Conditioned-medium of stem cells from human exfoliated deciduous teeth prevent apoptosis of neural progenitors

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KEYWORDS
Secretome; Stem cells from human exfoliated deciduous teeth; Apoptosis; Neural progenitor; Gamma-aminobutyric acid; Neuroregeneration

Abstract  Purpose: This study aimed to evaluate the neuroprotective ability of the conditioned medium of stem cells from human exfoliated deciduous teeth (CM-SHED) to prevent glutamate-induced apoptosis of neural progenitors.

Materials and methods: Neural progenitors were isolated from two-day-old rat brains, and the conditioned medium was obtained from a mesenchymal stem cell SHED. Four groups were examined: neural progenitor cells cultured in neurobasal medium with (N+) and without (N-) glutamate and glycine, and neural progenitor cells cultured in CM-SHED with (K+) and without (K-) glutamate and glycine.

Results: The expression of GABA A1 receptor (GABAAR1) messenger RNA (mRNA) in neural progenitor measured by real-time quantitative PCR. GABA contents were measured by enzyme-linked immunosorbent assay, whereas the apoptosis markers caspase-3 and 7-aminoactinomycin D were analysed with a Muse® cell analyzer. The viability of neural progenitor cells in the K+ group (78.05 %) was higher than the control group N- (73.22 %) and lower in the N+ group (68.90 %) than in the control group. The K+ group showed the highest GABA con-

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Neural death is a major symptom in neurodegenerative diseases such as Alzheimer’s, Parkinson’s, multiple sclerosis, and Huntington’s disease. Apoptosis results from a trigger that activates a signal transduction pathway leading to cell death. Some triggers for apoptosis in chronic neurodegenerative diseases include excitotoxicity mediated by increased extracellular glutamate levels, increased formation of free radicals, induced DNA damage, increased expression of genes encoding the p53 protein, and damage to the plasma membrane (Kermor, 2004, Taylor, 2008). Excitotoxicity is an essential component of the pathogenesis of brain damage during the perinatal period. This process initiates by elevated extracellular glutamate levels, which lead to hyperactivity of the glutamate receptors, increasing calcium (Ca^{2+}) influx and ultimately resulting in apoptosis (Allen et al., 2004, Almeida et al., 2005). Glutamate can be converted into gamma-amino butyric acid (GABA). Thus, GAD reduces extracellular glutamate levels. In mature neurons, GABA is a major inhibitory neurotransmitter of the central nervous system, whereas in neural progenitors, GABA can cause chlorine (Cl^-) efflux, resulting in depolarization which leads to neural excitation (Brooks-Kayal and Russek, 2012, Egusa et al., 2012). To perform its function, GABA must bind its receptor. The GABA A1 receptor (GABAAR1) is the most common GABA receptor in the brain, which may inhibit apoptosis when activated. Caspase-3 is expressed as its inactive form procaspase-3, which has a nitrosylated cysteine residue in its catalytic site. During apoptosis, denitrosylation of this cysteine residue converts nitrosylated cysteine residue in its catalytic site. During apoptosis, denitrosylation of this cysteine residue converts nitrosylated cysteine residue in its catalytic site. However, this process is not invasive, and cell availability is unlimited (Miura et al., 2003, Wei et al., 2012, Khademizadeh et al., 2019).

SHED also possess neuroregenerative and neuroprotective capabilities owing to the secretion of their metabolites into their conditioned medium (CM) (Kerkis and Caplan, 2012, Inoue et al., 2013). One such metabolite is GAD. In this study, we assessed the neuroprotective potency of the conditioned medium of stem cells from human exfoliated deciduous. This study provides a scientific basis for the use of CM-SHED to treat neurodegenerative diseases, which has not been previously reported.

