Inhibition of Carrageenin-induced Dental Inflammatory Responses Owing to Decreasing TRPV1 Activity by Dexmedetomidine

Gang Lu

Rizhao People's Hospital

Guanhua Zhu

Jingzhou Central Hospital

Maohua Xu

Rizhao People's Hospital

Xinggao Ping (✉ xinggaoping1025@163.com)

Rizhao People's Hospital <https://orcid.org/0000-0002-5876-4930>

Qingfeng Xiao

Xiangyang Central Hospital

Research

Keywords: Dental pulp cell, inflammation, Dex, TRPV1, cytokines

Posted Date: December 15th, 2019

DOI: <https://doi.org/10.21203/rs.2.18613/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background : Dexmedetomidine (Dex) is a highly selective agonist of the α_2 adrenergic receptor and a common sedative; however, its anti-inflammatory effect has been studied. In this study, the inhibitory effect of Dex on inflammation in dental pulp cells was assessed. To solve this problem, the effect of Dex on inflammation induced by carrageenan (Car) in human dental pulp cells (hDPCs) was studied. Car incubation induced a robust inflammatory response in the hDPCs as well as activation of PKA–STAT3 and PKC–nuclear factor kappa B (NF κ B) signaling pathways.

Results : Dex could reduce the expression of inflammatory cytokines in a dose-dependent manner. Meanwhile, the phosphorylation of PKA, PKC, STAT3, and NF κ B as well as the nuclear accumulation of STAT3 and NF κ B were significantly increased in Dex-treated Car-induced hDPCs. Western blotting results also showed that the phosphorylation level of transient receptor potential cation channel subfamily V member 1 (TRPV1) was downregulated owing to Dex treatment. Further, we found that the administration of the TRPV1 agonist capsaicin (Cap) reversed the effects of Dex on proinflammatory cytokines; however, the expression and activation of PKA–STAT3 and PKC–NF κ B signals were not altered owing to Cap administration.

Conclusions : These results indicate that Dex exerts a defensive role in dental pulp inflammation by regulating the TRPV1 channel and can be used as a potential target for human dental pulp inflammation intervention.

Introduction

Dental pulp inflammation is a general phenomenon, usually a sequela of dental caries or trauma 1. Clinically, it could cause severe pain 2, and if not controlled, it may eventually lead to fatal systemic inflammatory disease 3. Considering the sudden impact of pulpitis, it is important and necessary to study the pathological progress of pulpitis to ensure its effective treatment. However, several studies have been concerned about the effect of immune cells 4, such as macrophages, dendritic cells, and lymphocytes.

Human dental pulp cells (hDPCs) are the main cell types in dental pulp and play multiple roles in host defense and regeneration 5–7. HDPCs induced by proinflammatory mediators [including tumor necrosis factor alpha (TNF- α) and lipopolysaccharide (LPS)] could locally secrete a large number of cytokines to attract additional immune cells and initiate and regulate inflammation 8, 9.

During inflammation, primary nociceptive neurons (nociceptors) are sensitized and the pain sensation (hyperalgesia) is increased. The direct effect of inflammatory mediators such as prostaglandins (PGI₂ and PGE₂) and sympathetic amines (epinephrine and dopamine) on their receptors in the nociceptor membrane can cause sensitization. Transient receptor potential cation channel subfamily V member 1 (TRPV1), a ligand-gated ion channel, is involved in pain modulation 10. The flavonoid eriodictyol (an antagonist of the TRPV1 channel) supports this view by reducing nociceptive behavior 11. In addition, the nociceptor is partially characterized by the expression of TRPV1 12.

As a specific agonist of the α_2 adrenergic receptor, dexmedetomidine (Dex) is commonly applied for analgesia and sedation purposes in the operation room and intensive care unit 13, 14. Recently, it was reported that Dex has a protective effect against inflammation that is triggered by endotoxin 15, spinal cord injury 16, sepsis 17, or lung injury 18. Therefore, this study sought to study the protective effect of Dex on carrageenan (Car)-induced pulp inflammation and to determine the mechanism of inflammation of hDPCs.

