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The Effect Of MSC-Conditioned Medium (MSC-CM) Low Dosage on Urea Levels in Acute Renal Failure Rats

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ABSTRACT

Background: Acute renal failure (ARF) is associated with a high incidence of morbidity and mortality, as well as a high risk of developing chronic renal failure that is associated with elevation of urea level. The kidneys have an extraordinary ability to regenerate after injury and fully recover, and clinical options are limited to fluid management and dialysis procedures. The development of new strategies to increase the ability of kidney regeneration due to ARF, and to maintain kidney function both in the short term and in the long term is needed.

Objective: This study aims to determine the effect of low-dose MSC-CM on urea levels in ARF.

Method: In this study, 12 male gentamicin-induced Wistar rats were divided into 2 groups control (intravenous PBS administration) and treatment (intravenous MSC-CM low-dose at 0,2 cc). Parameters including urea-urine level were measured spectrophotometer and then analyzed by unpaired T-test.

Result: The results of this study showed that the mean urea levels between the control group (19.46 ± 0.56 mg / dL) and the treatment group (13.96 ± 0.73 mg / dL) were significantly different ($p < 0.05$).

Conclusion: The conclusion of this study indicated that there was an effect of low-dose of MSC-CM on urea levels in acute renal failure.

Keyword : Acute Renal Failure, MSC-CM, Urea Level

INTRODUCTION

Acute Renal Failure (ARF) still has a high incidence of morbidity and mortality and is at high risk of developing into chronic failure[1–3]. One of the assessments of kidney function is an increase in serum urea in the body[4,5]. High levels of urea can be used as an indicator of ARF[2]. There are two treatment options for kidney failure include dialysis (hemodialysis or peritoneal dialysis) and kidney transplantation that have side effect due to the body's immune system for a lot of different reasons[6]. It is important to realize that transplant patients have no control over most of these causes of transplant failure[7]. While the low blood pressure (hypotension) is one of the most common side effects of hemodialysis[8].

The development of new strategies in order to increase the ability to regenerate after ARF, as well as to maintain kidney function both in the short and long term is urgently needed[1,9]. Stem cell-based therapy is a promising option where this therapy requires cells with maximum ability to support cardiac regeneration[10,11]. The protective effects on organs by mesenchymal stem cells (MSC) and

and mesenchymal stem cell conditioned medium (MSC-CM) has been enhanced in recent decades, suggesting that either MSC or MSC-CM are capable of enhancing tubular regeneration through their paracrine effects[12,13]. However, until now, there are not many publications related to the effect of MSC-CM on urea in acute renal failure.

Acute Renal failure (ARF) is a major public health problem, it is associated with a high mortality rate, morbidity, and long-term risk of becoming a chronic disease[1,14]. In some developed countries, the prevalence of ARF is increasing[3]. About 15% of patients are hospitalized due to ARF and most of them are in critical condition[2,3]. In critically ill patients, the rate of ARF varies according to the population studied and the definition of ARF used is around 8–89% in pediatric patients and 7–25% in adults[13,14]. The Assessment of Worldwide Acute Kidney Injury, Renal Angina, and Epidemiology (AWARE) found a relationship between the incidence of ARF with morbidity and mortality in patients aged 3 months to 25 years based in the intensive care unit[14]. Cases of ALF if not handled properly, may give unfavorable results, besides that it can increase hospital stay, can develop into chronic home disease, and can increase mortality[5,15]. Based on several research references, in rat models of acute and chronic renal failure, the MSC-CM dose used was 0,2 cc as the lowest dose and 0,4 cc as the highest dose[15].

The paracrine effects of MSCs can also be produced by MSC-CM. This condition allows MSC-CM to be used for therapy as a substitute for MSC itself. MSC-CM contains various products of all bioactive molecules such as growth factors secreted by MSCs in culture media[16]. MSC-CM contains many growth factors such as IGF-1, TGF-β1, HGF, and VEGF[17]. MSC-CM therapy known was able to increase the regeneration of injured kidney tissue by suppressing cell infiltration reducing interstitial fibrosis and reducing changes in the structure of the glomerulus[18]. In vitro research conducted by Moghadasali et al. (2012) used the culture of proximal tubular epithelial cells, which are cells in the kidney induced by gentamicin, which were obtained as a result of cell repair after administration of MSC-CM from the bone marrow of MSCs[19]. Several studies have also shown that MSC-CM can improve podocyte apoptosis in STZ-induced type 1 diabetes models and provide glomerular repair in tissues in chronic disease models[20,21]. This study aimed to evaluate the efficacy of treatment with conditioned medium-mesenchymal stem cells (MSC-CM) in an acute renal failure model in rats of low doses of urea levels.

