Leptin stimulates DMP-1 and DSPP expression in human dental pulp via MAPK 1/3 and PI3K signaling pathways

Jenifer Martín-González,*†, Antonio Pérez-Pérez*, Daniel Cabanillas-Balsera*, Teresa Vilarino-García, Victor Sánchez-Margalef, Juan José Segura-Egea

†Department of Stomatology (Endodontics section), School of Dentistry, University of Sevilla, C/ Avicena s/n, 41009, Sevilla, Spain
*Department of Biochemistry and Molecular Biology, and Immunology, Virgen Macarena University Hospital, University of Sevilla, Av. Dr. Fedriani 3, 41071, Sevilla, Spain

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ABSTRACT

Introduction: To investigate the physiological function of leptin in human dental pulp, and to determine the specific pathways implicated in its effect.

Methods: Twenty-seven dental pulp samples were obtained from human third molars. Pulp samples were treated with or without human recombinant leptin. Leptin functional effect was analyzed in terms of regulation of the synthesis levels of DSPP and DMP-1, determined by immunoblot.

Results: Leptin stimulated DMP-1 and DSPP synthesis in all human dental pulp specimens. The stimulatory effect of leptin on DMP-1 and DSPP synthesis was partially prevented by blocking mitogen-activated protein kinase (MAPK 1/3) and phosphatidylinositol 3 kinase (PI3K) pathways, respectively.

Conclusions: The present study demonstrates the functional effect of leptin in human dental pulp stimulating the expression of DMP-1 and DSPP, both proteins implicated in dentinogenesis. Leptin stimulates DSPP expression via PI3K pathway and DMP-1 synthesis via MAPK 1/3 pathway. These results support the role of leptin in pulpal reparative response, opening a new research line that could have translational application to the clinic in vital pulp therapy procedures.

1. Introduction

Different harmful stimuli can cause damage to the dental pulp (Mattos, Soares, & Ribeiro Ade, 2014). When reversible pulpitis develops, vital pulp therapies (VPT) are indicated, applying capping agents directly or indirectly on pulp tissue, in order to protect the dental pulp and to preserve the health status of the tooth (Cohenca, Paranjpe, & Berg, 2013; Rosa, Cocco, & Silva, 2017). The stimulation of odontoblastic secretory activity, with subsequent tertiary dentine formation, is a key factor for the success of VPT. Two odontoblastic proteins, dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 (DMP-1) are positive regulators of hard tissue mineralization (Suzuki, Haruyama, & Nishimura, 2012), acting as nucleators of apatite crystal formation in the presence of collagen, with DSPP inducing highly organized intrafibrillar collagen mineralization and DMP-1 inducing the deposition of mineral particles along the collagen fibril axis (Deshpande et al., 2011). DMP-1 and DSPP also play important roles during odontoblast differentiation. It has been described their expression during stem cells from exfoliated deciduous teeth (SHED) differentiation into functional odontoblasts, and in functional odontoblastic-like cells derived from human iPSCs (Casagrande et al., 2010; Da Rosa, Piva, & da Silva, 2018; Xie et al., 2018).

On the other hand, leptin is a 16-kDa-glycosylated protein product of the Ob gene synthesized mainly in adipose cells (Ahima & Flier, 2000) to regulate weight control in a central manner (Flier, 1995). There is increasing evidence that leptin has systemic effects apart from those related to energy homeostasis, including regulation of neuroendocrine, reproductive, hematopoietic and immune functions (Ahima & Flier, 2000; Pérez-Pérez et al., 2015, 2017). In fact, leptin and its specific receptor (LEPR) are expressed in other tissues, such as placenta, stomach and skeletal muscle (Bado et al., 1998; Masuzaki et al., 1997; Pérez-Pérez et al., 2013). The expression of leptin (Martín-González, Sánchez-Jiménez et al., 2013) in human dental pulp has been described, being its production regulated by neuropeptides (El Karim, Linden, Irwin, & Lundy, 2009). Compelling evidence implicates leptin in dental pulp biology. Leptin acts as modulator of pulp mesenchymal stem cell differentiation (Um, Choi, Lee, Zhang, & Seo, 2010), being synthesized and secreted in vitro by pulp fibroblasts (El Karim et al.,...
2009). Human dental pulp expressed LEPR (Martín-González, Pérez-Pérez et al., 2013), and it has been reported the up-regulation of leptin (Martín-González, Sánchez-Jiménez et al., 2013) and LEPR (Martín-González, Pérez-Pérez et al., 2013) expression in inflamed pulp samples, suggesting that leptin can play a role in dental pulp inflammatory and immune responses. Finally, the findings that leptin promotes the expression of dentin DSPP in human dental pulp (Martín-González et al., 2015) and induces angiogenesis, odontogenic differentiation, and mineralization in human dental pulp cells (hDPCs) (Ngo et al., 2018), support the functional role of leptin in dental pulp reparative and immune responses.

