

## Brain Derived Neurotrophic Factor Levels in Aged Rats Post-Systemic Human Mesenchymal Stem Cell Administration

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### ABSTRACT

Brain-Derived Neurotrophic Factor (BDNF) levels were affected by aging. Brain BDNF levels were known to decrease along with advanced age thus correlated with any diseases such as cognitive impairment and Alzheimer. Mesenchymal Stem Cell (MSC) is one of the potential modalities actively investigated against age-related diseases. This study evaluated the effect of human MSC administration to brain BDNF levels in aged rats. Intravenous injection of 10 million per body weight human MSC were given four times in 3 months interval to 22-24 months old female and male Sprague–Dawley rats. As control group, aged rats were injected by normal saline at the same volume and frequencies. Moreover, young 3-6 months rats also examined as negative control. By the end of the experiment, we analyzed three rats from each group. Brain BDNF levels were measured by enzyme-linked immunosorbent assay and normalize to the protein levels. One-way ANOVA and LSD post hoc analysis was performed to compare the differences between groups. BDNF levels in male appeared similar between young, aged, and MSC treated groups. Meanwhile, control aged female groups had significantly lower BDNF levels compared to young ( $p = 0.019$ ) and MSC-treated aged rats ( $p = 0.001$ ). There was no difference of BDNF levels between young and MSC-treated aged in female rats ( $p = 0,068$ ). Both sex had similar BDNF levels ( $p = 0.249$ ) in control-aged groups. In contrast, female young and MSC-treated aged rats achieved significantly higher BDNF levels ( $p = 0.009$  and  $p < 0.001$ ) compared to the male groups, respectively. These results suggest that human mesenchymal stem cell intravenous injection can increase brain BDNF levels in female aged rats.

**Keywords:** BDNF, human mesenchymal stem cell, aged rats.

## INTRODUCTION

One of the major molecules in ageing is Brain-Derived Neurotrophic Factor (BDNF). Study in human suggested that plasma BDNF is a biomarker of impaired memory and general cognitive function in ageing women (Komulainen et al, 2008). BDNF is highly expressed in brain areas demonstrating functional plasticity (Tapia-Arancibia et al, 2008). Brain BDNF levels were known to decrease along with advanced age thus correlated with any diseases such as cognitive impairment and Alzheimer (Budni et al, 2015). Cognitive defects associated with aging might be related to BDNF system (Calabrese, 2013). Restoring BDNF levels may be promising therapeutic target in preventing age-related diseases. Mesenchymal Stem Cell (MSC) role in aging was being enormously investigated against age-related diseases. Human adipose tissue-derived MSC (ADMSCs) intravenous injection in aged male ICR mice (18-month-old) able to

## METHODS

The protocol of this study has been approved by the Animal Care and Use Committee Veterinary Hospital Faculty of Veterinary Bogor Agricultural Institute (N0: 21-2016 ACUC RSHP FKH-IPB). Female and male, 22-24 months old Spraque-Dawley rats were housed individually, fed ad libitum in 12-hour light/dark cycle. WJ-MSC used in this study was prepared according to De Bruyn et al (2011).

The aged rats were divided into 2 groups: positive control group and WJ-MSC treatment group. The WJ-MSC treatment groups

restore whole brain BDNF levels and showed cognitive improvement (Park et al, 2013). Human amniotic membrane-derived MSC and ADMSCs injection in 10-month-old male F344 rats elongate health span and life span along with neurotrophic factors recovery (Kim et al, 2015). Another source of MSC, Wharton-Jelly (WJ) MSC, which is easier to obtain may also has promising potential advantage for treatment of age-related diseases.

In human studies, plasma BDNF associated with general cognitive decline in ageing women but not in men (Komulainen et al, 2008). Moreover, Chan and Ye (2017) considered sex influences in BDNF levels on sex hormones, which were able to modulate the activities of BDNF. Thus, evaluating BDNF in both sexes might be valuable. This study evaluated the effect of human WJ-MSC administration to brain BDNF levels in female and male aged rats.

were further divided into 4 different doses:  $10^6$  per kg body weight four times at 3-month intervals,  $10^7$  per kg body weight four times at 3-month intervals,  $10^6$  per kg body weight twice at 6-month intervals, and  $10^7$  per kg body weight twice at 6-month intervals. Normal saline were injected to the positive control group in the same volume and intervals as WJ-MSC treatment groups.