MATERIAL AND METHODS

Ethical Clearance

This research was conducted at the Stem Cell and Cancer Research (SCCR) Laboratory, Faculty of Medicine, Sultan Agung Islamic University, Semarang. The population in this study were male Sprague Dawley rats kept in the enclosure of the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung with number 254/VII/2021/Komisi Bioetik.

Acute Renal Failure Animal Model

The sample size in this study was 12 rats, with 6 rats in each group. In this study, 2 groups were used where rats were induced by gentamicin at a dose of 60mg/kg BW intraperitoneally for 10 days. Furthermore, 3 rats were terminated and their kidney histopathology was examined to assess kidney damage. Each group became 5 rats. Furthermore, the control group was treated with PBS intravenously, while the treatment group received an injection of MSC-CM at a low dose of 0.2 cc intravenously.

Urine Collection and Urea Spectrophotometry Analysis

All treatments were carried out on the 11th day. Urea levels in urine were measured in each group after the 19th day. The concentration of the sample and standard against the blank was read using spectrophotometry. In units of mg/dL.

Statistical Analysis

Furthermore, the data were obtained, processed, edited, tabulated, and cleaned, then carried out descriptive data using the mean, median, and mode. Then test the normality of the data using the Shapiro-Wilk test. The data was obtained normally distributed so that the independent sample t-test was carried out differently. Processing of data analysis was carried out using SPSS 20.0 for Windows.

RESULTS

The results of this study showed that the mean urea levels between the control group (19.46 ± 0.56 mg/dL) and the treatment group (13.96 ± 0.73 mg/dL) were significantly different (Figure 1). The results of descriptive statistical tests in this study decreased the average level of urea in the treatment group compared to the control group. Based on the results of the independent sample T-test, (p-value = 0.000) which indicates a significant difference in the average urea level in the treatment group (Treatment) compared to the control group (Control). This shows that there is an effect of giving high doses of MSC-CM on urea levels in acute kidney failure compared to controls.

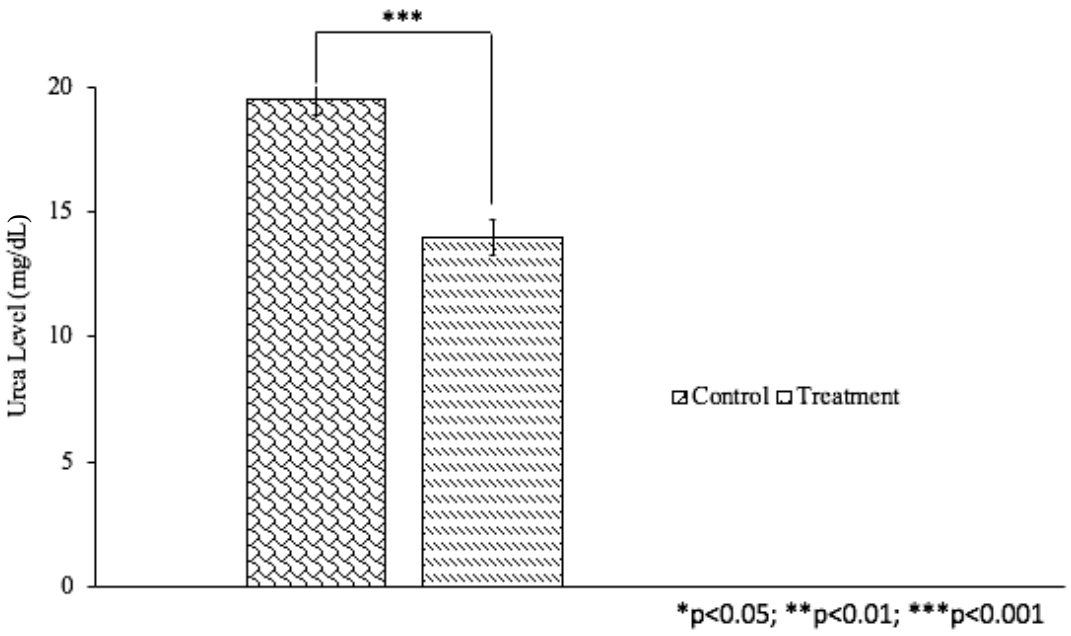

Figure 1. Spectrophotometry level of urea in the urine of; (Control) group rat model gentamicin-induced (Treatment) rat model gentamicin induced and treated with 0,2 cc MSC-CM. *** indicates a significant difference between the groups (p< 0.05; n = 6). The data represent mean ± standard deviation.

