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Short Communication

A Comparative Study on the Repair and Regeneration Ability of Dental Pulp Stem Cells in Young and Adult Rats Stimulated by Inflammation

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LM and YC wrote the first draft of this paper and also did the experimental tasks. SW did the analysis and wrote the results section. XZ revised the manuscript. MX finalized the paper and review the manuscript.

Key words
Regeneration, Dental pulp stem cells, LPS, Proliferation

ABSTRACT

The objective of this study was to compare the repair and regeneration ability of dental pulp stem cells (DPSCs) in young and adult rats after inflammatory stimulation. For this study, 15 young rats and 15 adult rats were selected, and juvenile dental pulp stem cells (JDPSCs) of young rats and adult dental pulp stem cells (ADPSCs) were isolated respectively. The JDPSCsof young rats were divided into groups A and B, respectively at 0 μ m/m and 1 μ of lipopolysaccharide (LPS) for 24 h. The ADPSCs of young rats were divided into groups A and B, respectively at 0 and 1 μ/mL of LPS for 24 h. The number of mineralized nodules and ALP activity in group B were significantly higher than those in group D. The protein expression of OCN and ALP in group B was significantly higher than that in group A. ALP protein expression in group D was significantly higher than that in group C. The protein expression of OCN and ALP in group B was significantly higher than that in group D. It is concluded that LPS can induce the proliferation of JDPSCs and ADPSCs, and the proliferation ability of JDPSCs is stronger than that of ADPSCs, and LPS could up regulate the expression of mineralization related proteins.

Dental pulp stem cells (DPSCs) have strong cell activity and multi-directional differentiation potential, it can participate in the repair process of dental pulp tissue damage and occupy an important position, which have become the focus of research in the oral professional field (Gnanasegaran *et al*., 2017; Ma *et al*., 2021). Active DPSCs implanted on biocompatible scaffold materials to form complexes and then inoculated under the skin of nude mice can produce a pulp-dentin complex-like structure. After applying this complex to pulpitis patients after pulpectomy, pulp tissue regeneration occurred 6 months later (Li *et al*., 2020; Jochums *et al*., 2021). Dental pulp ages with age. A study on the biological properties of DPSCs isolated and grown from the dental pulp tissue of 20-23, 42-45.and 62-66 years old shows that proliferation, osteogenesis/dentinogenesis, etc., decline with age (Siew Ching *et al*., 2017). DPSCs have changed biologically from typical dental pulp stem cells (Dou and Guan, 2003). The present study aims at comparing the repair and regeneration ability of DPSCs from different ages following inflammation.

Materials and methods

SPF SD rats were purchased from Guangdong Medical Laboratory Animal Center, production license number: SCXK (Guangdong) 2021-0001, including 15 young (4 weeks old) rats and 15 adult (6 months old) rats. All the rats were in good health, with complete dentition. No dental caries, periodontal disease, etc. were found.

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Table I. Comparison of OD values and S-phase cell proportions in each group ($\bar{x}\pm s$).

		JDPSCs		ADPSCs		F value	P value
		A	B	C	D		
OD	1d	0.41±0.01	0.56±0.05[ac]	0.37±0.01	0.44±0.01[b]	143.57	<0.001
	3d	0.48±0.03	0.56±0.06[ac]	0.46±0.02	0.49±0.03[b]	19.57	<0.001
	5d	0.50±0.02	0.55±0.03[ac]	0.49±0.02	0.52±0.02[b]	20.00	<0.001
	7d	0.53±0.03	0.52±0.04	0.51±0.01	0.52±0.01	1.48	0.229
S-phase cell proportions (%)		16.13±0.15	24.63±3.52[ac]	12.11±0.26	16.99±0.21[b]	130.98	<0.001

Compared with group A, [a]P<0.05; compared with group C, [b]P<0.05; compared with group D, [c]P<0.05

The rats were killed by anesthesia, their jaws were separated with intact mandibular incisors, and the pulp tissue was taken out. Juvenile dental pulp stem cells (JDPSCs) and adult dental pulp stem cells (ADPSCs) were then separated. When the growth fusion degree of JDPSCs and ADPSCs reached 80%~90%, passed them down, and then they were purified by limiting dilution method. JDPSCs of juvenile rats were divided into group A and group B, and were stimulated with lipopolysaccharide (LPS) at 0 μg/mL and 1 μg/mL, respectively for 24 h. The ADPSCs of adult rats were divided into group C and group D, and were stimulated with lipopolysaccharide (LPS) at 0μg/mL and 1μg/mL, respectively for 24h.The proliferation ability of JDPSCs and ADPSCs was detected by CCK-8 method. The JDPSCs and ADPSCs in the logarithmic growth phase were digested with 0.25% trypsin and centrifuged in a centrifuge. After 24 h of LPS stimulation, the cell cycle distribution of JDPSCs and ADPSCs was detected by flow cytometry. After 7 days of mineralization induction, the expression of mineralization-related proteins, such as ALP, Osteocalcin (OCN) were detected in JDPSCs and ADPSCs by western blot.

