

THE EFFECTIVENESS OF STEM CELL THERAPY IN MYOCARDIAL INFARCTION REVIEWED FROM UREUM, CREATININE AND KIDNEY HISTOPATHOLOGY LEVELS

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ABSTRACT

This study aims to study the effectiveness of single placental stem cell therapy or cardiomyocyte combination in pigs experiencing myocardial infarction in terms of urea, creatinine and kidney organ histopathology values. A total of 9 pigs were divided into three groups: without therapy (K1), single placental stem cell therapy (K2), and cardiomyocyte coculture combination therapy (K3). All pigs underwent circumflex artery ligation of the proximal coronary artery branch to stimulate cardiac ischemia and therapy was given after myocardial infarction and ST-elevation were formed. The pigs were then treated for eight weeks, euthanized and necropsied for kidney organ removal. The results showed a significant decrease in urea and creatinine values ($P < 0.05$) in K2 and K3 as compared to K1. Histopathology results showed a decrease in glomerular atrophy values (K2: 10.26 ± 0.93 and K3: 12.44 ± 1.21), tubular necrosis (K2: 13.67 ± 3.76 and K3: 17.23 ± 0.55), and tubular dilation (K2: 16.36 ± 1.26 and K3: 18.03 ± 1.76) and these values were significantly different than those observed in K1. It was concluded that the administration of placental stem cell therapy or combined with cardiomyocyte coculture could reduce urea, creatinine values, and improve kidney tissue histopathologically.

Key words: creatinine, histopathology, kidney, myocardial infarction, stem cell, urea

ABSTRAK

Penelitian ini bertujuan mempelajari efektivitas terapi stem cell placenta tunggal maupun kombinasi kardiomyosit pada babi yang mengalami infark miokardium ditinjau dari nilai ureum, kreatinin dan histopatologi organ ginjal. Sebanyak 9 ekor babi di bagi menjadi tiga kelompok, yaitu tanpa terapi (K1), terapi stem cell placenta tunggal (K2), dan terapi kombinasi kokultur kardiomyosit (K3). Seluruh babi dilakukan ligasi arteri circumflexa percabangan dari arteri coronary bagian proximal untuk menstimulasikan iskemia jantung dan pemberian terapi dilakukan setelah terbentuk infark miokardium dan ST-elevasi. Setelah itu, babi dirawat selama delapan minggu, selanjutnya dieuthanasia dan dinekropsi untuk pengambilan organ ginjal. Hasil penelitian menunjukkan penurunan nilai ureum dan kreatinin pada K2 ($13,36 \pm 3,90$ mg/dl) dan K3 ($15,75 \pm 3,43$ mg/dl). Hasil histopatologi ditemukan kongesti, atrofi glomerulus, nekrosis tubulus, dan dilatasi tubulus. Hasil persentase histopatologi menunjukkan penurunan nilai atrofi glomerulus (K2: $10,26 \pm 0,93$ dan K3: $12,44 \pm 1,21$), nekrotik tubulus (K2: $13,67 \pm 3,76$ dan K3: $17,23 \pm 0,55$), dan dilatasi tubulus (K2: $16,36 \pm 1,26$ dan K3: $18,03 \pm 1,76$). Berdasarkan uji analysis of variance (ANOVA) menunjukkan ada perbedaan yang nyata ($P < 0,05$). Disimpulkan bahwa pemberian terapi stem cell placenta maupun yang dikombinasi dengan kokultur kardiomyosit dapat menurunkan nilai ureum, kreatinin, dan memperbaiki jaringan ginjal secara histopatologi.

Kata kunci: kreatinin, histopatologi, ginjal, infark miokardium, stem cell, ureum

INTRODUCTION

Myocardial infarction is a condition in which an area of myocardial necrosis forms due to a blockage in the coronary artery that causes an imbalance in myocardial oxygen supply and demand. Left untreated, myocardial infarction can lead to heart failure (Heusch 2019). Heart failure is characterized by myocardial dysfunction that results in decreased cardiac output, thus the heart is unable to meet the tissue metabolic needs. This incident causes hypoperfusion which triggers damage and failure of non-cardiac organs such as the kidneys (Saleh and Ambrose 2018).