Results

Expression of proinflammatory cytokines induced by Car in hDPCs

To explore hDPC inflammation following Car treatment, the expression of proinflammatory cytokines in hDPCs was assessed. Results of qPCR and ELISA tests revealed that the messenger RNA (mRNA) and protein expressions of IL-1 β , IL-6, and TNF- α in HDPCs after Car treatment were higher than those in the control group ($P < 0.01$) (Figs. 1A and 1B).

Car-induced activation of PKA–STAT3 and PKC–nuclear factor kappa B (NF κ B) in induced hDPCs

Because the activation of the PKA–STAT3 and PKC–NF κ B pathways is crucial for triggering cytokine expression 19, 20, the expression and phosphorylation of PKA, STAT3, PKC, and NF κ B after Car treatment were studied. qPCR results suggested that Car upregulates the mRNA expressions of STAT3 and NF κ B ($P < 0.05$), while the expressions of PKA and PKC were not altered (Fig. 2A). Moreover, the WB results indicated that the phosphorylated levels of PKA, STAT3, PKC, and NF κ B were increased following Car treatment (Fig. 2B). Additionally, the nuclear localization of STAT3 and NF κ B was clearly increased due to Car incubation (Fig. 2C). These results suggest that Car treatment triggered inflammatory reactions in hDPCs by activating the PKA–STAT3 and PKC–NF κ B pathways.

Dex ameliorated the inflammatory responses triggered by Car

Previous studies have indicated that the local injection of Dex could reverse inflammation induced by Car 21, 22, Therefore, ELISA was utilized to detect the production of proinflammatory cytokines under Car stimulation and various concentrations of Dex. It was revealed from this that IL-1 β , IL-6, and TNF- α were downregulated in a dose-dependent manner at 2 h after Dex treatment in hDPCs ($P < 0.05$) (Figs. 3A, 3B, and 3C).

WB results showed that the phosphorylation of PKA, STAT3, PKC, and NF κ B was downregulated in hDPCs owing to stimulation using 5 μ M Dex ($P < 0.05$) (Fig. 4A). Meanwhile, we found that STAT3 and NF κ B were distributed in cytoplasm again after Dex treatment (Fig. 4B), suggesting that these two signaling pathways were blocked as a result of Dex treatment in hDPCs.

Dex desensitized TRPV1 channel in Car-treated hDPCs

Previous studies have suggested that TRPV1 (a substrate of different protein kinases) is activated by multiple inflammatory mediators, such as PKC 23 and PKA 24. Therefore, we attempted to examine the activation of TRPV1 during Car induction and Dex treatment. As qPCR data showed, TRPV1 expression did not significantly change following Dex treatment (Fig. 5A); however, its activity, which was indicated by CGRP release, was clearly reduced after Dex treatment (Fig. 5B). Furthermore, WB data indicated that the phosphorylation of TRPV1 was downregulated and confirmed that its expression did not change (Fig. 5C). The data suggested that TRPV1 was desensitized owing to Dex administration.

Effects of the TRPV1 agonist Cap on inflammation in hDPCs

To further examine the influence of TRPV1 on Dex-ameliorated and Car-induced inflammation in hDPCs, cells were coadministered with 5 μ M of capsaicin (Cap), a TRPV1 agonist. We found that CGRP release was obviously increased after Cap treatment ($P < 0.05$) (Fig. 6A). It was also indicated that the phosphorylation of TPRV1 was markedly increased after hDPCs were coadministered with Cap. Meanwhile, the activation of both the PKA–STAT3 and PKC–NF κ B pathways was not altered, suggesting that TRPV1 was located downstream of these two pathways (Fig. 6B). In addition, the sensitization of TRPV1 resulted in the restoration of proinflammatory cytokine production (Fig. 6C), suggesting that TRPV1 sensitization counteracts the effects of Dex on hDPC inflammation.