The fully active isoform of LEPR, through which leptin exerts its biological activity, is expressed in human dental pulp (Martín-González, Pérez-Pérez et al., 2013). The binding of leptin to LEPR results in auto-phosphorylation of LEPR and activation of several intracellular signalling cascades, including JAK/STAT (Janus kinase/signal transducer and activator of transcription), PI3K/AKT (phosphatidylinositol-3-kinase/protein kinase B) and ERK/MAPK 1/3 (mitogen-activated protein kinase) signalling pathways (Saxena et al., 2007). The intracellular signalling pathways activated by leptin have been studied in many different systems (Frühbeck, 2006). A recent study has found evidence of the involvement of the MAPK 1/3 signalling pathway in the effect of leptin on angiogenesis, odontogenic differentiation, and mineralization in hDPCs (Martín-González, Pérez-Pérez et al., 2013). However, the molecular mechanisms underlying the effects of leptin on DMP-1 and DSPP expression in dental pulp cells have not been definitively established. Characterizing leptin signaling pathways implicated in the stimulatory effect of leptin on protein expression in dental pulp is a necessary preliminary step to continue deepening and understanding its physiological role. Moreover, the development of therapeutic strategies involving leptin to act in non-collagenous protein synthesis during odontoblastic reactive dentinogenesis cannot be carried out without knowing its signal translation mechanisms. The aim of the present study was to investigate the specific pathways implicated in the stimulatory effect of leptin on protein expression in human dental pulp.

2. 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 Local Review Board.

2.1. Reagents

Human recombinant leptin was provided from R&D Systems (Minneapolis, MN, USA). Polyclonal rabbit anti-phospho-PKB (Ser472/473, 1:1500) and polyclonal rabbit anti-phospho-STAT3 (Tyr705, 1:1500) were purchased from Cell signaling Technology. Polyclonal rabbit anti-phospho-mitogen-activated protein kinases 1 and 3 (MAPK1/3; Thr202/Tyr204, 1:3000) were from Sigma-Aldrich. Monoclonal mouse anti-DSPP (LFBmB-21, 1:1500) and anti-DMP1 (LFBmB-31, 1:1500) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The monoclonal mouse anti-β-tubulin (1:5000) was provided from Sigma-Aldrich. Horseradish peroxidase-linked anti-mouse/anti-rabbit (1:10,000) immunoglobulins were purchased from Amersham Pharmacia (Amersham Pharmacia Biotech, Barcelona, Spain). MEK1 inhibitor (PD98059) and PIK3 inhibitor (Wortmannin) were purchased from Sigma-Aldrich.

2.2. Human dental pulp samples preparation and treatments

Human dental pulps from 25 human donors (22–32 years old), who gave their written informed consent, were obtained from 27 freshly extracted third molars. All teeth used in this study were caries- and restoration-free and without signs of periodontal disease.

The teeth were sectioned by using a Zekrya bur (Dentsply Maillefer, Tulsa, OK, USA) in a high-speed hand-piece irrigated with saline solution. The pulp tissue was obtained by using a sterile endodontic excavator, washed 2–3 times in sterile ice-cold phosphate-buffered saline (PBS) to remove excess blood. Each dental pulp was thoroughly rinsed with sterile ice-cold PBS.