Physiologically, the kidneys receive 25% of the blood from the heart, and any decrease in cardiac output can affect kidney perfusion, causing acute kidney injury (Makris and Loukia, 2016). Acute kidney injury is very common in heart failure patients with a prevalence ranging from 20% to 57% (Cleland *et al.* 2012).

Acute kidney injury is a decrease in the glomerular filtration rate that occurs suddenly within a few hours to

a few weeks or less than 3 months, which results in retention of nitrogenous metabolic waste, especially creatinine and urea (Basile *et al.* 2012). Decreased cardiac output can cause increased activity of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system. This condition causes increased glomerular arteriole vasoconstriction, renal hypoperfusion, and the formation of oxidative stress which causes damage to renal parenchymal tissue in the form of glomerular atrophy, tubular dilation and congestion (Ferenbach and Bonventre 2015). In this regard, myocardial infarction treatment is needed to improve heart function and stop myocardial damage, thereby restoring blood flow to non-cardiac organs such as the kidneys.

Geberally, several treatments for myocardial infarction practice invasive strategies, such as percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG), while the drugs used are anticoagulants, fibrinolytics, anti-platelets, and aspirin. These treatments still unable to stop progressive and

irreversible myocardial damage (Reddy *et al.* 2015). Therefore, the treatment that able to regenerate tissue is needed, such as the use of stem cells (Amin 2013).

Stem cells are unspecialized cells that have the potential to develop into specific cell types. Stem cells have the ability to differentiate into other cells and proliferate, thus possess ability to regenerate tissue (Saba *et al.* 2021). Placental stem cells are multipotent cells that have the potential to differentiate into cardiomyocytes and endothelial cells in damaged myocardium, thus reducing the extent of infarction and regenerating heart tissue. This condition causes improvement in heart function (Vadakke *et al.* 2019). Based on this fact, this study aims to study the effectiveness of single placental stem cell therapy or a combination with cardiomyocyte coculture on kidney dysfunction as reviewed from the urea, creatinine, and kidney histopathology values in cases of myocardial infarction.

MATERIALS AND METHODS

In this study, 9 pigs were divided into three treatment groups, each group consisting of three pigs that were divided randomly. K1 was the group without therapy, K2 was the group treated with single placental stem cells, and K3 was the group treated with a combination of cardiomyocyte coculture.

The surgical procedure of ligation of the circumflex artery branching from the proximal coronary artery was performed on all pigs to create myocardial infarction referring to Gunanti *et al.* 2021. Administration of placental stem cell therapy and cardiomyocyte coculture after myocardial infarction and ST-elevation were formed. In group K1, no therapy was given. In group K2, therapy was given using a single placental stem cell by injection intramyocardially (IM) into the proximal heart muscle. An average 6 million cells of placental stem cells were injected into the area of myocardial infarction. In group K3, placental stem cell therapy and a combination of cardiomyocyte coculture were given in the form of a cardio patch, then attached to the myocardial infarction area. All experimental animals were treated for eight weeks, then euthanized and necropsied to collect the kidney organs for histopathological examination. Kidney tissue was collected with a size of ± 1 cm, then put into 10% neutral buffer formalin (NBF) fixative solution.

Histopathology Preparation

Tissue samples with a thickness of 3 mm were inserted into a tissue cassette, then subjected to an automatic tissue processor to undergo a dehydration process using graded ethanol of 70%, 80%, 90%, 96% and absolute ethanol I and II, clearing using xylol three times and infiltration using liquid paraffin two times. The whole process took 24 hours. The next process was tissue embedding in liquid paraffin. Once frozen, sectioning of paraffin blocks with a thickness of 3-5 μ m was performed using a rotary microtome. The tissue was floated on a 45° C water bath and attached to a 1%

poly-L-Lysine adhesive glass object. The glass object was put into an incubator overnight at a temperature of 50-60° C and stained using HE staining.

