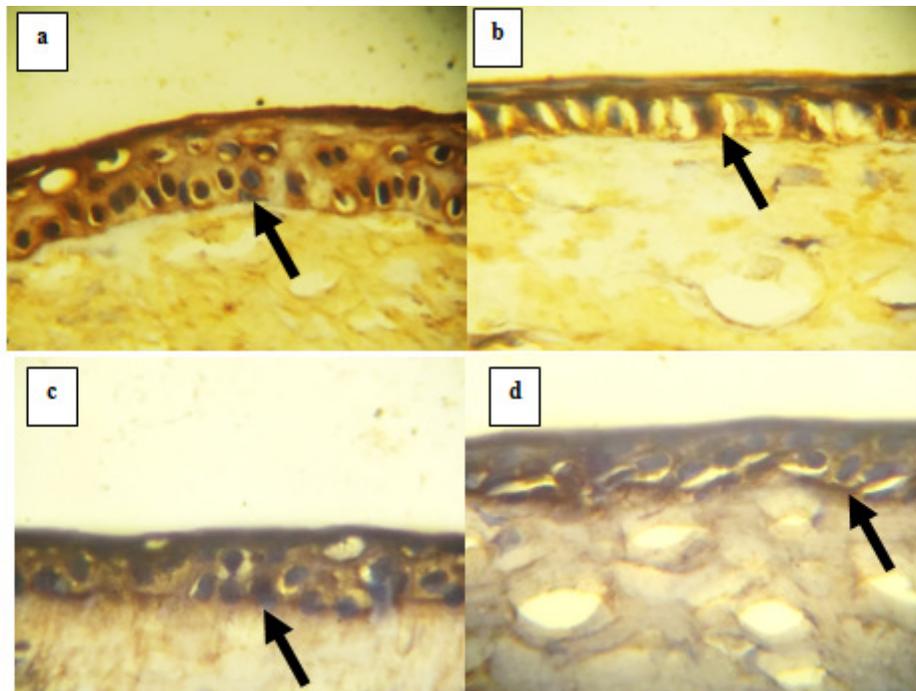


Fig. 2 : The expression of Na + / K + ATPase by examination of the Na + / K + ATPase α -1 antibody, seen with a 400 times magnification using a light microscope. (a) A2 and (b) A5 control groups, (c) B4 and (d) B10 treatment groups.

0.391 and p control = 0.851, $\hat{\alpha} > 0.05$) and no significant difference was found between the two groups ($p = 0.077$). Measurement of ECD was done 3 times with a specular microscope and the mean value was calculated. Data on corneal ECD showed that in the treatment group, the density was 2582.86 cells/mm² and in the control group, it was 2730.47 cells/mm². From the distribution data, the normal distribution was obtained in both groups (p

treatment = 0.221 and p control = 0.101, $\hat{\alpha} > 0.05$) and no difference was found between the two groups ($p = 0.110$).

Handheld slit lamp examination showed corneal edema, especially in the area of the incision towards the middle and this improved on the second and third days in both groups, as seen in Fig. 1. Inflammation and infection were not presented in either groups. Furthermore,

Table 1 : Distribution of axial length.

Group	n	Axial Length				P
		\bar{x}	SD	Minimum	Maximum	
Treatment	12	15.83	0.52	14.97	16.58	0.077
Control	12	15.41	0.60	14.61	16.79	

s significances = 0.077

Table 2 : Distribution Endothelial Cell Density (ECD).

Group	n	ECD				P
		\bar{x}	SD	Minimum	Maximum	
Treatment	12	2582.86	273.05	2076.33	3034	0.110

there were no complications in the iris and anterior chamber.

Corneal thickness (CCT) post treatment

Pachymetry data showed the mean measurement of corneal thickness was 358.83 ± 41.43 micro meters in the control group, while in the treatment group it was 371.75 ± 35.17 micro meters. Table 4 shows the average corneal thickness measurement (CCT) using a specular microscope. Corneal thickness data observed in the treatment group was 402.83 ± 33.43 micro meters and in the control group it was 388.21 ± 38.24 micro meters.

