STEM CELLS

Mesenchymal Stem Cells Administration in Aged Male Rats Increases Testosterone and Lower TNF-α Levels

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ABSTRACT

Background: **Treatment for sex hormone depletion along with chronic inflammation would be beneficial for aging males. Mesenchymal stem cells (MSC) are known for their immunomodulatory activities and differentiation ability in regenerative medicine. Whether MSC could influence both testosterone and pro-inflammatory marker in aging males is uncertain. This study aimed to explore the effects of human umbilical mesenchymal stem cell (hUCMSC) on testosterone, tumor necrosis factor-alpha (TNF-α), and creatinine levels in aged rats.**

Methods: **The hUCMSC was administered to aged male Sprague-Dawley rats (24 months old). After four injections of 1 million per kg body weight in 3-month intervals, the rats were sacrificed, and serum was collected for biochemical examinations.**

Results: **The hUCMSC administrations increased the testosterone level almost three-fold and decreased the TNF-α level. Moreover, the high testosterone level was strongly correlated with low TNF-α level ($p = 0.013$; $r = -0.863$) in aged male rats. These results were following our previous report, which showed that the hUCMSC increased the number of Leydig cells. Serum creatinine levels in the treatment group were slightly increased but were still within the normal limit.**

Conclusion: **The hUCMSC treatment in aged male rats tends to increase testosterone levels and lower TNF-α levels.**

KEY WORDS

aging, mesenchymal stem cell, testosterone, TNF-α

INTRODUCTION

Aging is associated with the decline of physiological functions such as reproduction system which is characterized by a decrease of sex hormones. Human testosterone levels are beginning to decrease gradually after the third decade of life. Decline of fertility caused by low testosterone in older men is also related to age-related morbidities and mortality[1]. Unexpectedly, exogenous testosterone therapy showed unsatisfactory results due to long-term safety issues[2]. Aside from testosterone depletion, aging is a complex process. Aging is also accompanied by higher pro-inflammatory cytokine in circulation such as tumor necrosis factor-alpha (TNF-α)[3].

Based on the above considerations, it appears that to increase testosterone levels and lowering TNF-α seems to be a sound strategy to maintain healthy aging. Mesenchymal stem cells (MSC) are multipotent stem cells that can restore damaged tissue and modulate immune response[4]. Based on their properties, MSC is considered a promising cell-based therapy for regenerative medicine. In aged male, MSC was potential therapy for erectile dysfunction and infertility. MSC was shown to be able to differentiate into sperm-like cells and restore infertility in male rats[5]. Moreover, MSC administration was found to be safe and able to improve physical health in the elderly by lowering TNF-α levels in the elderly[6]. Despite that, there was still limited evidence whether MSC is able to lower improve TNF-α altogether with improving testosterone in old age.

Human umbilical cord mesenchymal stem cells (hUCMSC) possess all the regenerative properties of MSCs. It is also easily obtained and expanded. Compared to induced pluripotent stem cells (iPSC), which were recently concerned for their tumorigenicity potential, MSC therapy in the elderly is formerly studied safe[6,7]. This study was aimed to explore the effects of hUCMSC administration on testosterone and TNF-α level in aged male rats. In addition to that, we also measured creatinine levels because testosterone was also known to have the potential to damage renal function[8]. Administration of human source of MSC to rats may also raise ethical problem due to possible xenogeneic reactions but is already considered harmless[9].

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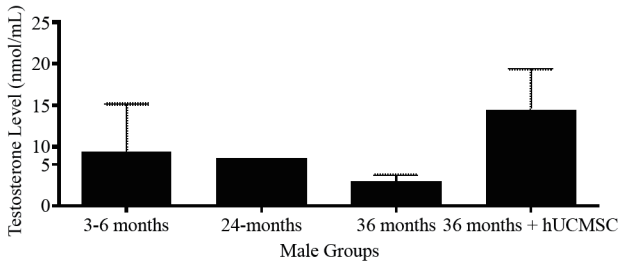

Figure 1: Testosterone Levels of Male Rats

Data are presented as mean ± standard error. The number of rats per group as follows: 3 young rats, 5 24-months old rats, 4 control groups (36 months), and 3 treatment groups (36 months + hUCMSC). As advancing age, the testosterone level was decreasing. The hUCMSC administration tend to increase the testosterone levels almost three times compared to control aged rats.