Human dental pulps were randomly distributed in tubes containing 1 ml of a DMEM-F12 medium (Invitrogen) 0% fetal calf serum (FCS), supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin, (3 replicates per treatment). Dental pulps were incubated at 37°C in 5% CO2 for 10 min to study the signal transduction pathways activated by leptin in the same medium supplemented or not with 0.1, 1 and 10 nM human recombinant leptin (R&D Systems, Minneapolis, MN).

On the other hand, dental pulps were also treated with or without leptin (10 nM) for 4 h in the presence or absence of the pharmacological inhibitor wortmannin (50 nM), an inhibitor of PIK3, or PD98059 (100 μM), an inhibitor of MEK, in the MAPK 1/3 pathway, to check the relative importance of both pathways in the protein synthesis stimulated by leptin. After all this, dental pulps were removed and resuspended in 75 μl of lysis buffer (1 x PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and 10 mg/ml phe- nylmethanesulfonyl fluoride (PMSF)) during 30 min at 4°C on an orbital shaker and later centrifuged at 10,000 g for 20 min. In the lysate process, lysis buffer was used first and then the orbital shaker for 30 min to homogenize the cell lysate. Any sonication was used. After centrifugation, the soluble cell lysates were used for Western blot analysis.

The sampling in the methodology was as follows:

<table>
<thead>
<tr>
<th>Treatments in Human Dental Pulp</th>
<th>Number of Human Dental Pulp per treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.- Controls (0 nM of leptin)</td>
<td>6</td>
</tr>
<tr>
<td>2.- 0.1 nM of leptin</td>
<td>3</td>
</tr>
<tr>
<td>3.- 1 nM of leptin</td>
<td>3</td>
</tr>
<tr>
<td>4.- 10 nM of leptin</td>
<td>3</td>
</tr>
<tr>
<td>5.- Inhibitor wortmannin</td>
<td>3</td>
</tr>
<tr>
<td>6.- Wortmannin + leptin (10 nM)</td>
<td>3</td>
</tr>
<tr>
<td>7.- Inhibitor PD98059</td>
<td>3</td>
</tr>
<tr>
<td>8.- PD98059 + leptin (10 nM)</td>
<td>3</td>
</tr>
</tbody>
</table>

2.3. Western blotting analysis

Total protein levels were determined by the bicinchoninic acid method (Thermo Scientific, Rockford, IL, USA), using bovine serum albumin as standard. SDS-stop buffer containing 100 mM of DTT was added to the pulp tissue samples, followed by 5 min boiling. In each lane 50 μg protein were loaded. The samples were then resolved by 12% SDS-PAGE and electrophoretically transferred onto nitrocellulose membranes (Hybond, Amersham Pharmacia). The membranes were blocked with buffered saline – 0.05% Tween 20 (PBST) containing 3% bovine serum albumin during 1 h at 23°C. Membranes were then immunoblotted overnight with polyclonal rabbit anti-phospho-STAT3 (Tyr705, 1:1500, Cell signalling Technology), with polyclonal rabbit anti-phospho-mitogen-activated protein kinases 1 and 3 (MAPK1/3; Thr202/Tyr204, 1:3000, Sigma-Aldrich), polyclonal rabbit anti-phospho-PKB (Ser472/473, 1:1500, Cell signalling Technology), monoclonal mouse anti-DSPP (LFBmB-21, 1:1500, Santa Cruz Biotechnology) and monoclonal mouse anti-DMP1(LFBmB-31, 1:1500, Santa Cruz Biotechnology).

Loading controls were performed by immunoblotting the same membranes with monoclonal anti-β-tubulin (1:5000, Sigma-Aldrich). Then, the blots were washed in PBST and further incubated with secondary antibodies using horseradish peroxidase-linked anti-mouse/anti-rabbit immunoglobulins (1:10,000, Amersham Pharmacia Biotech). Bound horseradish peroxidase was visualized by a highly sensitive chemiluminescence system (Super Signal; Pierce Biotechnology, Rockford, IL, USA). Quantification of protein bands was determined by...
densitometry using 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.

