Archives of Oral Biology 91 (2018) 9–16

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Exendin-4 relieves the inhibitory effects of high glucose on the proliferation and osteoblastic differentiation of periodontal ligament stem cells

Zijun Guo[a,1], Rui Chen[b], Fujun Zhang[b], Ming Ding[b], Ping Wang[a,*]

[a] Department of Stomatology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China
[b] Department of Oral and Maxillofacial Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

ARTICLE INFO

Keywords:
Periodontal ligament
Glucagon-like peptide 1
Cell proliferation
Osteogenesis

ABSTRACT

Background: With the impaired regenerative potential in patients with diabetes mellitus (DM), Periodontal ligament stem cells (PDLSCs) are regarded as an attractive source of stem cells for periodontal cytotherapy. Recent studies have shown that Exendin-4 (Ex-4) exerts cell-protective effects and bone remodeling ability on many types of cells. The aim of this study was to investigate whether Ex-4 alleviates the inhibition of high glucose on the proliferation and osteogenic differentiation of PDLSCs.

Methods: PDLSCs were incubated in medium supplemented with 5.5 mM D-glucose (NG), 30 mM D-glucose (HG), NG plus Ex-4, and HG plus different concentration (1, 10, 20, 100 nM) of Ex-4 respectively. Cell proliferation was detected by CCK-8 assay and cell cycle analysis. Osteogenesis was assessed by Alizarin Red S staining and evaluation of the mRNA expression of Runx2, ALP and Osx at day 7, 14 and 21. Intracellular level of reactive oxygen species (ROS) was detected using 5-(and-6)-chloromethyl-2′,7′-dichlorodihydro-fluorescein diacetate (CMH2DCF-DA).

Results: The proliferation ability, mineralized nodules forming capacity and the mRNA expression of Runx2, ALP and Osx of PDLSCs in HG group were decreased, the ROS level was increased compared to NG group. With the treatment of Ex-4, the HG-inhibited proliferation ability and osteogenic differentiation ability of PDLSCs were significantly reversed, the HG-increased ROS level could be down-regulated. Moreover, Ex-4 enhanced the osteogenic differentiation of normal PDLSCs.

Conclusions: Ex-4 alleviates the inhibitory effect of HG on the proliferation and osteoblastic differentiation of PDLSCs, and has a significant enhance in the osteoblastic differentiation of normal PDLSCs, giving new insights into the possible therapeutic method of diabetic periodontitis.

1. Background

Periodontitis is an inflammatory disease that causes pathological alterations in the tooth-supporting tissues including alveolar bone, periodontal ligament (PDL) and root cementum (Pihlstrom, Michalowicz, & Johnson, 2005). It is the main cause of tooth loss in adults and is closely related to multiple systemic disorders, such as retinal diseases, cardiovascular disease, especially diabetes mellitus (Pihlstrom et al., 2005). It has been reported that diabetes mellitus aggravates the condition of periodontitis, and vice versa (Bascones-Martinez, Gonzalez-Febles, & Sanz-Esporrin, 2014). The acceleration of alveolar bone loss is one of the main outcomes of diabetic periodontitis (Wu, Xiao, & Graves, 2015). The current therapies, such as open flap debridement or guided tissue regeneration (GTR) are able to control periodontal inflammation and, to some extent, to stimulate the reconstitution of bone in periodontal tissue (Chen, Shelton, Jin, & Chapple, 2009; Chen, An, Zhang, & Zhang, 2011). However, it has been revealed that diabetes mellitus interferes with bone formation and impairs periodontal healing (Jiao, Xiao, & Graves, 2015; Shirakata et al., 2014), thus, a well-content periodontal regeneration for diabetic patients remains a formidable challenge.