2. Materials and methods

2.1. Cultures of neural progenitors and CM-SHED

Neural progenitors were obtained from the brain of two-days-old Sprague-Dawley rats (Rattus norvegicus). SHED were isolated and characterized as previously reported (Inoue et al., 2013). CM-SHED was produced following a previously published method (Zainuri et al., 2018). Briefly, SHED culture which had reached 90% confluency, was added with osteocyte induction medium (Stempro osteogenesis differentiation kit Gibco A10072-01), adipocytes (Stempro adipogenesis differentiation kit Gibco A10070-01) and chondrocytes (Stempro Chondrogenesis differentiation kit Gibco A10071-01).

Neural progenitors were isolated as described in previous studies (Lee et al., 2009, Zainuri et al., 2018). The materials used for neural progenitor cell isolation were as follows: trypsin-ethylenediaminetetraacetic acid (EDTA) (T3924; Sigma Aldrich, Saint Louis, USA), phosphate-buffered saline (PBS) (10X), pH 7.4 (70011069; Gibco, Carlsbad, USA), Hanks’ balanced salt solution (HBSS) (14175-095; Gibco), sodium bicarbonate (NaHCO3) (S7277; Sigma Aldrich, Saint Louis, USA), sodium chloride (NaCl) (746398; Sigma Aldrich, Saint Louis, USA), potassium bicarbonate (NaHCO3) (S7277; Sigma Aldrich, Saint Louis, USA), sodium dihydrogen phosphate (Na2HPO4) (S3139; Sigma Aldrich, Saint Louis, USA), sodium dihydrogen phosphate (KH2PO4) (P5655; Sigma Aldrich, Saint Louis, USA), sodium bicarbonate (NaHCO3) (S7277; Sigma Aldrich, Saint Louis, USA), poly-d-lysine (P6407; Sigma Aldrich, Saint Louis, USA), antibiotic–antimycotic (100X) (15240062; Gibco, Carlsbad, USA), fetal bovine serum (FBS) (F4135; Sigma Aldrich, Saint Louis, USA), antibiotic–antimycotic (100X) (15240062; Gibco, Carlsbad, USA), and GlutaMAX supplement (35050; Gibco, Carlsbad, USA).

One of the treatment options under development for neurodegenerative diseases is using stem cells. Previous studies have reported that human adipose derived stem cells (hADSCs) are capable of differentiating directly into dopaminergic neurons (Spitzer, 2006). Stem cells from human exfoliated deciduous teeth (SHED) are adult stem cells derived from the deciduous dental pulp tissue. Stem cell lines were first isolated and characterized as previously reported (Inoue et al., 2013). SHED have the following advantages: the retrieval procedure is not invasive, and cell availability is unlimited (Miura et al., 2003, Wei et al., 2012, Khademizadeh et al., 2019).
Flow cytomtery characterization of SHED was carried out (Table 1). The polysialic acid neural cell adhesion molecule (PSA-NCAM) + and A2B5-PSA neural progenitor markers were characterized using A2B5 (+) Rat (APC) (130093582; Miltenyi Biotec Inc., Auburn, USA), PSA-NCAM( +) Rat (PE) antibody (130-120-437; Miltenyi Biotec Inc.), isotype phycoerythrin (PE) conjugated immunoglobulin M (IgM) (130099127; Miltenyi Biotec Inc.), and isotype allophycocyanin (APC) conjugated IgM (130102673; Miltenyi Biotec Inc.). On the first day after isolation, the neural progenitors were divided into the following four groups: neural progenitors cultured only in neurobasal medium (N-group), neural progenitors cultured in neurobasal medium-plus glutamate (10 μM) and glycine (1 μM) (N + group), progenitors cultured in neurobasal medium with CM-SHED without glutamate and glycine (K- group), and neural progenitors cultured in neurobasal medium with CM-SHED plus glutamate (10 μM) and glycine (1 μM) (K + group). After 24 h, GABA, GABAAR1 and GABAAR1 mRNA levels in neural progenitors cultured with CM-SHED (K + and K-) had higher viability than those cultured without CM-SHED (N + and N-). After treatment, the percentage of live neural progenitors in the K + group (78.05 %) was higher than that in the N- control group (73.22 %), the N + group had a lower viability (68.90 %).

