

Leptin Receptor Is Up-regulated in Inflamed Human Dental Pulp

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Abstract

Introduction: After leptin receptor (LEPR) identification in hematopoietic, immune system, and other tissues, a role for leptin regulating inflammation and immune response has been accepted. This study aims to describe the possible expression of LEPR in healthy human dental pulp and to compare it with LEPR expression in inflamed human dental pulp. **Methods:** Twenty-one pulp samples were obtained from freshly extracted caries-free and restoration-free human third molars. In 7 third molars (inflamed pulp group), inflammation was experimentally induced before extraction. Pulp samples were processed, and LEPR expression was determined by quantitative real-time polymerase chain reaction, and the amount of LEPR protein was analyzed by immunoblot. **Results:** All healthy and inflamed dental pulp samples expressed LEPR. Western blot analysis of human dental pulp revealed the presence of a protein with an apparent molecular weight of approximately 120 kDa, which corresponds to the estimated molecular weight of LEPR. The expression of LEPR mRNA was confirmed by quantitative real-time polymerase chain reaction analysis, and the size of the amplified fragment (338 base pairs for LEPR and 194 base pairs for cyclophilin) was assessed by agarose gel electrophoresis. The relative amount of LEPR in inflamed pulps was approximately 50% higher than in healthy pulps ($P < .05$). **Conclusions:** The presence of LEPR in human dental pulp tissues has been demonstrated for the first time. The up-regulation of LEPR expression in inflamed pulp samples suggests that leptin can play a role in inflammatory and local immune responses in human dental pulp. (*J Endod* 2013;39:1567–1571)

Key Words

Dental pulp stem cells, leptin, pulp immune response, pulp inflammatory response, pulp neuropeptides

Leptin is a 16-kDa protein hormone of 146 amino acids encoded by the *Ob* gene (1) and primarily synthesized and released from adipose tissue (2) and at lower levels by other tissues such as the stomach, skeletal muscle, placenta, and bone marrow (3). Initially, leptin was described as an adipocyte-derived signaling molecule playing a key role in metabolism and homeostasis, regulating the body weight through the control of energy intake and energy expenditure at hypothalamic level (4). According to its primary amino acid sequence that shows structural similarities to the long chain helical cytokine family, such as interleukin-2, interleukin-12, and growth hormone, leptin has been classified as a proinflammatory cytokine (1, 5). Consequently, a role for leptin regulating immunity, inflammation, and hematopoiesis has been accepted (6–9).

Leptin affects both innate and adaptive immunity, exerting an effect on T cells, monocytes, neutrophils, and endothelial cells. In innate immunity, leptin modulates the activity and function of neutrophils and mononuclear cells (8, 10). Moreover, leptin up-regulates both phagocytosis and the production of proinflammatory cytokines of the acute-phase response (11, 12). In adaptive immunity, leptin affects the generation, maturation, and survival of thymic T cells and the switch toward a proinflammatory Th1 immune response (13, 14). Consistent with this role of leptin in the mechanisms of immune response and host defense, leptin levels are increased in infectious and inflammatory stimuli such as lipopolysaccharide, turpentine, and cytokines (15).

Leptin receptor (LEPR) shows sequence homology to members of the class I cytokine receptor (gp130) superfamily (16), which includes the interleukin-6–type cytokine receptors, leukocyte inhibitory factor, and granulocyte colony-stimulating factor (17). LEPR is expressed in 6 isoforms as a product of alternative RNA splicing. According to its structural differences, the receptor's isoforms are divided into 3 classes: long, short, and secretory isoforms. Among all LEPR isoforms, only full-length isoform (LEPRb) is able to fully transduce the activation signals into the cell because its cytoplasmic region contains several motifs required for signal transduction (18, 19). According to the multifunctional role of leptin, this fully active isoform of LEPR is

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expressed not only in the hypothalamus, where it takes part in energy homeostasis, but also is present in peripheral tissues as well as in hematopoietic cells and in all types of immune cells involved in both innate and adaptive immunity (6, 8, 11, 13, 20, 21).

LEPR has been shown in mice monocytes and lymphocytes (11, 13). The presence of both the short and long isoforms of the LEPR has been confirmed in human peripheral blood T lymphocytes (both CD4 and CD8) by Western blot and flow cytometry analysis (8, 21).

In relation to oral tissues, leptin has been identified in both healthy and inflamed gingival tissues (22, 23), in crevicular fluid (24–26), and in cultured human pulp fibroblasts derived from extracted healthy molar teeth where leptin production seems to be regulated by neuropeptides (27). Moreover, LEPR immunoreactivity has been found in the gingival epithelium (23), and LEPR gene has been detected in periapical lesions in experimental rats (28), but no study has described yet the expression of LEPR mRNA and protein in these tissues.

Recently, the expression of leptin has been described in human healthy dental pulp (29). Moreover, experimentally induced dental pulp inflammation (after mechanical pulp injury with short exposure in mouth before tooth extraction) produces the up-regulation of leptin expression, suggesting a possible role of this cytokine-like hormone in mediating a tissue response to inflammation in dental pulp (29). Because there is no evidence of the expression of LEPR in normal human dental pulp so far, the aim of this study was to analyze the possible LEPR expression in human dental pulp tissue samples and, in addition, to compare the LEPR expression level in healthy and in experimentally induced inflamed dental pulps.