Conclusions

Many molecules can be used to induce the inflammation of hDPCs, including LPS, IL-6, IL-8, and TNF- α . More specifically, LPS can penetrate the dental pulp and induce inflammation and plays a key role in pulp infection 25. Proinflammatory cytokines, such as IL-6, IL-8, and TNF- α , are usually detected in inflammatory pulps and are considered key biomarkers and mediators for the diagnosis of pulp inflammation 26–28. In the current study, Car was used to induce the inflammation of hDPCs. A previous study revealed that the Car-induced acute inflammatory response can be examined to elucidate the inflammatory period related to phagocyte infiltration, excessive production of free radicals, and release of inflammatory mediators (e.g., TNF- α , cyclooxygenase-2, and inducible nitric oxide synthase) 29. An in vivo model showed that Car-induced acute inflammatory response reached its peak at 2–3 h after injection, with the inflammation later disappearing within 24–74 h 30. The inflammatory response induced by Car is considered to be a biphasic situation in which multiple mediators could induce the generation of inflammation 31. In this study, the robust generation of proinflammatory cytokines (i.e., IL-1 β , IL-6, and TNF- α) and the phosphorylation and nuclear localization of STAT3 and NF- κ B were observed after 100- μ M Car treatment for 2 h. These findings indicated the successful induction of dental pulp inflammation by Car.

Existing evidence has revealed that TRPV1 is involved in the occurrence and development of immune-related diseases and is a therapeutic target that is easily blocked by small molecules. For instance, currently, TRPV1 is considered to be an anticipated molecular target for the clinical evaluation of epilepsy therapy 32. Its antagonists are employed for the treatment of pruritus, inflammation-related pain, tissue

damage, ischemia, and other diseases 33, 34, and its agonists are used in a phase III clinical trial for the treatment of cluster headache, analgesia, and osteoarthritis 35, 36. Various inflammatory mediators can sensitize TRPV1, including neurotransmitters, cytokines and chemokines, lipids, peptides or small proteins, and growth factors. Many inflammatory mediators enhance the activity of TRPV1 37. A previous study demonstrated that TRPV1 can be activated by inflammatory mediators and corresponding receptors (i.e., PKC 23 and PKA 24). Both kinases can phosphorylate TRPV1 at different serine and threonine residues, resulting in TRPV1 sensitization. Alternatively, the calcium signaling pathway can be activated and TRPV1 phosphorylation can be induced by increased intracellular calcium in the TRPV1 channel, leading to downstream activation of STAT3 and NFκB and cytokine production 38. STAT3 and NFκB are both transcription factors involved in immune response and key factors regulating inflammation 19, 20. In the current study, the treatment of Dex led to the impaired inflammation and blockage of both the PKA–STAT3 and PKC–NFκB signaling axes as well as the deactivation of the TRPV1 channel. Further usage of the TRPV1 agonist Cap restored the generation of proinflammatory cytokines; however, the states of the PKA–STAT3 and PKC–NFκB signaling axes were not altered, indicating that TRPV1 activation (phosphorylation) is downstream of these two pathways.

To summarize, our study demonstrated the intrinsic mechanism underlying dental pulp inflammation in hDPCs. Car-induced inflammatory stimulation can induce the sensitization of TRPV1 by the PKA–STAT3 and PKC–NFκB pathways, which can actively regulate the inflammatory response by inflammatory cytokine overproduction. Meanwhile, Dex treatment in the induced hDPCs ameliorated the immune response via the deactivation of TRPV1 through the above mentioned two pathways. Previous studies have indicated that a local injection of Dex could reverse inflammation induced by Car [22, 23]. The present study's data show that Dex can be used as an additive for local anesthetics, which can not only improve the anesthetic potency of dental pulp inflammation but also induce anti-inflammatory potency.

Methods

Ethics

All experiments were approved by the Ethics Committee of Rizhao People's Hospital. All subjects signed informed consent forms.