2.4. Data analysis

Experiments were repeated separately at least three times to assure reproducible results. Results are expressed as means ± SD in arbitrary units (AU). Arbitrary units were calculated as normalized band intensity in Western blot analysis. Statistical analysis was performed using the GraphPad Prism computer program (GraphPad Software, San Diego, CA, USA) and the Mann–Whitney rank sum test. Significance levels were set at p < 0.05.

3. Results

3.1. Leptin activation of JAK-STAT3 signalling pathway in human dental pulp

Tyrosine phosphorylation of STAT3 was observed in response to human leptin stimulation in a dose-response manner (Fig. 1A, right panel). The relative amount of the phosphorylated form of STAT3 (P-STAT3) in stimulated pulps was significantly higher than in unstimulated pulps (p < 0.05). Activation of STAT3 was observed at 0.1, 1 and 10 nM leptin, reaching a maximum at 0.1 nM leptin. Western blot analysis revealed the presence in the pulp samples of a protein with apparent molecular weight of 93 kDa, corresponding to the estimated molecular weight of P-STAT3 (Fig. 1A, left panel).
3.2. Leptin activation of PI3K pathway in human dental pulp

Leptin dose-dependently stimulated the phosphorylation of the central kinase of PI3K pathway, i.e. PKB (Fig. 1B, right panel). The relative amount of phosphorylated form of PKB (P-PKB) in stimulated pulps was significantly higher compared to control pulps (p < 0.05). Activation of PKB was observed at 0.1, 1 and 10 nM leptin, reaching a maximum at 0.1 nM leptin. Western blot analysis revealed the presence in the pulp samples of a protein with apparent molecular weight of 60 kDa, which corresponds to the estimated molecular weight of the phosphorylated form of P-PKB (Fig. 1B, left panel).

Leptin activation of MAPK 1/3 pathway in human dental pulp

To investigate the effect of leptin on MAPK 1/3 pathways, the phosphorylation level of MAPK 1/3 was studied. Antibodies that specifically recognize the phosphorylated forms of the MAPK 1/3 (P-p42 MAPK 1/3, P-p44 MAPK 1/3) were used. Leptin 0.1, 1 and 10 nM stimulated tyrosine/threonine phosphorylation of MAPK 1/3 as assessed by specific immunoblot with the anti-phosphorylated MAPK 1/3 antibody (Fig. 1C, right panel). The relative amount of the phosphorylated forms of MAPK 1/3 in stimulated pulps was almost four-times higher than in unstimulated pulps (p < 0.05). Maximal phosphorylation was observed at 0.1 nM leptin. Western blot analysis revealed the presence in the pulp samples of two proteins with apparent molecular weights of 42 kDa and 44 kDa, which corresponds, respectively, to the estimated molecular weights of P-p42 MAPK 1/3 and P-p44 MAPK 1/3 (Fig. 1C, left panel).

3.3. Mechanism mediating the Leptin stimulation of DSPP and DMP-1 synthesis in human dental pulp

In order to investigate the signal transduction pathways of leptin in human dental pulp implicated in DSPP and DMP-1 synthesis, human dental pulp was stimulated with human leptin 10 nM for 4 h, in the absence and presence of pharmacological inhibitors of MAPK 1/3 and PI3K pathways.

Leptin 10 nM increased DMP-1 expression in human dental pulp in 10-fold (p < 0.05) (Fig. 2A). The stimulatory effect of leptin in DMP-1 expression was prevented by pretreatment with PD98059, an inhibitor of MAPK 1/3 pathway. Pretreatment with wortmannin, an inhibitor of PI3K pathway, reduced by 50% DMP-1 expression.

Leptin 10 nM also increased DSPP expression in human dental pulp (12-fold, p < 0.05) (Fig. 2B) and this effect was partially prevented by pretreatment with wortmannin, an inhibitor of PI3K pathway. Pretreatment with the inhibitor of MAPK 1/3 pathway PD98059 did not affect DSPP expression.