Materials and Methods

The study was carried out with the understanding and written consent of each subject and according to the principles of the World Medical Association Declaration of Helsinki. The protocol was approved by the Ethical Board of the University.

Human Dental Pulp Samples

Human dental pulps from 19 healthy, nonsmoking, human donors (22–32 years old), who gave their written informed consent, were obtained from 21 freshly extracted third molars, as previously described (30). All teeth used in this study were caries-free and restoration-free and without signs of periodontal disease.

Fourteen third molars were simply extracted and processed (healthy pulp group). However, in 7 third molars (inflamed pulp group), inflammation was induced before extraction by using the method described previously by Caviedes-Bucheli et al (31). Briefly, the inflammatory process was generated by mechanical exposure of the pulp chamber by using a no.1 round carbide bur in a high-speed handpiece without irrigation. After a period of 10 minutes, the teeth were extracted and processed.

The extracted teeth were washed with 5.25% sodium hypochlorite after extraction to eliminate remains of periodontal ligament that could contaminate the pulp sample, and they were kept at -80°C until use. The teeth were then sectioned by using a Zekrya bur (Dentsply Maillefer, Tulsa, OK) in a high-speed handpiece irrigated with saline solution. The pulp tissue was obtained by using a sterile endodontic excavator, washed 2–3 times in sterile phosphate-buffered saline to remove excess blood, and placed in an Eppendorf tube. Each sample was divided into 2 parts, one for the Western blotting analysis and the other one for the RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) assay.

Antibodies and Reagents

The monoclonal mouse anti- β -tubulin (1:1000) and the polyclonal rabbit antibodies against the long isoform of leptin receptor (C-terminal) were provided by Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-linked anti-mouse/anti-rabbit (1: 10,000) immunoglobulins were purchased from Amersham Pharmacia (Barcelona, Spain).

Western Blotting Analysis

The pulp tissue samples were incubated in 75 μL lysis buffer containing 20 mmol/L Tris, pH 8, 1% Nonidet P-40, 137 mmol/L NaCl, 1 mmol/L MgCl_2 , 1 mmol/L CaCl_2 , 1 mmol/L dithiothreitol, 10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride, and 0.4 mmol/L sodium orthovanadate at 4°C for 30 minutes on an orbital shaker and then centrifuged at 13,000 rpm for 15 minutes. The supernatants were transferred to new tubes. Total protein levels were determined by the bicinchoninic acid method (Thermo Scientific, Rockford, IL) by using bovine serum albumin as standard. We added sodium dodecylsulfate-stop buffer containing 100 mmol/L dithiothreitol to the pulp tissue samples, followed by boiling for 5 minutes. Fifty milligrams protein was loaded in each lane. The samples were then resolved by 10% sodium dodecylsulfate—polyacrylamide gel electrophoresis and electrophoretically transferred onto nitrocellulose membranes (21). The membranes were blocked with buffered saline–0.05% Tween 20 (PBST) containing 3% bovine serum albumin for 1 hour at 23°C . The blots were then incubated with primary antibody for 1 hour, washed in PBST, and further incubated with secondary antibodies by using horseradish peroxidase-linked anti-mouse/anti-rabbit immunoglobulins. Bound horseradish peroxidase was visualized by a highly sensitive chemiluminescence system (Super Signal; Pierce Biotechnology, Rockford, IL). The bands obtained in the blots were scanned and analyzed by the PCBAS 2.0 program. The relative optical density of the different bands was normalized by the corresponding intensity of the tubulin immunoblot in each individual experiment.

RNA Extraction and qRT-PCR Assay and Agarose Gel Electrophoresis

Abundance of LEPR mRNA was determined by qRT-PCR. Total RNA was extracted from human dental pulp tissue samples by using TRIsure (Bioline USA Inc, Taunton, MA) reagent (32). Concentration and purity of the isolated RNA were estimated spectrophotometrically at 260 and 280 nm. For cDNA synthesis, 5 μg total RNA was reverse transcribed at 55°C for 1 hour by using the Transcriptor First Strand cDNA synthesis Kit (Roche, Mannheim, Germany). Quantitative real-time PCR was performed by using the primers based on the sequences of the National Center for Biotechnology Information GenBank database (Table 1). Quantitative RT-PCR Master Mix Reagent kit was obtained from Roche (Fast Start universal SYBR Green), and PCRs were performed on a Mini-Opticon (Bio-Rad, Hercules, CA). A typical reaction contained 10 $\mu\text{mol/L}$ forward and reverse primer and 3 μL cDNA, and the final reaction volume was 25 μL . The reaction was initiated by preheating at 50°C for 2 minutes, followed by heating at 95°C for 10 minutes. Subsequently,

TABLE 1. Sequence of PCR Primers Used in This Study

Gene	Primer sequences
Leptin receptor	
Forward:	5'-ATAGTTCAGTCACCAAGTGC-3'
Reverse:	5'-GTCCTGGAGAACTCTGATGTC-3'
Cyclophilin	
Forward:	5'-CTTCCCCGATACTTCA-3'
Reverse:	5'-TCTTGGTGCTACCTC-3'

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