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Response of human umbilical cord mesenchymal stem proliferation during exposure to curcumin extract variations in dose and time

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Abstract

Mesenchymal stem cells can be isolated from various body tissues, including umbilical cord tissue or mesenchymal stem cells (hUC-MSC). hUC-MSC cells have various essential features, such as immunomodulators and low immunogenicity. Efforts to explore the potential of hUC-MSC cells cannot be separated from the expansion of these cells with in vitro culture techniques. In vitro culture must be able to maintain the characteristics and functional properties of hUC-MSC cells so that they retain their natural abilities. Curcumin is an active compound from turmeric and can be extracted from the rhizome plant Curcuma longa L. Its potential has been extensively studied as a therapeutic agent due to its activity targeting various molecular signaling pathways.
In contrast, the proliferation of hUC-MSC cells is needed to support the success of therapy. This study proved that curcumin at a dose of < 10 µM could maintain the proliferation cells of hUC-MSC at 3 days and seven days of exposure. Meanwhile, curcumin at concentrations of 0.5 µM and 1µM significantly increased the proliferation of hUC-MSC cultures at three days and seven days of exposure. This finding can be considered for using curcumin as a culture supplement to maintain the growth and proliferation of cells in the in vitro culture of hUC-MSC cells.

Keywords; hUC-MSC, curcumin, cell culture, mesenchymal, proliferation

INTRODUCTION

Regenerative medicine has experienced a very rapid growth trend in the medical world. These advances involve interdisciplinary sciences such as molecular biology, stem cells, tissue engineering, biomaterials, drug discovery, and various wound-healing technologies. One of the agents in regenerative medicine is stem cells (Chen et al., 2012).

Mesenchymal stem cells in humans are very abundant. One source is from umbilical cord tissue or human umbilical cord mesenchymal stem cells (hUC-MSC). Mesenchymal stem cells originating from the umbilical cord are a type of perinatal stem cell. Cord stem cells have a three to four-times higher proliferation rate than mesenchymal stem cells from bone marrow and adipose tissue. (Marino et al., 2019). The hU-MSC has low immunogenicity characteristics because of the absence of HLA-DR surface protein and common expression of HLA protein. Which simultaneously induces the proliferation of regulatory T cells, where these cells will suppress immune activity in response to alloantigens. (Marino et al., 2019) These immunosuppressive characteristics make hUC-MSC cells potentially a safer therapeutic agent. Curcumin is a secondary metabolite compound that is an active compound and has been known to regulate specific signaling molecules so that their activity can affect physiological functions at the cellular level (Hewlings & Kalman, 2017). Curcumin is reported to increase proliferation by reducing oxidative stress (Attari et al., 2015). Oxidative stress can inhibit proliferation, improve the aging process (senescence), and inhibit immunomodulation regulation. (Denu & Hematti, 2016). Curcumin can suppress the rate of apoptosis by decreasing the expression of the protein caspase-3, causing an increase in the proliferation of neuronal stem cells. In addition, curcumin also modulates proapoptotic genes such as p38 and JNK in (Wanjiang et al., 2022).
The involvement of various factors in the pro-proliferative activity of curcumin in stem cells has been studied, such as the effect of dose (Wanjiang et al., 2022) and the impact of exposure time (Attari et al., 2015). However, research with exposure time variable uses Neural stem Cell (NSC). This study explored curcumin's role in improving the quality of hUC-MSC cells by combining time and dose variables on proliferative activity.

METHODS

Material

This study used the consumables and reagents: serological pipettes 5 ml, 15 ml, 20 ml falcon tube, microtip, microtube, shrink filter 0.22 mol/L, T75 and T25 flask (Corning Costar), -MEM GlutaMax (GibcoTM Cat. (Cat No. 12103C), PBS Free-Water, Alcohol, TGF-β2 (Sigma Cat No. T2815), WST-1 reagent (Sigma Cat No. 5015944001), hUC-MSC cells (cell culture collection Eijkman BRIN Research Center)

Cell Expansion

hUC-MSC cells from the Eijkman BRIN Research Center's cell culture collection were cultured in vitro using -MEM media standard with L-Glutamine (0.03%) and anti-anti (1%). Passage 3 cells were plated on a 6-well plate with a cell density of 3 x 105/well as much as three wells. Cells were observed every day until the second day the cells had reached 85% confluent and were passed so that they became passage four cells. The media was changed every two days.
The result of the cell calculation is 1.5 x 106. Cells were then expanded using a T75 flask until they were confluent again on day 2 of culture, then passed and counted again using the same method. The cell count results amounted to 2.1 x 106; then, culture treatment can be carried out using curcumin extract.