This study found a difference in the treatment group between pre and post phacoemulsification, where there was an increase in initial corneal thickness from 371.75 ± 35.17 micro meters to 421.75 ± 29.10 micro meters, with a p value = 0.001. This was observed in the control group as well, where the initial corneal thickness moved from 358.83 ± 41.43 micro meters to 420.83 ± 40.45 micro meters, with a p value = 0,000. This proved that there was a change in thickness before and after. Table 5 shows parametric statistical tests using paired t-test, since the data was normally distributed on measurements with a specular microscope machine. It appears that there is a difference in the treatment group between pre and post phacoemulsification, as there was an increase in initial corneal thickness from 402.83 micro meters $\pm 33, 43$ micro meters to 439.72 micro meters ± 21.00 micro meters, with p value = 0.003. This was seen as well in the control group, where the initial corneal thickness shifted from 388.21 micro meters ± 38.24 micro meters to 427.58 micro meters $\pm 36, 95$ micro meters, with p = 0.000. This proves that there is a change in thickness before and after the action. However, using the independent t-test, data analysis between the two groups found no significance in the difference in corneal thickness changes between treatment and control groups (treatment group 36.89 micro meters ± 33.87 micro meters and control group 39.38 micro meters ± 20.94 micro meters,

with $p = 0.564$, $\alpha > 0.05$) the action. However, data analysis between the groups using independent t-test found no significant difference in changes in corneal thickness differences between the treatment and control groups (50.00 ± 36.45 micro meters and 62.00 ± 27.45 micro meters, $p = 0.372$)

Na+/K+ ATPase expression of endothelial cells

The expression of Na+/K+ ATPase is calculated based on the percentage of the number of endothelial cells expressing Na + / K + ATPase shown in Figure 2, where positive cells are painted brown in the endothelial cell membrane. As many as 3 visual fields were measured with a magnification of x400 with a light microscope and the average calculation was performed. Table 7 shows the mean percentage of Na+ / K + ATPase α -1 expression of corneal endothelial cells in the control group was $62.63\% \pm 15.79\%$ while in the treatment group, it was $62.86\% \pm 15.76$, $p = 0.973$.

Rabbit eyes were examined using a handheld slit lamp on the first, second and third day, especially to see the condition of corneal clarity and inflammation in BMD. On the first day, corneal edema was found mainly in the central cornea and in the temporal area where the main incision was. Clinical edema appeared more frequently in the control group than in the treatment group. The same study conducted by Kim *et al* (2015) shows that endothelial damage after phacoemulsification occurs mainly in temporal paracentral areas. The study mentioned that endothelial damage post phacoemulsification occurred mainly due to mechanical trauma from the entry and exit of instruments at the main incision. Edema was reduced and less visible on both the second day and third day in the two groups. This was in accordance with a study by Juan *et al* (2013), which states that corneal edema after cataract surgery can be reduced and improved until the seventh post-operative day. The selection of rabbits was in accordance with the criteria of Garcia *et al* (2015) with their age exceeding 12 months because of the effect of low endothelial cell mitosis in the group, making it suitable to be used as a research model for treatment of corneal endothelium. The peak mitosis of corneal endothelial cells occurs on the 3rd day after treatment (Garcia *et al*, 2015).

In this study, the percentage of Na+ / K + ATPase α -1 expression of corneal endothelial cells in the control group was $62.63\% \pm 15.79\%$ while in the treatment group it was $62.86\% \pm 15.76\%$. Statistical tests used paired t-test because the data were normally distributed (p control = 0.424 and p treatment = 0.087, $\alpha > 0.05$). Based on the paired-t-test, different test results obtained the percentage

of expression Na⁺ / K⁺ + ATPase α -1 in the two groups did not get a significant difference, $p = 0.973$ ($\alpha < 0.05$).

In a study by He *et al* (2016) painting of corneal endothelial IHC cells using antibodies were similar to this study. There was no counting of colored cells, and observations were made using a x40 magnification fluorescein microscope. In this study, the difference in intensity of painting is different between corneal endothelium and epithelium, stroma and descemet membrane, where endothelial cells are thick colored on cell membranes and greenish in color. In contrast, our study used as much as three fields of view when using a light microscope with the average calculation of the percentage of cells colored with antibodies and it was carried out by an anatomical pathologist (He *et al*, 2016).