With properties which are very similar to those of mesenchymal stem cells (MSCs), Periodontal ligament stem cells (PDLSCs) have been confirmed to be existed in human PDL, and have the ability to differentiate into bone, cartilage, dental tissue, or neural tissue (Seo et al., 2004). Currently, there is mounting evidence that PDLSCs have been regarded as the best candidates for periodontal cytotherapy (Chen, Sun, Lu, & Yu, 2012; Ding et al., 2010; Flores et al., 2008; Hynes, Menicanin, Gronthos, & Bartold, 2012; Lin, Gronthos, & Bartold, 2009; Liu et al., 2008; Lu, Xie, Zhao, & Chen, 2013; Park, Jeon, & Choung, 2011; Rios,

* Corresponding author at: Department of Stomatology, The First Affiliated Hospital of Chongqing Medical University, Youyi Road 1, Chongqing, 400016, China.
E-mail addresses: candyzijun@163.com (Z. Guo), submitwp@sina.com, cqcnwp@sina.com (P. Wang).
[1] Chongqing Medical University, Yixueyuan Road 1, Chongqing, 400016, China.

https://doi.org/10.1016/j.archoralbio.2018.03.014
Received 3 December 2017; Received in revised form 8 March 2018; Accepted 26 March 2018

Lin, Oh, Park, & Giannobile, 2011; Tsumanuma et al., 2011; Uematsu et al., 2013), especially for the reconstruction of bone defects (Ge et al., 2012). However, researchers have found that high glucose (HG) levels may vastly alter the viability, self-renewal and differentiation ability of PDLSCs (Kato et al., 2016; Kim, Park, Yeo, Choi, & Suh, 2006; S.Y. Kim et al., 2013; Li & Li, 2016; Liu et al., 2015). Therefore, how to rescue the proliferation and osteogenic differentiation of PDLSCs under HG condition still remains a significant unmet medical need.

Exendin-4 (Ex-4), a gastrointestinal hormone, sharing 53% homology with human glucagon-like peptide-1 (GLP-1), is primarily used to improve glucose control for diabetic patients by acting on the glucagon-like peptide-1receptor (GLP-1R) (Kreymann, Williams, Ghatei, & Bloom, 1987; Nielsen, Young, & Parkes, 2004; Sanz et al., 2010; Tahrani, Bailey, Del Prato, & Barnett, 2011), which has been found to be existed in bone mesenchymal stem cells (BMSCs) and adipose-derived stem cells (ADSCs) (Jeon et al., 2014; Sanz et al., 2010). In recent years, Ex-4 is believed to promote the proliferation or survival of many kinds of cells (Chang et al., 2013; Li et al., 2005), especially for MSCs (Zhang et al., 2016; Zhou et al., 2015). Moreover, it has been proved to be an critical modulator of bone growth and remodeling (J.Y. Kim et al., 2013; Yamada et al., 2008). Recent reports state that Ex-4 could promote osteogenic differentiation of BMSCs (Lu et al., 2015; Ma et al., 2013; Meng et al., 2016; Sun et al., 2016). However, little information is available regarding the influences of Ex-4 on PDLSCs, even the damaged PDLSCs under HG condition. With the similar stem cell properties between BMSCs and PDLSCs, as well as the wide range of biological effects of Ex-4, we hypothesized that Ex-4 might somehow alleviates the inhibition of HG on the biology of PDLSCs.

To prove our hypothesis, we have investigated the effect of HG on the proliferation and osteogenic differentiation of PDLSCs, and characterized the impact of Ex-4 on this process.

2. Materials and methods

2.1. PDLSCs isolation and culture

Following informed consent and approvement of the University's Human Ethical Committee, healthy human premolars were extracted and collected from normal adult patients (18–25 years of age) undergoing orthodontic therapy in the Chongqing Key Laboratory of Oral Diseases and Biomedical Science, Affiliated Stomatology Hospital of Chongqing Medcial University.

PDL primary cell culture was carried out according to previously described methods (Yang et al., 2013; Zhang et al., 2012) with minor modification. Briefly, teeth were washed twice in sterile PBS (hyclone, Los Angeles, CA, USA), and PDL tissues were gently scraped from the middle third of the root surface and then digested with 1 mg/mL of collagenase type I (Sigma-Aldrich, St. Louis, MO, USA), for 15 min at 37 °C.Upon completion of digestion, the tissue were attached to 25T cell culture flash (Excel Bio, Australia), with a thin layer of fetal bovine serum (FBS) (Natocor, Argentina), and then the dish were moved to incubator at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. After 24 h, a-minimum essential medium (a-MEM) (hyclone, Los Angeles, CA, USA), containing 10% (v/v) FBS, 100 U/mL of penicillin and 100 mg/mL of streptomycin (Gibco BRL, Grand Island, NY, USA) were added until cells grew out from the tissue patch and approached confluence. After two passages, cells were subjected to magnetic isolation with antibodies to detect STRO-1 (mesenchymal stem cell marker) antigen (Millipore, Billerica, MA, USA) and magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). All experiments were carried out with passage 3–5 cells.