Curcumin Treatment

Curcumin was dissolved with 0.01% DMSO for culture treatment. Culture treatment of hUC-MSC cells was carried out using 96 healthy plates with various doses of 0.5 M, 1 M, 5 M, and 8 M. Positive control used growth factor TGF-β2 and negative control only culture media. All treatment groups were cultured for 3 and 7 days, and their morphology was observed.

WST-1 Proliferation Test

All hUC-MSC cultured cells were cultured for 72 hours at 37°C with 5-6.5% CO2 content. The culture results were harvested, and the proliferation results were analyzed using the WST-1 assay. The WST-1 assay used WST-1 reagent (Roche, No. Cat. 11644807001), which was added to 20 l of cultured cells and incubated for 4 hours at +37°C and 5 to 6.5% CO2. Proliferation results were measured with an ELISA reader at a wavelength of 450 nm. The actual absorbance is then made into a linear regression curve. After that, the actual absorbance is entered into the x value in the Lambert-Beer equation.

Statistic test

The absorbance data was then processed by statistical tests using SPSS. The data were statistically tested using the Shapiro-Wilk normality test, Levene's homogeneity, and One-Way ANOVA, followed by further statistical analysis (Post-Hoc) using the Tukey HSD method. Significance was obtained at p-value <0.05.

RESULTS AND DISCUSSION

The cell culture results after passage 4 obtained a total of 2.1 x 106 and reached 85-90% confluence (figure 1). The number of cells obtained was sufficient to be used in subsequent treatments. According to Liu et al., cultured cells reaching a confluence above 80% can be passed through or receive further treatment (Liu et al., 2020).

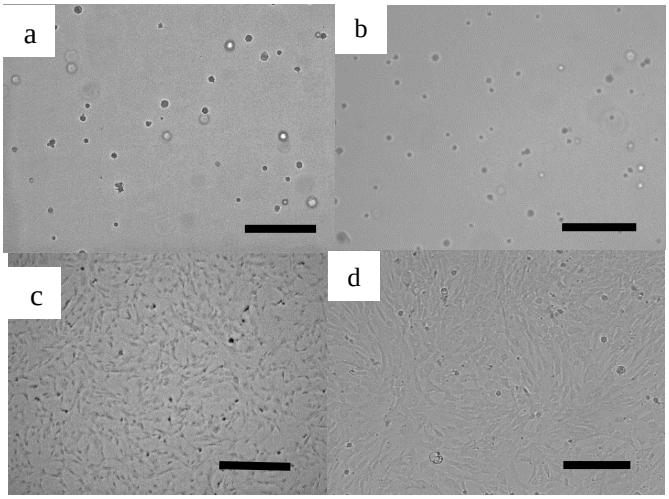

Figure 1. Cell culture results in a) Cell culture at passage 3, b) cell culture at passage 4 c) cell culture at 85% confluency d) cell culture at 90% confluence (100 X magnification) (bar graph 100 um)

Cell cultures were treated with curcumin to stimulate cell proliferation. Curcumin (diferuloylmethane) is an active compound found in rhizomes (tubers), namely Curcuma longa L. This compound has been widely reported to have activities that affect biological processes in cells, including anti-oxidative stress, anti-inflammatory, anti-proliferative, anti-inflammatory, and anti-inflammatory properties. Cancer is an immunomodulator and stimulates the differentiation process in stem cells. In this study, human umbilical cord mesenchymal stem cells (hUC-MSC) were cultured with the addition of curcumin with varying concentrations, and the response was observed on days 3 and 7. The same thing was done by Atari et al., who stimulated the proliferation of mesenchymal stem cells. With curcumin until day 7 (Attari et al., 2015).

The results of the WST-1 assays as a cell proliferation test showed that on the 3rd day after culture, the color was lighter than the cell culture on the 7th day. The results indicated an increase in cell proliferation from day seven compared to day 3, seen by the color absorption rate of fading (figure 2)