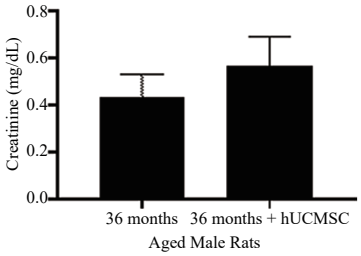

Figure 3: Creatinine Levels of Aged Male Rats

Data are presented as mean ± standard error. The number of rats per group were as follows: 4 control group (36 months) and 3 treatment group (36 months + hUCMSC). The hUCMSC treated aged rats had slightly higher creatinine level than control but still within normal limit.

MATERIAL AND METHODS

Animals

This study obtained ethical approval from Ethics Committee Faculty of Veterinary, Bogor Agricultural University (21-2016 ACUC RSHP FKH-IPB). Sprague-Dawley rats were used and kept in animal house of Faculty of Veterinary, Bogor Agricultural University; they were assigned into four groups of 6 rats each, i.e., young (3 months), old (24 months), control (36 months), and treatment group. The treated group was 24 months old rats injected 4 times with hUCMSC in a year while the control group was injected with normal saline. Twenty- four-month old rats are considered equivalent to 60 years old humans[10].

Mesenchymal Stem Cell

Treatment in this study used human umbilical cord mesenchymal stem cells (hUCMCS) which were isolated as previously mentioned[11]. Human umbilical cord-derived MSC was chosen because it was easier to obtain, easier to expand, and less immunogenic for xenogeneic research[4,12]. The hUCMCSs were administered to the treatment group through intravenous injection, 1 million per kg body weight, every three months for a year. One-year treatment was given until 36 months old, which were known as the maximum lifespan of laboratory rats[10]. This hUCMSC dose was chosen based on studies of MSC for treating age-related diseases[13]. Three months after the last injection, the rats were sacrificed.

Testosterone, Tumor Necrosis Factor-a (TNF-α), and Creatinine level

We collected the blood from the tail vein and examined the biochemical parameters. Serum was used to measure testosterone, TNF-α, and creatinine levels. Testosterone level was determined using chemiluminescent microparticle immunoassay (Abbott) while TNF-α level was measured using enzyme-linked immunosorbent assay (Elabscience). Creatinine was determined by a spectrophotometric method using DiaSys kit (Diagnostic Systems GmbH). All parameters were presented as mean ± standard error.

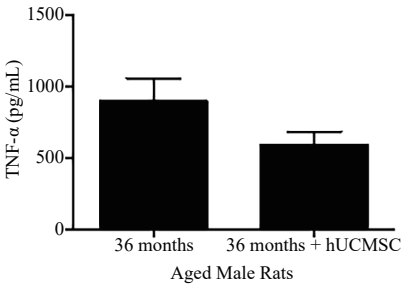

Figure 2: TNF-α Levels of Aged Male Rats

Data are presented as mean ± standard error. The number of rats per group were as follows: 4 control group (36 months) and 3 treatment group (36 months + hUCMSC). Aged rats treated with hUCMSC had higher TNF-α level than control.

Data Analysis

Data were presented and analyzed using GraphPad Prism 9 software. Kruskal-Wallis test was used to evaluate the differences in testosterone levels between four groups. Comparisons between two aged male groups were analyzed using the Mann-Whitney test while the correlation between testosterone and TNF-α was determined by the Spearman test. Differences between groups were considered statistically significant when $p < 0.05$.

RESULTS

As expected, our study showed the decline of testosterone level as advancing age (Figure 1). In addition, one year of hUCMSC administration tends to increase the testosterone levels in the treated rats (14.49 ± 4.87 nmol/L) compared with control group (3.01 ± 0.77 nmol/L). Despite the trends, the differences of testosterone levels among groups were not statistically significant ($p = 0.121$).