4. Discussion

The present study demonstrates the functional effect of leptin in human dental pulp stimulating the expression of DMP-1 and DSPP, both proteins implicated in dentinogenesis. These results support the role of leptin in pulpal reparative response, opening a new research line that could have translational application to the clinic in vital pulp therapy procedures.

The methodology used in this investigation is the same previously described in human placenta (Pérez-Pérez, Maymó, Gambino, Dueñas, Goberna, Varone, Sánchez-Margalet et al., 2009). The wortmannin and PD98059 concentrations used were not toxic and comparable to those used in other similar tissues (Pérez-Pérez, Maymó, Gambino, Dueñas, Goberna, Varone, Sánchez-Margalet et al., 2009; Slomiany & Slomiany, 2005)

Although the three LEPR signalling pathways, ERK/MAPK 1/3, PI3K and JAK/STAT-3, are triggered by leptin in human dental pulp, the major pathway whereby leptin stimulates DMP-1 expression seems to be the MAPK 1/3 one, also intervening to a lesser extent PI3K signalling pathway. Since the inhibition of MAPK 1/3 pathway completely abolishes the effect of leptin on the DMP-1 expression, the partial effect in the presence of the PI3K inhibitor may be mediated by the well known cross-talk between PI3K-PKB and Ras-Raf-MAPK 1/3 pathway (Yart, Chap, & Raynal, 2002). Thus, PI3K may activate Raf, the activator of MEK (MAPK 1/3). On the other hand, the stimulation of DSPP expression seems to be only mediated by the PI3K signaling pathway.

Previously published results have demonstrated that leptin is synthesized and secreted in cultured human dental pulp fibroblasts derived from extracted healthy molar teeth (Vaisse et al., 1996), as well as in human healthy and inflamed dental pulp (Martín-González, Sánchez-Jiménez et al., 2013). Moreover, it has been found that dental pulp leptin levels increase during experimentally induced pulpsitis (Martín-González, Sánchez-Jiménez et al., 2013). In addition, LEPR is up-regulated in inflamed dental pulp (Martín-González, Pérez-Pérez et al.,

**Fig. 2.** Inhibition of leptin-mediated DSPP and DMP-1 synthesis in the presence of the pharmacological inhibitors wortmannin (W) and PD98059 (PD) in human dental pulp.

Human dental pulps were treated with or without leptin (10 nM) for 4 h in the presence or absence of wortmannin (50 nM), a pharmacological inhibitor PI3K pathway, or PD98059 (100 μM), an inhibitor of MAPK 1/3 signaling pathway, and then hDPCs were processed as described in Materials and Methods. The effect of leptin on DMP-1 synthesis was completely blocked by pretreatment with PD98059, and partially blocked by pretreatment with wortmannin (A). The effect of leptin on DSPP synthesis was blocked by pretreatment with wortmannin (B). The amount of protein was controlled by immunoblotting with anti-tubulin. Densitograms with standard errors are shown. *p < 0.05 versus control. Results are mean ± SEM of three independent experiments run in triplicate. Protein synthesis is estimated as absolute units. kDa, kilodalton.
2013), and there has been provided evidence that leptin promotes odontoblastic differentiation and suppress adiagenic differentiation in dental pulp stem cells (DPSCs) (Martín-González et al., 2015). The stimulatory effect of leptin in protein secretion by hDPCs have also been demonstrated. Thus, leptin induces DSPP (Martín-González et al., 2015; Ngo et al., 2018), DMP-1 (Ngo et al., 2018) and vascular endothelial growth factor (VEGF) expression in hDPCs (Ide, Tokuyama, & Shimozuma, 2011). If all these findings are combined with the demonstration that hDPCs express leptin and LEPR (El Karim et al., 2009; Martín-González, Sánchez-Jíménez et al., 2013, 2013b), there is sufficient basis to suggest that leptin could play a role in defensive and reparative responses of dental pulp against deep dentin carious lesions. The probable action of leptin as modulator of pulp immune and inflammatory responses (Martín-González, Pérez-Pérez et al., 2013), raises the possibility of using it in the treatment of reversible pulpitis. Future research should analyze the possible application of leptin in VPT.