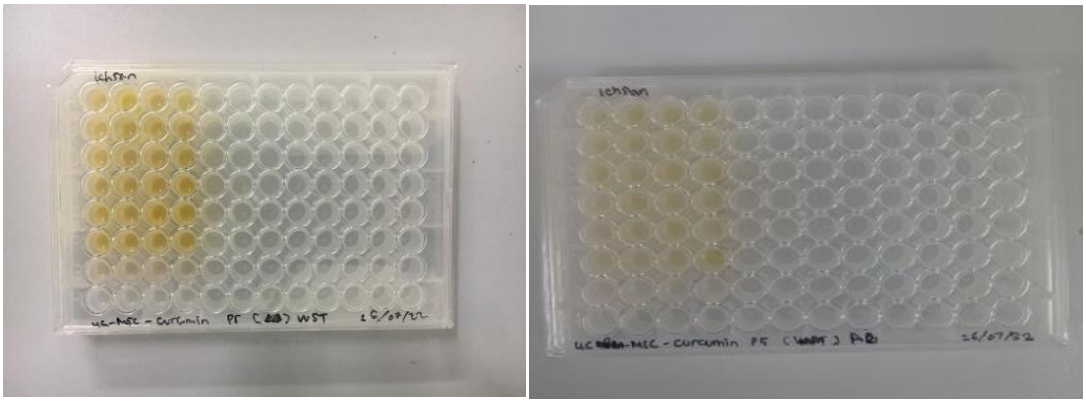

Figure 2. The cell culture was given by WST-1 reagent and incubated for 4 hours, changing color to yellow. a) treatment group on day 3. b) treatment group on day 7

The results of the WST-1 assay with the ELISA test were calculated semi-quantitatively. The number of proliferating cells is depicted in the form of bar charts in Figures 3 and 4. Section 3(a) shows the number of proliferating cells on day 3 of curcumin treatment. Each treatment group (control group and curcumin treatment group) showed statistical tests that were not significantly different ($p > 0.05$). Figure 3(b) shows a bar chart showing the number of proliferating cells on day 7 of curcumin treatment. The results of the homogeneity test statistical test showed that there was no significant difference between the treatment group and the control group. However, the 5uM treatment group showed a higher number of proliferating cells than the other groups, as well as on the 3rd day of treatment after curcumin administration. In the 8 M dose of curcumin treatment group, there was a decrease in the number of proliferating cells on the 3rd and 7th days of curcumin treatment.

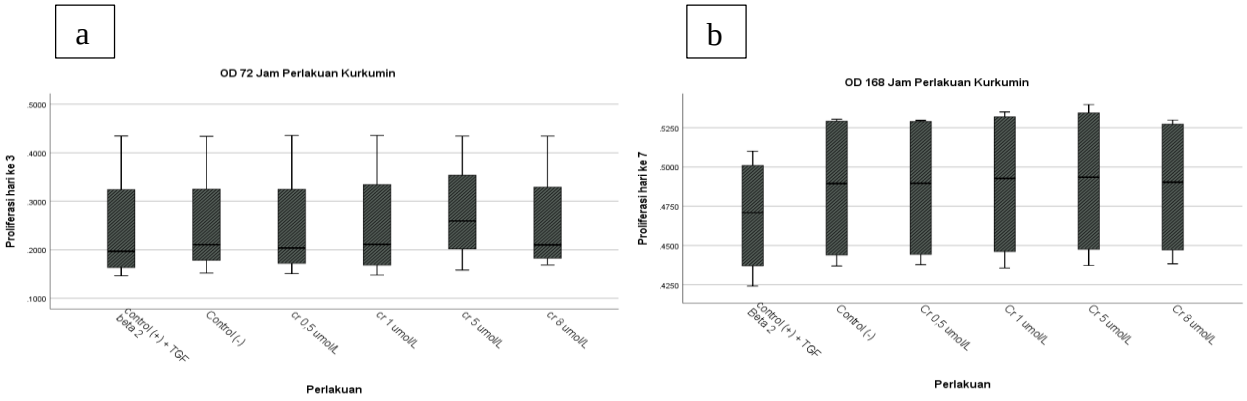

Figure 3. Shows a bar chart of the comparison of proliferation rates at different doses, namely 0.5, 1, 5, 8 M a) Proliferation rate to dose variation at 72 hours b) Proliferation rate to dose variation at 168 hours

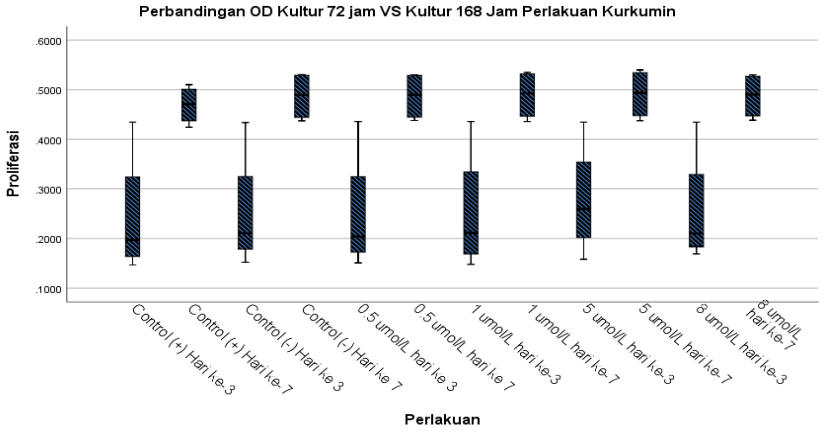

Figure 4. Comparison of the proliferation rate of hUC-MSC cells on day 3 and day 7 at each treatment concentration. A significant increase in concentration occurred in the negative control samples, 0.5 mol/L, and 1 mol/L ($p < 0.05$)

The results show that the variation of curcumin dose in all treatment samples in the 3rd and 7th-day groups compared to the positive control showed that the interpretation of curcumin dose in hUC-MSC culture did not have a significantly different proliferative effect. Although the graph shows an upward trend, the significance is not statistically proven to be different. However, the impact of curcumin showed its role in maintaining the stability of proliferation in hUC-MSC.