Separation and culture of hDPCs

Healthy permanent premolars for orthodontic or impacted third molars were collected from subjects (aged 18–26 years). As mentioned above, hDPCs were separated and cultured using an enzymatic method 39. The dental pulp tissue was separated, cut into small pieces, and digested at 37°C for 20 min with 3 mg/mL of type I collagenase (Gibco, USA). Next, the chopped pulp tissue was cultured at 37°C in Dulbecco's modified Eagle medium (DMEM) containing 20% fetal bovine serum, 100 U/mL of penicillin (Gibco, USA), and 100 mg/mL of streptomycin with 5% CO₂. The medium was replaced every 3 days.

After the cells achieved 80% confluence, they were separated by trypsin/ethylenediaminetetraacetic acid (Gibco, USA) and sub-cultured at a ratio of 1:2.

Dex incubation

Dex (350 μ M) was frozen at -20°C and diluted with DMEM/F-12 to a specified concentration if necessary. Before Dex treatment, cells were allowed to reach approximately 70%–80% confluence. The cells were exposed to Dex at different concentrations (0, 0.1, 1, 5, or 10 μ M) for 2 h to determine the most appropriate concentration. Cells were then divided into the following groups: control group, in which the cells were incubated without Dex treatment in a humidified environment at 37°C with 5% CO_2 ; the Car group, in which the cells were incubated with 100 μ M of Car for 2 h; and the Dex/Car group, in which the cells were pretreated with 5 μ M of Dex and 100 μ M of Car for 2 h.

Drug administration

To induce an inflammatory response, Car (100 μ M) was incubated with cells for 2 h. To promote TRPV1 activity, Cap (5 μ M) was incubated with cells for 2 h.

Immunofluorescence assay (IFA)

hDPCs were inoculated in a 24-well plate and fixed with 4% polyformaldehyde for 15 min. hDPCs were permeated for 30 min with 0.1% Triton X-100, cultured at ambient temperature for 15 min with 10% goat serum, and treated at 4°C with the primary antibody overnight. hDPCs were washed with PBS thrice and incubated at ambient temperature for 1 h with the secondary Cy3-labeled antibody in dark conditions. Then, the cells were dyed with 4',6-diamidino-2-phenylindole for 15 min. Images were obtained at 400 $\times$ magnification using a fluorescent microscope.

Western blotting (WB)

The cell lysis buffer was used for the lysis of hDPCs. Protein was determined using a bicinchoninic analysis kit, separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and then transferred to a polyvinylidene fluoride or polyvinylidene difluoride membrane. Tween 20 was added to bovine serum albumin (BSA; 5%) phosphate buffer to block nonbinding sites on the membrane for 1 h. Protein was cultured at 4°C overnight with the primary antibody, and the secondary antibody was bound to peroxidase (Amersham ECL). The protein bands were stained, and the gray values were measured on a C-DiGit Blot Scanner.

RNA extraction and quantitative real-time polymerase chain reaction (qPCR)

The total RNA was extracted with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) used as an internal standard. Next, under the following conditions, RNA was detected by qPCR (using an SYBR-Green Kit) in a 20- μ L system: predenaturation (95°C , 10 min), denaturation (95°C , 15 s, 40 cycles), annealing

(60°C, 30 s), and extension (72°C, 30 s). Quantitative analysis was based on the 2- $\Delta\Delta$ CT method and normalized according to GAPDH.

Enzyme-linked immunosorbent assay (ELISA)

According to the manufacturer's protocol, the concentrations of interleukin (IL)-1 β , IL-6, TNF- α , and CGRP in the cell culture supernatants were analyzed using an ELISA kit. An automated microplate reader (SpectraMax® M5) was used for the measurement of the optical density (OD) at 450 nm. Based on the OD value and the standard substance concentration, each sample concentration was detected.

Statistical analysis

The results of our study are presented as means $\pm$ standard deviations. Comparisons were analyzed by analysis of variance or a two-tailed Student's *t*-test. A P-value of <0.05 was considered to indicate a statistically significant difference.