2.2. PDLSCs identification

Colony-forming efficiency and multiple differentiation capacities of PDLSCs were determined to further confirm their stem cell properties. PDLSCs (1×10^3) were seeded into 60-cm^2 cell culture dishes (Excel Bio, Australia) and cultured in growth medium. After 7 days, the cells were observed, aggregates of > 50 cells were scored as a colony. Osteogenic differentiation was initiated by a switch to osteogenic induction medium (OIM): α-MEM containing 5% (v/v) FBS, 50 μg/mL ascorbic acid, 1 μM dexamethasone, and 3 mM β-glycerophosphate (all from Sigma-Aldrich). After 7, 14, 21 days, Alizarin Red staining (Solabio, Beijing, China) was applied to evaluated the mineralization nodules. Differentiation into adipocytes was initiated with an adipogenesis induction medium (AIM):2 mM glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, 10 uM insulin, 1 uM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 200 uM indomethacin (all from Sigma-Aldrich). After 2 weeks, Oil Red O (Solabio, Beijing, China) was applied to identify the lipid droplets. All experiments were repeated 3 times.

2.3. Cell counting kit-8 assays

PDLSCs were plated in 96-well microplates at 1×10^4 cells/ml in normal culture medium (100 μL/well). After 24 h, cells were incubated in medium supplemented with 5.5 mM D-glucose (NG as a control), 30 mM D-glucose (HG), NG plus 10 nM Ex-4, and HG plus different concentrations of Ex-4 (Sigma-Aldrich, St. Louis, MO, USA) (1, 10, 20, and 100 nM) respectively for 1, 3, 5, and 7 days. At each time point, assays were performed after plating by the addition of 100 μL of fresh medium in 10 μL of the Cell Counting Kit-8 (CCK-8) (Dojindo, Japan) solution for another 2 h at 37 °C. The absorbance was measured at a wavelength of 450 nm. The assay was repeated 3 times.

2.4. Cell cycle analysis

PDLSCs were incubated under NG, NG plus 10 nM Ex-4, HG and HG plus 10 nM Ex-4 respectively. Then, cells were harvested and fixed with ice-cold 70% ethanol overnight. The fixed cells were rinsed and stained with propidium iodide for 30 min in the dark. The Cell Quest software were used for cell cycle analyses. The assay was repeated 3 times.

2.5. Alizarin Red staining

PDLSCs were incubated with OIM under NG, NG plus 10 nM exendin-4, HG and HG plus 10 nM exendin-4 conditions respectively for 7, 14, 21 days. The medium was changed every other day. 21 days later, the cells were fixed with 4% paraformaldehyde and stained with 0.2% Alizarin Red S (pH 8.3) (Solabio, Beijing, China). Unbound and non-specifically bound stain was removed by copious rinsing with distilled water, and stained calcium nodules were identified microscopically. Finally, the mineralized nodules were dissolved by hexadecylpyridinium chloride, and absorbance was quantitatively measured at 560 nm for statistical analysis. The assay was repeated 3 times.

2.6. Quantitative real-time-polymerase chain reaction (RT-PCR)

The mRNA expression of Runt-related transcription factor-2 (Runx2), alkaline phosphatase (ALP) and osterix (Osx) (Sangon, Shanghai, China) in human PDLSCs was determined after 7-day, 14-day and 21-day osteogenic induction respectively. Total RNA of PDLSCs was extracted using Trizol reagent (Invitrogen, USA). Then, with appropriate amounts of enzymes and fluorescent dyes as recommended by the supplier, 500 ng of total RNA was converted to cDNA using a Revert Aid First Strand cDNA Synthesis Kit (Takara, Bio, Otsu, Japan). Finally, the reaction mixture (20 μL) contained 2 uL diluted cDNA, 0.8 uL of each diluted primer, and 10 uL SYBR Premix Ex Taq II kit (Takara, Bio, Otsu, Japan), as well as 6.4 uL distilled water. The quantitative PCR System (Bio-Rad, Hercules, CA, USA) was programmed as follows: denaturation at 95 °C for 2 min followed by 39 cycles at 95 °C for 5 s and 60 °C for 30 s. The primers used in the present study were listed in