The molecular mechanisms underlying the effect of leptin on hDPCs are not fully understood. Previous results had shown that leptin stimulates protein synthesis (Pérez-Pérez, Maymó, Gambino, Dueñas, Goberna, Varone et al., 2009) by stimulating MAPK 1/3 and PI3K pathways in different systems (Pérez-Pérez et al., 2008; Takahashi et al., 1996). Likewise, the results reported by Martín-González et al. (2015) and Ngo et al. (2018), have demonstrated that leptin can stimulate DSPP, DMP-1 and VEGF expression in human dental pulp, through the activation of MAPK 1/3 signaling pathway (Ngo et al., 2018).

The results of the present study show that leptin activates in hDPCs the same three signaling pathways (MAPK 1/3, PI3K and JAK-STAT) coupled to LEPR in other systems (Kellerer et al., 1997; Liu et al., 2014; Sánchez-Margale et al., 2003; Szanto & Kahn, 2000; Vaisse et al., 1996; Wang et al., 1997; Zhao et al., 2006).

Mitogen-activated protein kinase (MAPK 1/3) is a well-known signaling pathway activated by leptin receptor (Pérez-Pérez et al., 2008). MAPK 1/3 are an essential component in many physiologic processes, such as cell growth, proliferation, differentiation, and apoptosis. In dental pulp, MAPK 1/3 is activated during odontoblast stimulation in tertiary dentinogenesis and is one of the known kinases that is required for late-stage odontoblastic differentiation and angiogenic potential of DPSCs (Qin et al., 2014). Recently it has been demonstrated that MTA, Biodentine, Bioaggregate and calcium silicate cements are able to stimulate odontoblastic differentiation and mineralization nodule formation by activating the MAPK 1/3 pathway. MAPK 1/3 pathway plays an important role in regulating the angiogenic behavior of dental pulp cells cultured on MTA. Therefore, the present results showing that leptin strongly activates the MAPK 1/3 pathway in hDPCs are in agreement with those previous findings already mentioned and with those recently published by Ngo et al. (2018), who have found that leptin stimulates angiogenesis, odontogenic differentiation, and mineralization in hDPCs via activating the MAPK 1/3 pathway. All these findings support the stimulating role of leptin in odontoblastic differentiation and in dentinogenesis (Bado et al., 1997).

In relation with the PI3K signalling pathway, it has been implicated in the regulation of many cellular processes, including resistance to apoptosis, cell motility, differentiation, and proliferation (Pérez-Pérez, Maymó, Gambino, Dueñas, Goberna, Varone et al., 2009). The results of the present study, showing leptin activation of PI3K pathway in hDPCs, are in agreement with previous studies reporting that leptin activates PI3K pathway in myotubes, β-cells, hepatocytes and PBMC (Kellerer et al., 1997; Vaisse et al., 1996; Wang et al., 1997). In dental pulp, toll-like receptor 2 (TLR2) signaling through the PI3K pathway is necessary for lipoteichoic acid (LTA) -induced VEGF expression in pulp cells. Interaction between lipopolysaccharide (LPS) and toll-like receptor 4 (TLR4) also signaling through the PI3K pathway in DPSCs.

On the other hand, the present study has shown that leptin activate JAK-STAT signaling pathway in hDPCs. This pathway plays critical, non-redundant roles in mediating cellular transcriptional responses to cytokines, and in cell activation, survival and proliferation (Pérez-Pérez, Maymó, Gambino, Dueñas, Goberna, Varone et al., 2009; Takahashi et al., 1996). It has been shown that leukemia inhibitory factor (LIF) inhibits the odontoblastic differentiation of DPSCs via the JAK-STAT3 signaling pathway (Takahashi et al., 1996).