Among all the of experiments, the highest and lowest level of GABA was observed in the K + and N + group, respectively (Fig. 2). There was a significant difference (p < 0.05) of GABA content between the K + group and the other groups (Fig. 3).

The expression of GABAAR1 content was observed to be significantly higher in the N + group than the other groups (p < 0.05) (Fig. 4).

Among all the of experiments, the highest and lowest level of GABA was observed in the K + and N + group, respectively (Fig. 2). There was a significant difference (p < 0.05) of GABA content between the K + and N-group (73.22 %), the N + group had a lower viability (68.90 %). The neural progenitors’ biomarkers PSA-NCAM + and A2B5- were characterized using flow cytomtery. Among the five fractions, only fractions two, four, and five had a population of PSA-NCAM + and A2B5- cells above 80 %.

This study has been approved by the National Board of Health Research and Development Jakarta, Indonesia (approval number LB.02.01 / 5.2 / KE.055).

3. Results

The neural progenitors’ biomarkers PSA-NCAM + and A2B5- were characterized using flow cytomtery. Among the five fractions, only fractions two, four, and five had a population of PSA-NCAM + and A2B5- cells above 80 %.

The expression of GABAAR1 mRNA in the K + group was higher than that in the N- group (p < 0.05), whereas in the N + and K- groups, the levels were lower than the N- group (p < 0.05).

4. Discussion

In the present study, we found that neural progenitors cultured with CM-SHED were more viable than those cultured without CM-SHED were. This is presumably due to the role of CM-

Table 1 Flow cytometry characterization of stem cells from human exfoliated deciduous teeth (SHED).

| Marker   | Passage (%) |
|----------|-------------|
|          | P2 | P3 | P4 | P5 | P7 | P8 |
| CD90+    | 99.7 | 98.7 | 99.9 | 99.9 | 97.4 | 100.0 |
| CD73+    | 99.3 | 98.6 | 99.9 | 99.9 | 97.4 | 100.0 |
| CD105+   | 70.3 | 86.6 | 99.7 | 99.8 | 95.1 | 60.9 |
| CD90+ _CD73+ | 99.2 | 98.6 | 99.1 | 99.5 | 97.4 | 100.0 |
| CD90+ _CD73+ _ | 66.2 | 86.5 | 96.6 | 99.2 | 85.8 | 61.0 |
SHED in protecting neural progenitors from damage induced by glutamate. This result is consistent with a previous study, which reported CM-SHED to suppress neuron death by glutamate induction and by several factors in CM-SHED that are involved in various neuroregenerative mechanisms, such as neuroprotection, axon extension, neurotransmission, inflammatory suppression, and microglia regulation (Lee et al., 2009). Inoue et al. reported that CM-SHED could increase the migration and differentiation of endogenous neural progenitor cells, thereby reducing ischemic brain damage (Inoue et al., 2013). Other studies have reported that the addition of CM-SHED might inhibit apoptosis, reduce tissue loss in the brain, and improve neurological function in mice with ischemic hypoxia (Mita et al., 2015, Wang et al., 2018). Another study reported that the upregulation of the phosphoinositide-3-kinase (PI3K)/Akt pathway prevents neuronal apoptosis via regulation of BCL2 and BAX expression (Yamagata et al., 2013). Yamaza et al. reported the ability of SHED to express...
the transforming growth factor-β1 (TGF-β1) and TGF-β2 receptor, platelet-derived growth factor (PDGF) receptor, extracellular signal-regulated kinases (ERK), phosphorylated ERK, Akt, and phosphorylated Akt (Yamaza et al., 2010).

This study showed no significant difference regarding GABA content between the K- and N- groups, which were not subjected to glutamate induction. This result might be caused by normal glutamate content in the CM. The addition of glutamate and CM-SHED containing GAD catalyzes the glutamate-to-GABA conversion, resulting in high GABA content. Miura et al. found that SHED could express the GAD enzyme (Miura et al., 2003). Another study reported the ability of SHED to express neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) (Esmaeili et al., 2014). One previous study reported that BDNF might enhance the release of GABA via the mitogen-activated protein kinase (MAPK) signaling pathway and cyclic adenosine monophosphate-response element binding protein (CREB) transcription factors (Yamagata et al., 2013).