In a study by Nakahara *et al* (2013) *in vitro* on day 14 mentioned that along with the level of proliferation of endothelial cells treated with MSC conditioned media (from bone marrow tissue), the function of Na⁺ / K⁺ + ATPase will follow to improve. In this study the IHC staining method using a fluorescein microscope and western blot analysis to quantitatively assess Na⁺ / K⁺ + ATPase expression, obtained significant results on the 14th day post-treatment. Whereas in our study we used the 3rd day benchmark because in theory, the peak of mitosis occurred on the 3rd day after treatment. Examination by western blot analysis method was also used in the study of Lu *et al* (2010), where the treatment group in an *in vitro* study where human corneal endothelial cells that were cultured using embryonic stem cell conditioned media of rat animals on days 3 and 4 after treatment. This resulted in proliferation and increased expression of Na⁺ / K⁺ + than in the control group (Nakahara *et al*, 2013; Lu *et al*, 2010).

Research by Rolev *et al* (2017), looked at the expression of Na⁺ / K⁺ + ATPase using transverse corneal fragments. Anti-body immunohistochemical staining of Na⁺ / K⁺ + ATPase was done and it was read using a light microscope with x100 magnification, and the expression of Na⁺ / K⁺ + ATPase qualitatively read. Whereas in our study, a x400 magnification was used and the percentage calculations were performed. If observed clinically, the anterior segment in the control group on the first day after treatment appeared to have more edema in the cornea when compared to the treatment group and it improved on days 2 and 3 after treatment. This is consistent with the study of Usta *et al* (2017) where corneal thickness was significantly increased on the first day after phacoemulsification in the control and treatment groups. In this study, using autologous serum for treatment, the treatment group

experienced a difference in corneal thickness on the first and seventh days (Usta *et al*, 2017).

There was a difference in the treatment group between pre and 3rd day post phacoemulsification, where there was an increase in initial corneal thickness from 371.75 micro meters \pm 35.17 micro meters to 421.75 micro meters \pm 29, 10 micro meters, with $p = 0.001$. This also occurred for the control group, where the initial mean corneal thickness was 358.83 micro meters \pm 41.43 micro meters and it shifted to 420.83 micro meters \pm 40.45 micro meters, with $p = 0.000$. This proves that there is a change in thickness before and after the action. However, data analysis between groups using independent t-test found no significant difference in changes in corneal thickness between treatment and control groups.

In a previous study, we did not get a journal about the measurement of corneal thickness in rabbits after phacoemulsification ultrasound exposure by giving LMSC conditioned media *in vivo*. Research by Usta *et al* (2017) mentioned a significant difference on the seventh day after phacoemulsification ultrasound exposure, followed by autologous administration of rabbit serum with $p = 0.048$. This was comparable to the phacoemulsification control group alone, whereas on the first day after phacoemulsification, the two groups were not statistically significant with $p = 0.031$. The measuring instrument used in the above treatment is by using a non-contact specular microscope. In our study, the tools we used were non-contact with specular microscopy and corneal contact with ultrasound pachymetry. Homogenization between groups before the treatment was carried out.

To the best of our knowledge, there has been no similar study examining the effect of LMSC conditioned media on corneal thickness and expression of Na⁺ / K⁺ + ATPase corneal endothelial cells *in vivo* in experimental animals. Several studies have looked at the effect of conditioned media on corneal endothelium using sources from mouse embryonic stem cells, human bone marrow mesenchymal stem cells and human adipose stem cells. All this research was carried out *in vitro*. Whereas studies that observed *in vivo* proliferation and morphology of endothelial cells in experimental animals used other treatments in the form of Rho-associated kinase (ROCK) inhibitors and autologous serum.