Declarations

Ethics approval and consent to participate: All experiments were approved by the Ethics Committee of Rizhao People's Hospital. All subjects signed informed consent forms.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: No funding was received.

Authors' contributions: GL, GZ, QX analyzed and interpreted the patient data regarding the hematological disease and the transplant. GL, GZ, MX, XP performed the histological examination of the kidney. QX was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements: None.

References

1. Boyle M, Chun C, Strojny C, Narayanan R, Bartholomew A, Sundivakkam P, et al. Chronic inflammation and angiogenic signaling axis impairs differentiation of dental-pulp stem cells. *PLoS One*. 2014; 9: e113419.
2. Lin J-J, Du Y, Cai W-K, Kuang R, Chang T, Zhang Z, et al. Toll-like receptor 4 signaling in neurons of trigeminal ganglion contributes to nociception induced by acute pulpitis in rats. *Scientific reports*. 2015; 5: 12549.
3. Serhan C N and Petasis N A. Resolvins and protectins in inflammation resolution. *Chemical reviews*. 2011; 111: 5922.
4. Renard E, Gaudin A, Bienvenu G, Amiaud J, Farges J, Cuturi M, et al. Immune cells and molecular networks in experimentally induced pulpitis. *Journal of dental research*. 2016; 95: 196.
5. Lee S-I, Min K-S, Bae W-J, Lee Y-M, Lee S-Y, Lee E-S, et al. Role of SIRT1 in heat stress-and lipopolysaccharide-induced immune and defense gene expression in human dental pulp cells. *Journal of endodontics*. 2011; 37: 1525.
6. Xiong H, Wei L and Peng B. IL-17 stimulates the production of the inflammatory chemokines IL-6 and IL-8 in human dental pulp fibroblasts. *International endodontic journal*. 2015; 48: 505.
7. Zhao Y, Wang C-L, Li R-M, Hui T-Q, Su Y-Y, Yuan Q, et al. Wnt5a promotes inflammatory responses via nuclear factor κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) pathways in human dental pulp cells. *Journal of Biological Chemistry*. 2014; 289: 21028.
8. Feng Z, Li Q, Meng R, Yi B and Xu Q. METTL 3 regulates alternative splicing of MyD88 upon the lipopolysaccharide-induced inflammatory response in human dental pulp cells. *Journal of cellular and molecular medicine*. 2018; 22: 2558.
9. Horst O V, Horst J A, Samudrala R and Dale B A. Caries induced cytokine network in the odontoblast layer of human teeth. *BMC immunology*. 2011; 12: 9.
10. Basbaum A I, Bautista D M, Scherrer G and Julius D. Cellular and molecular mechanisms of pain. *Cell*. 2009; 139: 267.
11. Rossato M F, Trevisan G, Walker C I B, Klafke J Z, de Oliveira A P, Villarinho J G, et al. Eriodictyol: a flavonoid antagonist of the TRPV1 receptor with antioxidant activity. *Biochemical pharmacology*. 2011; 81: 544.
12. Caterina M J, Schumacher M A, Tominaga M, Rosen T A, Levine J D and Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997; 389: 816.
13. Cui J, Zhao H, Yi B, Zeng J, Lu K and Ma D. Dexmedetomidine attenuates bilirubin-induced lung alveolar epithelial cell death in vitro and in vivo. *Critical care medicine*. 2015; 43: e356.
14. Gertler R, Brown H C, Mitchell D H and Silvius E N. Dexmedetomidine: a novel sedative-analgesic agent. *Baylor university Medical center proceedings*. Taylor & Francis. 2001; 14: 13.