Hematoxylin and Eosin Staining

Staining was started with the process of deparaffinization of the slide using xylol I for 5 minutes and xylol II for 2 minutes, then continued with rehydration with alcohol of decreasing concentration from absolute alcohol I and II, 96% alcohol I and II, 90% alcohol each for 2 minutes and running water. The slide was soaked in Mayer's haematoxyllin dye for 7 minutes, washed with running water, put in lithium carbonate once and washed with running water. The slides were immersed in eosin for 7 minutes, washed with running water, then rehydrated with 96% alcohol I and II, absolute I and II, each dipped twice. The next stage, the sample was cleared with xylol I, II, and III for 3 minutes each, then mounted with Entelan® and covered with a cover glass. The stained tissue was ready to be examined under a microscope with 10 fields of view. Each field of view was calculated for the number of glomerular atrophy, tubular necrosis, and tubular dilation using ImageJ software.

Blood Serum Examination

Blood was collected through the jugular vein (3 mL) into a plain vacuum tube, left for 30 minutes in a cool box, then centrifuged for 15 minutes at a speed of 2000 rpm. Serum was collected and put into a microtube for analysis using a blood chemistry analyzer to measure urea and creatinine values as parameters of kidney function. The effectiveness of single placenta stem cell therapy and cardiomyocyte coculture combination in pigs with myocardial infarction was evaluated based on reatinine and urea values as well as histopathology of kidney tissue.

Data analysis

Urea, creatinine and histopathology values of kidney tissues were analyzed using one way analysis of variance (ANOVA) and further analysis using Tukey's test.

RESULTS AND DISCUSSION

Urea and Creatinine Values

The urea and creatinine values (mg/dL) of the kidneys of experimental pigs with myocardial infarction treated with single placental stem cells and a combination of cardiomyocyte coculture are presented in Table 1.

Statistical results showed that urea and creatinine values had significant difference ($P < 0.05$) between the group of animals without therapy (K1) and those treated with single placental stem cells (K2). In group K1, after ligation of the proximal circumflex artery and left without therapy, it was assumed that myocardial infarction occurred which resulting in myocardial dysfunction (Savira *et al.* 2020). Myocardial infarction can spread and reduce blood flow to all organs of the

body including the kidneys and causing hypoxia (Chen *et al.* 2022).

In group K2, the placental stem cell therapy was assumed can reduce myocardial tissue damage due to neovascularization, resulting in mild damage. Placental stem cells will repair myocardial tissue by secreting vascular endothelial growth factor (VEGF) and angiopoietin (Ang-1) which play a role in cell proliferation, endothelial cell migration, and neovascularization (Zhang *et al.* 2021). This condition improved blood flow to the myocardium, resulting in a decrease in the area of myocardial infarction, which then causes blood flow to the kidneys return to normal.

The urea value of group K3 was lower and not significantly different ($P>0.05$) compared to K1, while the creatinine value of K3 was lower and significantly different ($P<0.05$) compared to K1. The decrease in urea and creatinine values was probably due to the improvement of blood flow to the kidneys as a

consequence of improvement of heart function. Stem cells induced into the myocardium will migrate to the infarct area due to stimulation of chemo attractive molecules released by damaged tissue such as stromal cell derived factor-1 (SDF-1). Stem cells will express chemokine receptors, namely cxc chemokine receptor-4 (CXCR-4) which plays a role in detecting chemoattractive molecules that promote stem cells to migrate to the infarct area (Bajdak *et al.* 2023). Placental stem cells then initiate the myocardial repair process by differentiation and paracrine signaling (Shafei *et al.* 2017).