In our study, we took corneal measurements on the 3rd day after ultrasound exposure because researchers wanted to know the expression of Na⁺ / K⁺ + ATPase and corneal thickness at the peak of mitosis 3-4 days post-action. It can be said that the treatment was given in the form of intracameral LMSC conditioned media

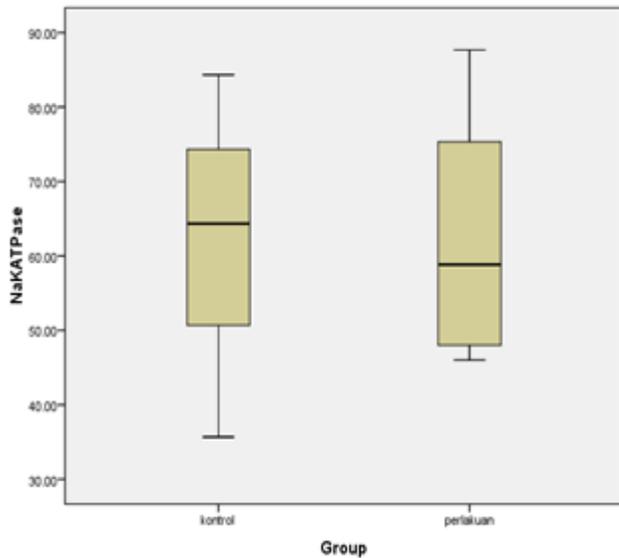


Fig. 3 : Bar diagram of percentage expression of Na + / K + ATPase α -1 corneal endothelial cells in the control and treatment groups.

Table 3 : Distribution of CCT measured by ultrasound pachymetry.

Group	n	CCTpachymetry(mikro meter)				P
		Average	SD	Minimum	Maximum	
Treatment	12	371.75	35.17	310	452	0.419*
Control	12	358.83	41.43	298	429	

Significanses = 0.419

Table 4 : Distription of CCT measured by specular microscope.

Group	n	CCT specular microscope (mikro meter)				P
		Rerata	SD	Minimum	Maximum	
Treatment	12	402.83	33.43	369.00	474.00	0.329*
Control	12	388.21	38.24	335.00	454.00	

Significanses = 0.329

injection. It has not been able to provide a significant influence on both the parameters of corneal thickness and expression of Na + K + ATPase in corneal endothelial cells. This is thought to occur because the observation time is too short, as well as the dose of administration (according to *in vitro* studies) and the pharmacology/ pharmacodynamics were not yet fully clear.

Research conducted by Nakahara *et al* (2013) observed the effect of bone marrow MSC-conditioned media in various concentrations on the proliferation of corneal EDC and the functional effects of endothelial cells, including the expression of Na+ / K + ATPase pumps. The results showed that the cells that were positively colored with Ki-67 were 8.2% in the control group and 15.8% in the treatment group, as well as the Na + / K + ATPase function following the amount of proliferation that was well expressed. In our research

tree, a Ki-67 calculation was performed with the condition of observing the field of view in a small amount that is obtained an average of 4.5 cells per three fields of view in the treatment group and an average of 1.5 cells per three fields of view and significant significance. This affected the calculation of the number percentage of cells expressed by Na + / K + ATPase α -1 antibodies. The phosphorylation process is characterized by a process of proliferation and expression of barrier functions, one of which is a Na+ / K + ATPase pump. The study of Nakahara *et al* (2013) also indicated that conditioned media induced proliferation of human corneal endothelial cells with different effects based on dose. This study uses a dilution effect test and states that conditioned media at full concentration have the same effect as 10% conditioned media while concentrated media at 1% and 3% have lower proliferation effects.

There has not been any similar study examining the effect of LMSC conditioned media on corneal thickness and expression of Na+ / K + ATPase cells in human corneal endothelial cells and their functions. The results showed human corneal endothelial cells proliferated with polygonal shape on day 2 of the conditioned media group. Endothelial cells could be sub-cultured to passage 6 without increasing cell volume. The usual culture media group proliferated on the 3rd day with slightly larger cell sizes, and in cell subcultures they only reached passage 2. The number of cells with Ki-67 is positive and the percentage of cells entering S and G2 phases is higher in the conditioned media group. The function of the Na + / K + ATPase pump is also expressed qualitatively.

The study of Nakahara *et al* (2013) also indicated that conditioned media induced proliferation of human corneal endothelial cells with different effects based on dose. This study uses a dilution effect test and states that conditioned media at full concentration have the same effect as 10% conditioned media while concentrated media at 1% and 3% have lower proliferation effects. In contrast, the research conducted by Lu *et al* (2010) observed the effect of rat embryonic stem cell conditioned media on the proliferation of human corneal endothelial cell cultures and their functions. The results showed that human corneal endothelial cells proliferated with a polygonal shape on day 2 of the conditioned media group, endothelial cells could be sub-cultured to passage 6 without increasing the cell volume. Whereas in the usual culture media group, human endothelial cells proliferate on the 3rd day with slightly larger cell sizes and in cell

Table 5 : Corneal Thickness (CCT) Changes by measurements using a specular microscope machine pre and post phacoemulsification between groups.