An important finding of the present study is the characterization of the signaling pathways implicated in leptin stimulation of DSPP and DMP-1 expression in hDPCs, two specific markers for odontogenic differentiation (Suzuki et al., 2012). The major pathways whereby leptin exerts this effect seem to be the MAPK 1/3 one in DMP-1 expression and the PI3K one in DPP expression. The identification of the pathways was carried out analyzing the effect of pharmacological inhibitors of MAPK 1/3 and PI3K pathways on leptin stimulation of DSPP and DMP-1 expression, and checking the relative importance of both pathways.

Pretreatment of hDPCs with PD98059, an inhibitor of MAPK 1/3, completely blocked the stimulating effect of leptin on DMP-1 expression, indicating that the main signaling pathway whereby leptin stimulates DMP-1 expression is MAPK 1/3. However, DMP-1 expression was also partially decreased by wortmannin, an inhibitor of PI3K pathway, indicating that this signaling pathway was be also implicated in leptin stimulation of DMP-1 expression in hDPCs. PI3K is also important for leptin action in other cells, such as lymphocytes (Sánchez-Margale et al., 2003), placenta (Pérez-Pérez et al., 2016), or breast cancer cells (Pérez-Pérez et al., 2016).

On the other hand, pretreatment of hDPCs with wortmannin, an inhibitor of the PI3K signaling pathway, blocked leptin stimulation of DSPP expression, whereas PD98059, an inhibitor of the MAPK 1/3 pathway did not affect DSPP expression. These results suggest that the main signaling pathway of leptin stimulation of DSPP expression in hDPCs is PI3K. This finding does not agree with the results of Ngo et al. (2018), who found that MAPK 1/3 activation was involved in leptin-mediated DSPP and VEGF expression in hDPCs. These authors used the MAPK 1/3 inhibitors U0126, SP600125, and SB202190 to assess the implication of MAPK 1/3 signaling pathway in leptin stimulation of DSPP and VEGF expression in hDPCs. U0126 is an extracellular signal-related kinases (ERK) inhibitor, SP600125 is a c-Jun N-terminal kinase (JNK) inhibitor, and SB202190 is a p38 mitogen-activated protein kinase (MAPK) inhibitor (Ngo et al., 2018). On the contrary, in the present study the inhibitor of the MAPK 1/3 pathway used was PD98059, a potent and selective inhibitor of MAP kinase kinases (MAPKK), MEK1 and MEK2 (Alessi, Cuenda, Cohen, Dudley, & Saltiel, 1995). It binds to the inactive form of MAPKK and prevents activation by upstream activators such as c-Raf.

The findings of this study open a new line of research that could have translational application to the clinic in vital pulp therapy. In dentinogenesis non-collagenous proteins (NCPs), such as DMP-1 and DSPP, secreted by odontoblasts, are believed to actively promote and control the mineralization of collagen fibers and crystal growth within predentin. DSPP, a member of the SIBLING (Small Integrin-Binding Ligand, N-linked Glycoprotein) family of NCPs, is the most abundant NCPs, playing a main role in the biomineralization process of predentin. Mutations in the DSPP gene are associated with dentinogenesis imperfecta (DGI), an autosomal dominant disorder causing dentin hypomineralization and significant tooth decay. Phosphorylated form of DSPP induces highly organized intrafibrillar collagen mineralization (Suzuki et al., 2012).

5. Conclusions

The stimulation of DSPP and DMP-1 expression in hDPCs by leptin coupled to PI3K and MAPK 1/3 signaling pathways, respectively, showed in this investigation, suggest that leptin-LEPR interaction in odontoblasts induces protein synthesis in hDPCs. These findings, together with previous findings showing that LEPR is up-regulated in inflamed human dental pulp (Pérez-Pérez et al., 2013), strongly support...
the stimulatory role of leptin in dental pulp reparative response. During reversible pulpitis, leptin could act stimulating odontoblastic reactive dentinogenesis, increasing non-collagenous protein synthesis (DMP-1 and DSPP), collaborating to maintain dental pulp health after VPT procedures.

Conflicts of interest

The authors deny any conflicts of interest.

Ethical approval

The study was carried out according to the principles of the World Medical Association Declaration of Helsinki. The protocol was approved by the Ethical Board of the University of Sevilla, SPAIN.

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See references for further details.