The expression of GABAAR1 mRNA was highest in the K+ group compared to that in the other groups. This is probably due to the GABA-mediated activation of the MAPK pathway and phosphorylation of CREB at the serine 133 residue. CREB transcription factors are active when bound to other CREB family members, forming a heterodimer structure. Thereafter, they bind to the cAMP response element (CRE) in the promoter region of GABAAR1, which has a TGACGTCA motif, resulting in GABAAR1 mRNA transcription. It has been proven that the administration of muscimol, a GABAAR1 agonist, causes a subunit change from γ2 to non-γ2 in the GABAAR1 synaptic region, resulting in decreased GABAergic synapse strength (Yamaza et al., 2010). In the present study, the lowest GABA content was found in the N+ group, presumably due to the binding of GABA to

Fig. 2 Effect of conditioned medium of stem cells from human exfoliated deciduous teeth (CM–SHED) with or without the addition of glutamate and glycine on gamma-aminobutyric acid (GABA) levels in neural cell culture. *Significant difference, p < 0.05.

Fig. 3 Effect of conditioned medium of stem cells from human exfoliated deciduous teeth (CM-SHED) with or without the addition of glutamate and glycine on the level GABA A1 receptor (GABAAR1) protein in neural cell culture. *Significant difference, p < 0.05.
GABAAR1. GABAAR1 content was highest in the N+ group. Another possibility for the reduced GABA content in the N+ group is the negative feedback from GABAAR1. It has been reported that GABAAR1 activation lowers the GAD65 content via the BDNF/ tropomyosin receptor kinase B (TrkB) signaling pathway. Decreased levels of GAD65 cause a reduction in GABA (Obrietan et al., 2002). The high concentration of GABAAR1 in the N+ group was also thought to be due to BDNF, which increases GABAAR1 protein expression on the cell surface via the TrkB, protein kinase C (PKC), and PI3K pathways. In each pathway, BDNF exerts positive feedback on GABAAR1 (Matsuzaki et al., 1999). The present study reports a decreased GABAAR1 mRNA expression in the N+ group compared to that in the control group. This decrease could be either because the protein was not required, or may be induced by BDNF that decreases the transcription of GABAAR1 mRNA via the Janus kinase/ signal transducer and activator of transcription (JAK/STAT) pathway. BDNF activates the phosphorylation process in STAT3 signaling, thereby initiating the formation of inducible cAMP early repressor (ICER), which binds to inactive, phosphorylated CREB (pCREB), thus inhibiting the binding of pCREB to CRE in the promoter of GABAAR1 (Matsuzaki et al., 1999, Lund et al., 2008, Porcher et al., 2011).

There was no significant difference in the content of GABAAR1 protein between the N- and K- groups, because GABA content was similar in both the groups. Khalilov et al. reported a dynamic change in progenitor neuron GABAAR1 regulation before and after the peak of giant depolarizing potentials (GDPs) (Khalilov et al., 2015). The increase in GABA reported in the K+ group may be due to postoperative GDP inhibition of GABAAR1 activity, compensating for excitatory glutamate activity and resulting in neuroprotection.

In the present study, the suspected postoperative GDP inhibition of GABAAR1 activity in the N+ group could not overcome the excitation from glutamate induction, leading to apoptosis. This study ‘Conditioned-Medium of Stem Cells from Human Exfoliated Deciduous Teeth Prevent Apoptosis of Neural Progenitors’ obtained ethical approval from National Board of Health Research and Development with the ethical approval number LB.02.01 / 5.2 / KE.055.

5. Conclusion

CM-SHED may effectively prevent glutamate-induced apoptosis of neural progenitors, as indicated by the expression level of GABAAR1 mRNA being highest in the K+ group. This study reveals that stem cells from human exfoliated deciduous teeth are a resource, which may be used for regenerative therapy in the medical-dental field.

Ethical statement

This study obtained ethical approval from National Board of Health Research and Development with the ethical approval number LB.02.01 / 5.2 / KE.055.