DISCUSSION

In this study, it was found that the administration of low-dose MSC-CM (0.2 cc) was able to significantly reduce urea levels compared to the control group. Several other studies are similar to the results of this study[22–24]. In this study, compared with the administration of MSC-CM in mice induced by acute kidney failure with cisplatin, the BUN and serum creatinine levels significantly decreased in the negative control group. Research conducted by Birtwistle et al. (2021) explained that the administration of MSC-CM both induced under hypoxic conditions and in normal culture was able to reduce kidney damage parameters, namely serum creatinine in the AKI model induced by cisplatin compared to the control group[14]. Research conducted by Tsuji et al. (2018) proved that presenting MSC-CM can improve kidney function in rats with acute renal failure models by reducing the rate of tissue damage and tubular cell apoptosis[13]. Different results were found in the research conducted by Zhao et al. (2020) explained that a significant decrease in BUN levels occurred in the MSC treatment group and not the MSC-CM group in the AKI rat model induced by ischemia-reperfusion injury[25].

Gentamicin is an aminoglycoside antibiotic that is widely prescribed to effectively treat patients with infections, but the associated side effects of oxidative stress and injury limit its long-term use[2]. The onset of renal failure is usually slower and the daily increase in serum creatinine tends to be lower than for other causes of acute renal failure[3]. Serum creatinine and blood urea nitrogen typically increase 7-10 days after initiation of aminoglycoside therapy[5]. Gentamicin as an aminoglycoside drug can cause damage to renal tubular cells such as disruption of the lysosomal membrane structure, mitochondria, and plasma[26]. Gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubular region[27].

Mesenchymal stem cell conditioned medium (MSC-CM) is a product development of stem cells that utilize the secretome of MSC to take advantage of the role of conventional MSC therapy in healing various tissue damage[28]. Several contents of MSC-CM based on proteomic studies gave the results that IGF-1 levels were 1515.6 ± 211.8 pg/mL; VEGF levels 465.8 ± 108.8 pg/mL; TGF-β1 levels were 339.8 ± 14.4 pg/mL and HGF levels were 20.3 ± 7.9 pg/mL[29]. In the process of repairing kidney injury, VEGF becomes an additional factor in renoprotection, while IGF-1 and HGF play a role in the acute regeneration of the post-injured kidney[30]. Exposure to gentamicin causes necrosis of the proximal tubule, thus giving MSC- In vitro CM in human kidney cell culture has been shown to increase tissue repair by increasing cell viability and accelerating cell migration after gentamicin-induced cell toxicity[23].

MSC-CM significantly reduced tubular cell apoptosis, improved kidney function, and increased survival in mice suffering from acute kidney injury[19,29,30]. MSC-CM efficiently produces nephrotoxicity by reducing the influx and capacity of dendritic cells and T lymphocyte inflammatory cytokines[31,32]. In addition, the role of nitric oxide is responsible for the MSC-CM-mediated reno-protective effect[33,34]. MSC-CM injection significantly increased cisplatin-induced acute kidney injury and inflammation as indicated by decreased levels of serum creatinine, IL-1, and IL-6 and the presence of activated neutrophils in the injured kidney[21,35,36]. This study indicates that low-dose MSC-CM affects improving kidney function compared to the control group which is in line with previous researches [32,37,38]. At the pre-clinical stage for experimental animals, presenting MSC-CM was able to provide improvement in kidney function parameters damaged by gentamicin induction[22,39,40]. The constraint of this research is the MSC isolation technique to obtain MSC-CM which is so complex[33,41,42]. This study has limitations where the researcher did not analyze the involvement of various molecules found by MSC-CM in this study, so the regeneration mechanism in this study still refers to the theory or previous studies. There was no examination of the histology of the tissue, such as the tubular injury score so tissue repair was only seen from the functional function of the kidney through a decrease in urea levels.

CONCLUSION

Administration of MSC-CM can reduce urea levels in acute renal failure rat models. Further research can be explored further by assessing the tubular injury score after MSC-CM administration in rat models of acute kidney failure with various doses

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AUTHORS' CONTRIBUTIONS

R.W.S.I. conducted the majority of the experiments, analyzed data, and prepared the manuscript. A.R.K. conducted data analysis and provide suggestions for the project. N.A.C.S. supervised the project, conceived the experiments, and wrote most of the manuscript.

COMPETING INTERESTS

The authors declare that there was no conflict of interest.

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