The research data were analyzed by SSPSS23.0, and the measurement data such as the OD value of JDPSCs, ADPSCs, and the proportion of cells in S phase were expressed as ($\bar{x}\pm s$). One-way analysis of variance was used for comparison between multiple groups, and SNK-a test was used for comparison between two groups. P<0.05 was regarded as statistically significant.

Results

Table I shows the effect of LPS on growth as determined by OD values, proportion of S phase cells, formation of mineralized nodules, ALP activity, and expression of OCN in JDPSCs and ADPSCs. The growth of cells, proportion of S phase cells were found significantly higher in groups B and D in comparison to control group. Moreover, the formation of mineralized nodules and ALP activity in group B were significantly higher than those in group A (P<0.05). There was no significant difference in ALP activity between group D and group C (P>0.05). The formation of mineralized nodules and ALP activity in group B were significantly higher than those in group D (P<0.05, Table II).

Table II. Comparison of the formation of mineralized nodules between two groups ($\bar{x}\pm s$).

Groups		The formation of mineralized nodules	ALP (mol/L)
JDPSCs	A group	0.19±0.02	0.63±0.01
	B group	0.78±0.05[ac]	0.94±0.04[ac]
ADPSCs	C group	0.31±0.02	0.59±0.03
	D group	0.58±0.05[b]	0.65±0.04
F		731.380	367.020
P		<0.001	<0.001

Compared with group A, [a]P<0.05; compared with group C, [b]P<0.05; compared with group D, [c]P<0.05

Table III. Comparison of ALP and OCN protein expression in each group.

Group	n	OCN	ALP
A	10	0.76±0.06	0.69±0.07
B	10	1.18±0.10[a]	0.89±0.09[ac]
C	10	1.16±0.15	0.71±0.03
D	10	1.02±0.05	0.97±0.01[b]
F		29.46	40.44
P		<0.001	<0.001

Compared with group A, [a]P<0.05; compared with group C, [b]P<0.05; compared with group D, [c]P<0.05.

The protein expression of OCN and ALP in group B was significantly higher than that in group A. The expression of ALP protein in group D was significantly higher than that in group C. The protein expression of OCN and ALP in group B was significantly higher than that in group D (Table III).

Discussion

Aging slows tissue regeneration. Weakening stem cell function and micro environmental changes may be to blame (Cui *et al*., 2017). Cytokines can alter stem cell-related gene expression and govern self-renewal and multi-differentiation in the stem cell microenvironment (Zhang *et al*., 2017). Inflammation changes DPSCs' biological characteristics (Hu *et al*., 2019). LPS induction is a popular approach to promote inflammation *in vivo* or *in vitro* (You *et al*., 2021). Inflammation promotes dental pulp stem cell regeneration in young/adult rats.

It has been shown that 1g/mL LPS increases BDNF and stimulates astrocyte development.

Zhang *et al*. (2017) and Liu *et al*. (2014) evaluated the effect of *Escherichia coli* LPS and *Streptococcus* mutants lipoteichoic acid on human dental pulp stem cells. We examined the cell cycle distribution of DPSCs and ADPSCs. LPS influences JDPSCs and ADPSCs' cell cycles, according to flow cytometry. JDPSCs grew faster than ADPSCs atter LPS induction suggesting that LPS can increase cell proliferation. Sall levels of bacterial toxic chemicals can cause DPSCs to travel to injured areas, mature into odontoblasts, generate restorative dentin, and build defenses (Wang *et al*., 2019). Proliferation and mineralization of DPSCs can repair and restore inflamed dental pulp (Yang *et al*., 2017). Mineralized nodules imply osteogenic/dental DPSCs. ALP can hydrolyze organophosphates and oversaturate phosphorous ions in fluids, causing excessive salt deposition that builds mineralized tissues (Ásgeirsson and Andrésson, 2001), ALP indicates early dentin differentiation and DPSCs' differentiation capacity. LPS can produce mineralized nodules in JDPSCs and ADPSCs, and JDPSs displayed stronger osteogenic/dentinogenic differentiation potential after LPS induction. OCN affects apatite crystallization in dentin and bone formation. It's a diabetic and male reproductive disease hormone (Ruoslahti *et al*., 1982). LPS upregulates mineralization-related proteins in JDPSCs and ADPSCs, according to western blot analysis.

Conclusion

LPS can induce the proliferation of JDPSCs and ADPSCs, and the proliferation ability of JDPSCs is stronger than that of ADPSCs; meanwhile, LPS can up-regulate the expression of mineralization-related proteins (ALP, OCN) in the mineralization-induced microenvironment, and the up-regulation of JDPSCs is higher than that of ADPSCs; indicating that after inflammation stimulation, JDPSCs showed stronger repair ability.

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IRB approval

This research was carried out with the approval of Wuxi Stomatological Hospital Committee.

Ethical statement

Research experiments conducted in this article with animals or humans were approved by Ethical Committee and responsible authorities of our research organization(s) following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Statement of conflict of interest

The authors have declared no conflict of interest.

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