CRediT authorship contribution statement

Masagus Zainuri: Data curation, Formal analysis, Writing – original draft. Jan Purba: Data curation, Formal analysis, Investigation. Sri WA Jusman: Conceptualization, Supervision, Resources, Validation. Endang W Bachtiar: Project administration, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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References

Allen, N.J., Rossi, D.J., Attwell, D., 2004. Sequential release of GABA by exocytosis and reversed uptake leads to neuronal swelling in simulated ischemia of hippocampal slices. J. Neurosci. 24, 3837–3849.

Almeida, R.D., Manadas, B.J., Melo, C.V., Gomes, J.R., Mendes, C.S., Graos, M.M., Carvalho, R.F., Carvalho, A.P., Duarte, C.B., 2005. Neuroprotection by BDNF against glutamate-induced apoptotic cell death is mediated by ERK and PI3-kinase pathways. Cell Death Diff. 12, 1329–1343.

Asadollahi, A., Asadi, M., Hosseini, F.S., Ekhhtiari, Z., Biglar, M., Amanlou, M., 2019. Synthesis, molecular docking, and antiepileptic activity of novel phthalimide derivatives bearing amino acid conjugated anilines. Res. Pharm. Sci. 14, 534.

Banke, T.G., Traynelis, S.F., 2003. Activation of NR1/NR2b NMDA Receptors. Nat. Neurosci. 6, 144–152.

Brooks-Kayal, A.R., Russe, S.J., 2012. Regulation of GABAA receptor gene expression and epilepsy. Jasper’s Basic Mech. of the Epilepsies [Internet]. 4th edition.

Bufone, F., Minghetti, L., 2003. SHED: stem cells from human exfoliated deciduous teeth. Stem Cell Res. Ther. 21677 2012, 1–11.

Bull. 88, 617–623.

Chen, C., Liu, Y., Shi, Y., Gronthos, S., 2012. Stem cells in dentistry–part I: stem cell sources. J. Prosthdont. Res. 56, 151–165.

Esmaeili, A., Alifarja, S., Nourbakhsh, N., Talebi, A., 2014. Messenger RNA expression patterns of neurotrophins during transdifferentiation of stem cells from human-exfoliated deciduous teeth into neural-like cells. Avicenna J. Med. Biotech. 6, 21.

Finucane, D.M., Bossy-Wetzel, E., Waterhouse, N.J., Cotter, T.G., Green, D.R., 1999. Bax-induced caspase activation and apoptosis via cytochrome release from mitochondria is inhibitable by Bel-xL. J. Biol. Chem. 274, 2225–2233.

Inoue, T., Sugiyama, M., Hattori, H., Wakita, H., Wakabayashi, T., Ueda, M., 2013. Stem cells from human exfoliated deciduous tooth-derived conditioned medium enhance recovery of focal cerebral ischemia in rats. Tissue Eng. Part A 19, 24–29.

Kerkis, I., Caplan, A.I., 2012. Stem cells in dentistry–part I: stem cell sources. J. Prosthdont. Res. 56, 151–165.

Kemper, P., Liman, J., Weishaupt, J.H., Bähr, M., 2004. Neuronal apoptosis in neurodegenerative diseases: from basic research to clinical application. Neurodegener. Dis. 1, 9–19.

Khademizadeh, M., Messripour, M., Ghasemi, N., Attar, A.M., 2019. Differentiation of adult human mesenchymal stem cells into dopaminergic neurons. Res. in Pharm. Sci. 14, 209.

Khaliilov, I., Minlebaev, M., Mukhtarov, M., Khazipov, R., 2015. Dynamic changes from depolarizing to hyperpolarizing GABAergic activities during giant depolarizing potentials in the neonatal rat hippocampus. J. Neurosci. 35, 12635–12642.

Lee, H.Y., Greene, L.A., Mason, C.A., Manzini, M.C., 2009. Isolation and culture of post-natal mouse cerebellar granule neuron progenitor cells and neurons. Jove (J. Vis. Exp.) e990.