The TNF-α levels in aged male rats are shown in Figure 2. Aged rats treated with hUCMSC had a TNF-α level (683.3 ± 742 pg/mL) which were not significantly different with control (895.8 ± 51.8 pg/mL) ($p = 0.57$). In aged male rats, there was a strong negative correlation between testosterone and TNF-α level ($p = 0.013$; $r = -0.863$)

Creatinine levels in aged male rats are shown in Figure 3. The hUCMSC treated aged rats had slightly higher creatinine level (0.57 ± 0.1206 mg/dL) than control (0.43 ± 0.096 mg/dL), but not statistically significant ($p = 0.6286$).

DISCUSSION

Aging was accompanied by decreasing reproductive ability. This declined was associated with a low level of sex hormone in the elderly. Reproductive system aging in male rats is started at 6 months old. Testosterone level was found to decrease by 50% in 6 months old compared to 3 months old rats. Furthermore, the testosterone level in 24 months old male rats was declined significantly[14]. As expected, the testosterone level in our male rats was decreased as advancing age. The hUCMSC injections were able to restore the testosterone level in 36 months old male rats. The testosterone level was raised almost three times after hUCMSC administrations compared to control. We previously reported that the hUCMSC injection was able to recover testis histopathology features in aged rats[15,16]. The hUCMSC administration increased the number of Leydig cells, caused the seminiferous tubule became wider and the interstitial area also became narrower. All these improvements in testis were following higher testosterone levels found in the treatment group. The mechanisms of MSC regenerative effect in

the testis were considered to be mainly due to secreting paracrine factors by the triggered resident stem cells[17]. Other possible mechanisms are direct cell-to-cell communication, mitochondrial transfer, and differentiation. Locally transplanted hUCMSC to the testis showed that the stem cells differentiate into normally functioning Leydig-like cells thus recover serum testosterone levels in a male rat hypogonadism model[18]. Furthermore, MSC secretome was also found to induce testosterone production by Leydig cells[19].

The hUCMSC injections in aged rats lower the TNF-α level. This result was in accordance with MSC's ability to suppress inflammation. MSC was able to reduce inflammation by inhibition of T effector cells and regulatory T cells which later reduced pro-inflammatory cytokines production[20]. This notion is supported by an inverse correlation found between testosterone and TNF-α level in aged male rats. A high testosterone level caused by hUCMSC might further contribute to lower TNF-α. Previous studies showed that testosterone also influences the immune system. Testosterone was mostly known as an immunosuppressant[21]. The anti-inflammatory effect of testosterone exerts via inhibiting the expression of pro-inflammatory cytokines includes TNF-α through the nuclear factor kappa-B (NF-κB) pathway and also by increasing anti-inflammatory cytokines (IL-10) synthesis[20]. Our findings may also add more support for hUCMSC therapy for the emerging COVID-19 disease, which involves a highly inflammatory state.

Since hUCMSC cound increase testosterone level and that testosterone was reported to be harmful to the kidney[8], we also measured the creatinine levels of the rats. Testosterone can influence the creatinine level through its ability to induce podocyte damage and apoptosis along with the presence of testosterone receptors in the kidneys[22]. In our study, hUCMSC treated aged group had a somewhat higher creatinine level than the control. Nonetheless, serum creatinine levels in both aged male groups were still within the physiological limit of Sprague-Dawley rats[23]. Data from human studies are also inconsistent[24,25]. Additionally, mesenchymal stem cells also confer renal protection effect to some extent[26].

The limitation of our study is the small number of samples due to the natural death of old rats. Nonetheless, our study showed that the hUCMSC administrations could be beneficial as a safe rejuvenating agent in term of male testosterone replenishment.

CONCLUSION

Mesenchymal stem cell administration in aged male rats can increase testosterone levels together with decrease TNF-α. Elevated testosterone levels does not affect serum creatinine levels.

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