15. Taniguchi T, Kidani Y, Kanakura H, Takemoto Y and Yamamoto K. Effects of dexmedetomidine on mortality rate and inflammatory responses to endotoxin-induced shock in rats. *Critical care medicine*. 2004; 32: 1322.
16. Can M, Gul S, Bektas S, Hanci V and Acikgoz S. Effects of dexmedetomidine or methylprednisolone on inflammatory responses in spinal cord injury. *Acta Anaesthesiologica Scandinavica*. 2009; 53: 1068.
17. Tasdogan M, Memis D, Sut N and Yuksel M. Results of a pilot study on the effects of propofol and dexmedetomidine on inflammatory responses and intraabdominal pressure in severe sepsis. *Journal of clinical anesthesia*. 2009; 21: 394.
18. Yang C-L, Tsai P-S and Huang C-J. Effects of dexmedetomidine on regulating pulmonary inflammation in a rat model of ventilator-induced lung injury. *Acta Anaesthesiologica Taiwanica*. 2008; 46: 151.
19. Chang J, Zhang C, Tani-Ishii N, Shi S and Wang C-Y. NF- κ B activation in human dental pulp stem cells by TNF and LPS. *Journal of dental research*. 2005; 84: 994.
20. Takeda K and Akira S. STAT family of transcription factors in cytokine-mediated biological responses. *Cytokine & growth factor reviews*. 2000; 11: 199.
21. Sukegawa S, Higuchi H, Inoue M, Nagatsuka H, Maeda S and Miyawaki T. Locally injected dexmedetomidine inhibits carrageenin-induced inflammatory responses in the injected region. *anesthesia & analgesia*. 2014; 118: 473.
22. Walker S M, Howard R F, Keay K A and Fitzgerald M. Developmental age influences the effect of epidural dexmedetomidine on inflammatory hyperalgesia in rat pups. *Anesthesiology: The Journal of the American Society of Anesthesiologists*. 2005; 102: 1226.
23. Premkumar L S and Ahern G P. Induction of vanilloid receptor channel activity by protein kinase C. *Nature*. 2000; 408: 985.
24. Bhawe G, Zhu W, Wang H, Brasier D, Oxford G S and Gereau IV R W. cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. *Neuron*. 2002; 35: 721.
25. Parolia A, Gee L S, Porto I and Mohan M. Role of cytokines, endotoxins (LPS), and lipoteichoic acid (LTA) in endodontic infection. *J Dent Oral Disord Ther*. 2014; 2: 1.
26. Elsalhy M, Azizieh F and Raghupathy R. Cytokines as diagnostic markers of pulpal inflammation. *International endodontic journal*. 2013; 46: 573.
27. Song F, Sun H, Wang Y, Yang H, Huang L, Fu D, et al. Pannexin3 inhibits TNF- α -induced inflammatory response by suppressing NF- κ B signalling pathway in human dental pulp cells. *Journal of cellular and molecular medicine*. 2017; 21: 444.
28. Zanini M, Meyer E and Simon S. Pulp inflammation diagnosis from clinical to inflammatory mediators: a systematic review. *Journal of endodontics*. 2017; 43: 1033.
29. Huang M-H, Wang B-S, Chiu C-S, Amagaya S, Hsieh W-T, Huang S-S, et al. Antioxidant, antinociceptive, and anti-inflammatory activities of *Xanthii Fructus* extract. *Journal of*