After one year, the aged rats were sacrificed. Blood and organs were collected and stored for further analysis. Brain BDNF levels were measured by enzyme-linked immunosorbent assay (Elabscience Catalog No:

E-EL-R1235) and normalize to the brain protein levels. BDNF levels are expressed in pg/mg protein. The results were compared to young rats 3-6 months old. One-way ANOVA and LSD

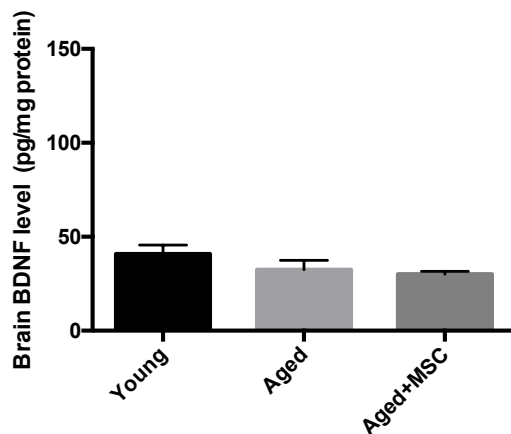
## RESULTS

At the end of our experiment, the mortality rate of the WJ-MSc treatment groups was high. Thus in this report, we analyzed one of

post hoc analysis was performed to compare the differences between groups. Statistic analyses were prepared using SPSS 20.

the WJ-MSc treatment groups dose  $10^7$  per kg body weight four times at 3-month intervals. We evaluated three rats from each group.

A.



B.

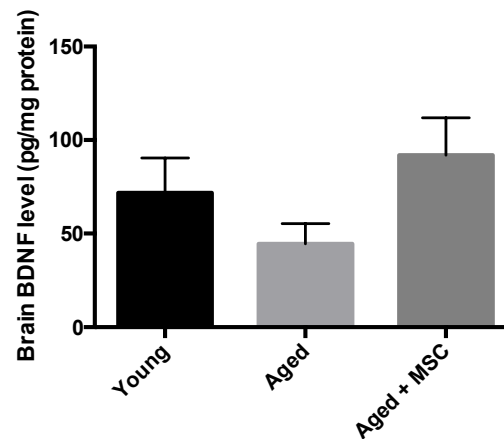


Figure 1. BDNF levels in A: male rats; B: female rats (n = 3). Values are presented in mean  $\pm$  SD.

BDNF levels in male appeared similar between young, aged, and MSC treated groups (Figure 1). Meanwhile, control aged female groups had significantly lower BDNF levels compared to young ( $p = 0.019$ ) and MSC-treated aged rats ( $p = 0.001$ ). There was no difference of BDNF levels between young and MSC-treated aged in female rats ( $p = 0.068$ ). Both sex had similar BDNF levels ( $p = 0.249$ ) in control-aged groups. In contrast, female young and MSC-treated aged rats achieved significantly higher BDNF levels ( $p = 0.009$  and  $p < 0.001$ ) compared to the male groups, respectively.

## DISCUSSION

Our research showed the differences in male and female aged rats after repetitive WJ-MSc intravenous injection. Brain BDNF levels in female aged rats markedly increased after human WJ-MSc intravenous injection but not in the male rats. This sex difference response might be related to the role of sex hormones as previously studied (Chan and Ye, 2017). Estrogen receptors are found in the brain (hippocampus and frontal lobes), these two areas are important for cognitive function. Research

about the estrogen role in BDNF expression is still in controversial.

Other factors could also influences BDNF expression in the brain. Senescence causes loss of endogenous rhythms of BDNF expression. This is associated with structural, molecular, and cellular changes of the aged brain thus lead to alterations in circadian clock (Coria-Lucero 2016). The location of the brain sample measured for BDNF also may influence the

## CONCLUSION

In conclusion, WJ-MSC intravenous injection increased brain BDNF levels in female aged rats.

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