Group	CCT Specular Microscope		P	CCT Changes
	Pre Phaco-emulsification	Post Phaco-emulsification		
Treatment	402.83±33.43 (369.00–474.67)	439.72±21.00 (405.00–471.67)	0.003	36.89±33.87 (-30.00–85.33)
Control	388.21±38.24 (335.00–454.00)	427.58 ± 36.95 (361.33 – 475.00)	0.000	39.38 ± 20.94 (19.00 – 80.00)
P	0.329	0.336		0.564

Significanses = 0.564

Table 6 : Corneal Thickness (CCT) changes by measurements using an ultrasound pachymetry machine pre and post phacoemulsification between groups.

Group	CCT Ultrasound Pachymetry		P	CCT Changes
	Pre Phaco-emulsification	Post Phaco-emulsification		
Treatment	371.75±35.17 (310.00–452.00)	421.75±29.10 (378.00–482.00)	0.001	50.00±36.45 (-31.00–112.00)
Control	358.83±41.43 (298.00–429.00)	420.83±40.45 (349.00–474.00)	0.000	62.00±27.41 (22.00 – 124.00)
P	0.419	0.950		0.372

Significanses = 0,372

Table 7 : Distribution of Na⁺/K⁺ ATPase expression of cornea endothelial cells post phacoemulsification.

Group	n	Expression Na ⁺ /K ⁺ ATPase -α1(%)				P
		Average	SD	Minimum	Maximum	
Control	12	62.63	15.79	35.67	84.33	0.973*
Treatment	12	62.86	15.76	46.00	87.67	

Significanses = 0.973, α> 0.05

subcultures they only reached passage 2. The number of cells with Ki-67 is positive and the percentage of cells entering S and G2 phases is higher in the conditioned media group. The function of the Na⁺ / K⁺ + ATPase pump is also expressed qualitatively.

Research on the metabolic function of post proliferating endothelial cells conducted by Hoppenreijts *et al* (1994) states that bFGF induces mitotic responses in bovine and human corneal endothelial cell cultures. While research by Nakahara *et al* (2013) also observed the pathway effect of MSC-conditioned media, the results showed that the phosphorylation of ERK1/2 increased 15 minutes after cells were given MSC-conditioned media. The amount of p27 then increased after administration of a PI 3-kinase inhibitor in the final phase of G1. These results indicate that the MSC conditioned media uses the PI 3-kinase signal pathway to regulate cell cycle progression through p27 and cyclin D. This phosphorylation process is also needed for Na⁺ + K⁺

ATPase pumps with an amount of 1.6 × 10⁶ per endothelial cell fulfilled ATP energy. The effect expected by researchers of conditioned media is mainly from the content of growth factors secreted by LMSC and contained in LMSC conditioned media, more specifically FGF-2. However, one of the limitations of this study is that we did not conduct specific research regarding the content of the LMSC conditioned media so that the pharmacokinetics and pharmacodynamics were not specifically identified.

This study has limitations, some of which are that the observation periods in this study were relatively short and only up to the 3rd day. Within the 1st to 3rd days, the thickness of the cornea was examined so that no significant difference was found. The mechanism of the administration of LMSC-conditioned media which did not have an exact dosage, route of administration, repeat administration and pharmacodynamics and pharmacokinetics were not yet clear. The duration of corneal endothelial tissue exposed to conditioned media is shorter than in vitro studies, due to its active production of aqueous humor and excretion. This study also did not use immunohistochemical staining techniques, using inlead a fluorescein microscope and western blot analysis as a standard in quantitative calculations of Na⁺ / K⁺ + ATPase expression.

CONCLUSION

In conclusion, LMSCs-CM exerts clinically improvement but delayed in functional recovery regarding to Na⁺/K⁺ + ATPase expression in corneal endothelial cells in day-3 after treatment.

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