- Ethnopharmacology. 2011; 135: 545.
30. Molina C and Herrero J F. The influence of the time course of inflammation and spinalization on the antinociceptive activity of the $\alpha 2$ -adrenoceptor agonist medetomidine. *European journal of pharmacology*. 2006; 532: 50.
 31. Vinegar R, Schreiber W and Hugo R. Biphasic development of carrageenin edema in rats. *Journal of pharmacology and experimental therapeutics*. 1969; 166: 96.
 32. Nazıroglu M. TRPV1 channel: a potential drug target for treating epilepsy. *Current neuropharmacology*. 2015; 13: 239.
 33. Moran M M, McAlexander M A, Bíró T and Szallasi A. Transient receptor potential channels as therapeutic targets. *Nature reviews Drug discovery*. 2011; 10: 601.
 34. Rowbotham M C, Nothaft W, Duan W R, Wang Y, Faltynek C, McGaraughty S, et al. Oral and cutaneous thermosensory profile of selective TRPV1 inhibition by ABT-102 in a randomized healthy volunteer trial. *Pain*. 2011; 152: 1192.
 35. Kissin I and Szallasi A. Therapeutic targeting of TRPV1 by resiniferatoxin, from preclinical studies to clinical trials. *Current topics in medicinal chemistry*. 2011; 11: 2159.
 36. Miller F, Björnsson M, Svensson O and Karlsten R. Experiences with an adaptive design for a dose-finding study in patients with osteoarthritis. *Contemporary clinical trials*. 2014; 37: 189.
 37. Ma W and Quirion R. Inflammatory mediators modulating the transient receptor potential vanilloid 1 receptor: therapeutic targets to treat inflammatory and neuropathic pain. *Expert opinion on therapeutic targets*. 2007; 11: 307.
 38. Kong W-L, Peng Y-Y and Peng B-W. Modulation of neuroinflammation: role and therapeutic potential of TRPV1 in the neuro-immune axis. *Brain, behavior, and immunity*. 2017; 64: 354.
 39. Jing L, Rong-yin T and Yan L. Study of primary culture method of human pulp cell [J]. *Chinese Journal of Conservative Dentistry*. 2006; 6:

Figures

Figure 1

Car-triggered inflammation in hDPCs. hDPCs were treated with both Car and PBS for 2 h. After lysis, the expressions of IL-1 β , IL-6, and TNF- α were assessed by (A) qPCR and (B) ELISA. Data are expressed as means $\pm$ standard deviations. **P < 0.01, ***P < 0.001.

Figure 2

Car activated both PKA–STAT3 and PKC–NFκB signaling in hDPCs. (A) Cell lysates were subjected to qPCR to assess the expressions of PKA, PKC, STAT3, and NFκB. (B) WB was performed to probe with antibodies for phosphorylation and expression PKA, PKC, STAT3, and NFκB in cell lysates. (C) IFA detected the cellular location of STAT3 and NFκB in hDPCs. Data are expressed as means ± standard deviations. *P < 0.05.

Figure 3

. Dex ameliorated the generation of proinflammatory cytokines in hDPCs. hDPCs were cotreated with Car or different concentrations of Dex (0, 0.1, 1, 5, or 10 μM) for 2 h. The expressions of IL-1β (A), IL-6 (B), and TNF-α (C) at 2 h after Car and Dex treatment were detected by ELISA. Data are expressed as means ± standard deviations. **P < 0.01, ***P < 0.001.

Figure 4

Dex deactivated both PKA–STAT3 and PKC–NFκB signaling in hDPCs. hDPCs were cotreated with Car (100 μM) or Dex (5 μM) for 2 h. (A) WB was performed to probe with antibodies for the phosphorylation and expression of PKA, PKC, STAT3, and NFκB in cell lysates. (B) IFA detected the cellular location of STAT3 and NFκB in hDPCs.

Figure 5

Dex desensitized the TRPV1 channel in hDPCs. (A) qPCR revealed the expression of TRPV1 in hDPCs. (B) The release of CGRP in cell culture medium was determined at 2 h after Car and/or Dex cotreatment by ELISA. (C) WB analyses determined the expression and phosphorylation of TRPV1 in hDPCs. Data are expressed as means $\pm$ standard deviations. ** $P < 0.01$.

Figure 6

Cap treatment restored Car-induced inflammation in hDPCs. hDPCs were cotreated with Car (100 μ M), Dex (5 μ M), and/or Cap (5 μ M) for 2 h. (A) The release of CGRP in cell culture medium was determined at 2 h after Car and/or Dex cotreatment by ELISA. (B) WB analyses were used to examine the phosphorylation and expression of PKA, PKC, STAT3, NF κ B, and TRPV1 in cell lysates. (C) ELISA was conducted to assess IL-1 β , IL-6, and TNF- α expression. Data are expressed as means $\pm$ standard deviations. ** $P < 0.01$, *** $P < 0.001$.