The urea and creatinine values of the animal group treated with single placental stem cells (K2) were not significantly different ($P>0.05$) from those treated with a combination of cardiomyocyte coculture (K3). There was no difference in values in groups K2 and K3 since both placental stem cells and cardiomyocyte coculture were equally able to repair the myocardial infarction

Table 1. Urea and creatinine values (mg/dL) of experimental pigs with myocardial infarction treated with single placental stem cells and a combination of cardiomyocyte cocultures

Group	Urea ($\bar{x}\pm SD$)	Creatinin ($\bar{x}\pm SD$)
K1	17.36 \pm 4.24 ^b	1.96 \pm 0.52 ^b
K2	13.36 \pm 3.90 ^a	1.45 \pm 0.29 ^a
K3	15.75 \pm 3.43 ^{ab}	1.51 \pm 0.25 ^a

^{a,b}Different superscripts in the same column indicate significant differences ($P<0.05$). K1= No therapy/positive control, K2= Single placental stem cell, K3= Cardiomyocyte coculture combination

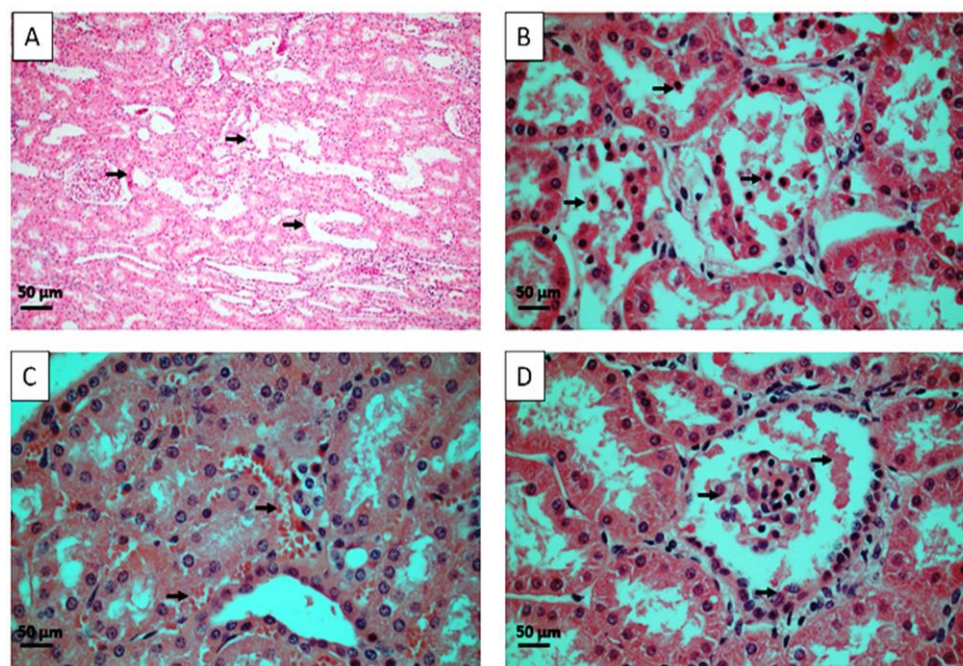


Figure 1. Renal lesions of pigs treated with single placental stem cells and cardiomyocyte coculture combination. A= Tubular dilation in K1, B= Epithelial cell necrosis and desquamation in K2, C= Congestion in K1, D= Glomerular atrophy and protein deposits in Bowman's space in K3. K1= No therapy/positive control, K2= Single placental stem cells, and K3= Cardiomyocyte coculture combination. HE staining, bar: 50µm

Table 2. Histopathological changes in pig kidneys (%) that experienced myocardial infarction and were treated with single placental stem cells and a combination of cardiomyocyte coculture