Furthermore, on day 7, only concentrations of 0.5 and 1 M experienced a significant increase compared to the day three treatment with the same dose. At the same time, the amounts of 5 and 8 M did not experience a significant increase. The results prove that different doses also regulate the response of curcumin, although it takes other time. These results are in line with studies conducted by Attari et al which showed an increase in NSC cell proliferation at a concentration of 0.1; 0.5; and 1 M and against BM-MSC at a concentration of 0.5 M with an exposure time of 72 hours after culture compared to control (Attari et al., 2015). At the same time, 5 and 10 M doses decreased proliferation at 72 hours of exposure.

Meanwhile, another report also said that curcumin decreased proliferation in BM-MSC at a concentration of 10 M (Yang et al., 2021). This dose was used based on a study that reported that curcumin at doses < 5 M could increase proliferation in NSC and BM-MSC cells (Attari et al., 2015). The same report also said that the quantity of 0.1, 0.5, and 2.5 M increased proliferation in NSCs (Ma et al., 2018).

Meanwhile, at doses of 5 and 8 M on culture day three, curcumin did not increase significantly with day 7 ($P > 0.05$). BM-MSC cells cultured with curcumin were also reported to have increased proliferation after seven days of culture at a concentration of 0.1 – 1 M (Yang et al., 2021). In the same study, curcumin concentrations of 5 and 8 M increased apoptosis after seven days of culture, although at 7-day intervals, proliferation still occurred. However, it will gradually decrease at concentrations > 5 M.

Based on the time assay, the curcumin extract exposure was divided into two groups: the 72 hours or three-day test group and the 168 hours or seven-day test group. Curcumin at concentrations of 0.5 and 1 M on day 7 experienced a significant increase when compared to day 3. This study is also in line with a survey conducted by (Attari et al., 2015) which saw an increase in proliferation on day three compared to day two after culture on 0.5 and 1 M treatments.

Based on the variation of curcumin dose, all treatment samples in the 3rd and 7th-day groups were compared with the positive control. It was found that the interpretation of curcumin dose in hUC-MSC culture did not have a significant proliferative effect. Although graphically, there is an increase, this is not statistically proven. However, the impact of curcumin which has an increasing trend towards positive and negative controls, although not significant, allows curcumin to play a role in maintaining the stability of proliferation in hUC-MSCs. Furthermore, on day 7, only concentrations of 0.5 and 1 M experienced a significant increase from the observation on day 3. In contrast, doses of 5 and 8 M did not experience a significant increase. The results prove that different doses also regulate the response of curcumin, although it takes other time.

Curcumin is thought to play a role in maintaining functional properties in cells, although, in this study, it is not known how the role of curcumin controls this process. However, the effect of curcumin in maintaining the proliferation of hUC-MSC cells can be seen even though it is not directly affected by dose variations at the same time. The results s is in line with research conducted by (Attari et al., 2015) which found that curcumin at treatment concentrations < 10 M increased proliferation in bone marrow mesenchymal stem cells (BM-MSC). Likewise, the study (So et al., 2008) said that curcumin at a dose of 500 nM induced proliferation 24 hours after culture.

As a comparison of curcumin treatment on hUC-MSC proliferation, positive control and negative control were used. The positive control was given the addition of TGF 2, and the negative control was not given any treatment other than the growth medium. However, the results obtained were that the negative control experienced a significant increase from culture day 3 to day seven compared to the positive control ($P < 0.05$).

Curcumin at concentrations of 0.5 and 1 M on day 7 experienced a significant increase when compared to day 3. This study is also in line with a survey conducted by (Attari et al., 2015) which saw an increase in proliferation on day three if compared to pa.

CONCLUSION

Curcumin can stimulate the proliferation of hUC-MSC cells in a dose-dependent manner where the doses that significantly increase proliferation were 0.5 and 1 mol/L or low (< 5 mol/L). Second, the time-dependent manner of curcumin also affected the proliferative response of hUC-MSC to different doses. The exposure time variables given were day three and day 7, with a significant increase in the rate of proliferation in the treatment with low curcumin concentrations of 0.5 and 1 mol/L. Curcumin affects stimulating the proliferation of hUC-MSC cells related to the exposure time.

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