Lund, I.V., Hu, Y., Raol, Y.H., Benham, R.S., Faris, R., Russe, S.J., Brooks-Kayal, A.R., 2008. BDNF selectively regulates GABA receptor transcription by activation of the JAK/STAT pathway. Sci. Signal. 1, ra9–ra9.

Matsuzaki, H., Tamatani, M., Mitsuda, N., Namikawa, K., Kiyama, H., Miyake, S., Tohyama, M., 1999. Activation of Akt kinase inhibits apoptosis and changes in Bel-2 and Bax expression induced by nitric oxide in primary hippocampal neurons. J. NeuroChem. 73, 2037–2046.

Mita, T., Furukawa-Hibi, Y., Takeuchi, H., Hattori, H., Yamada, K., Hibi, H., Ueda, M., Yamamoto, A., 2015. Conditioned medium from the stem cells of human dental pulp improves cognitive function in a mouse model of Alzheimer’s disease. Behav. Brain Res. 293, 189–197.

Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L.W., Robey, P.G., Shi, S., 2003. SHED: stem cells from human exfoliated deciduous teeth. Proc. Nat. Acad. Sci. 100, 5807–5812.

Nechushtan, A., Smith, C.L., Hsu, Y.T., Youle, R.J., 1999. Conformation of the Bax C-terminus regulates subcellular location and cell death. EMBO J. 18, 2330–2341.

Obrietan, K., Gao, X.B., Van Den Pol, A.N., 2002. Excitatory Actions of GABA Increase BDNF Expression via a MAPK-CREB-Dependent Mechanism—A Positive Feedback Circuit in Developing Neurons. J. Neurophysiol. 88, 1005–1015.

Pawlowski, J., Kraft, A.S., 2000. Bax-induced apoptotic cell death. Proc. Nat. Acad. Sci. 97, 529–531.

Porcher, C., Hatchett, C., Longbottom, R.E., McAninch, K., Sihra, T.S., Moss, S.J., Thomson, A.M., Jovanovic, J.N., 2011. Positive feedback regulation between γ-aminobutyric acid type A (GABAA) receptor signaling and brain-derived neurotrophic factor (BDNF) release in developing neurons. J. Biol. Chem. 286, 21667–21677.

Smaili, S.P., Ureshino, R., Rodrigues, L., Rocha, K., Carvalho, J., Oseki, K., Bincoletto, C., Lopes, S., Hirata, H., 2011. The role of mitochondrial function in glutamate-dependent metabolism in neuronal cells. Curr. Pharm. Des. 17, 3865–3877.

Spitzer, N.C., 2006. Electrical activity in early neuronal development. Nature 444, 707–712.

Taylor, R.C., Cullen, S.P., Martin, S.J., 2008. Apoptosis: controlled demolition at the cellular level. Nature Rev. Mol. Cell Biol. 9, 231–241.

Wang, J., Gan, Y., Han, P., Yin, J., Liu, Q., Ghanian, S., Gao, F., Gong, G., Tang, Z., 2018. Ischemia-induced neuronal cell death is mediated by chemokine receptor CX3CR1. Sci. Rep. 8, 1–11.

Wei, X.W., Yan, H., Xu, B., Wu, Y.P., Li, C., Zhang, G.Y., 2012. Neuroprotection by co-activation of GABA receptors by preventing caspase-3 demitrosylation in KA-induced seizures. Brain Res. Bull. 88, 617–623.

Yamagata, M., Yamamoto, A., Kako, E., Kiyama, H., Matsuzaki, H., Tamatani, M., Mitsuda, N., Namikawa, K., Kiyama, H., Miyake, S., Tohyama, M., 1999. Activation of Akt kinase inhibits apoptosis and changes in Bel-2 and Bax expression induced by nitric oxide in primary hippocampal neurons. J. NeuroChem. 73, 2037–2046.

Zainuri, M., Bachtiar, E.W., 2018. Establishing methods of stem-cell conditioned medium of stem cells from human exfoliated deciduous teeth prevent apoptosis of neural progenitors.