Group	Glomerular atrophy (%) ($\bar{x}\pm SD$)	Tubular necrosis (%) ($\bar{x}\pm SD$)	Tubular dilation (%) ($\bar{x}\pm SD$)
K1	20.44 \pm 2.78 ^b	30.68 \pm 6.30 ^b	29.90 \pm 2.52 ^b
K2	10.26 \pm 0.93 ^a	13.67 \pm 3.76 ^a	16.36 \pm 1.26 ^a
K3	12.44 \pm 1.21 ^a	17.23 \pm 0.55 ^a	18.03 \pm 1.76 ^a

^{a,b}Different superscripts in the same column indicate significant differences ($P<0.05$). K1= No therapy/positive control, K2= Single placental stem cell, K3= Cardiomyocyte coculture combination

area. Cardiomyocyte coculture is a type of adult stem cell that has limited proliferation capacity, low cardiomyogenesis, and a decrease in the number of cells (Soejitno and Pande 2011). Zaruba *et al.* (2010) reported that administration of cardiomyocytes triggered most of the cells to undergo necrosis and decreased cell engraftment when transplanted into the heart of infarcted adult rats.

Renal Histopathology

Histological changes in the renal parenchyma due to myocardial infarction are glomerular atrophy, tubular dilatation, tubular necrosis and congestion (Figure 1). Glomerular atrophy is characterized by the presence of a shrinking glomerular tuft, expansion of Bowman's space, and protein deposits in Bowman's space. Tubular necrosis was characterized by the presence of necrotic and desquamated epithelial cells. Tubular dilatation is characterized by the widening of the tubular lumen. Changes in the interstitium were only found in congestion which was characterized by an increase in the number of erythrocytes in the blood vessels. The percentage of histological changes in the kidneys of pigs with myocardial infarction, treated with single placental stem cells and a combination of cardiomyocyte coculture are presented in Table 2.

The results of statistical analysis showed that the percentage of glomerular atrophy, tubular necrosis and tubular dilation after single placental stem cell therapy (K2) and cardiomyocyte coculture combination (K3) decreased and was significantly different ($P < 0.05$) compared to the group without therapy (K1). This indicated that single placental stem cells and cardiomyocyte coculture combination were able to reduce renal parenchymal damage indirectly through improving renal circulation due to increased heart function. Stem cells induced in the myocardial infarction area can reduce cardiomyocyte necrosis through cardioprotective molecules secreted by stem cells: hypoxia inducible factor-1 (HIF-1) and insulin growth factor-1 (IGF-1) (Muller *et al.* 2018). Stem cells increase the activation of the enzyme heme oxygenase-1 (HO-1) (Kirby *et al.* 2018). HO-1 is an antioxidant enzyme that causes a decrease in the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 and ROS in damaged tissues (Shan *et al.* 2019). This condition causes a reduction in the expansion of myocardial infarction and increases tissue regeneration.

The therapy in this study was carried out after the ST-elevation that caused kidney damage was still in the mild category. Mild damage could facilitate the process of kidney regeneration. The kidneys had the ability to repair tubules that are damaged by ischemia. Living tubular epithelial cells can enter the cell cycle to proliferate (Canaud and Bonventre 2015). Epithelial cells were normally in the G0 phase, which was the epithelium in a state of rest. When there was stimulation in the form of ischemia or activation of growth factors, the epithelium enters the cell cycle (Lazzeri *et al.* 2018). When the kidneys experience ischemia, it would activate the extracellular regulated kinase (ERK) signal

which could increase tubular epithelial proliferation (Jang *et al.* 2013). Epithelial cells that undergo proliferation could replace vanished cells and re-form epithelial cell polarity, thereby improving the functional integrity of the nephron (Feng *et al.* 2015).

CONCLUSION

Single placental stem cell therapy or cardiomyocyte coculture combination in pigs with myocardial infarction could reduce urea and creatinine values. Lesions found in kidney tissue after myocardial infarction formation were congestion, glomerular atrophy, dilation and tubular necrosis. Single placental stem cell therapy or cardiomyocyte coculture combination were both able to improve circulatory disorders to the kidney organ.

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