The Role of Heme Oxygenase-1 in the Proliferation and Odontoblastic Differentiation of Human Dental Pulp Cells

Sun-Ju Kim, PhD,†* *Kyung-San Min, DDS, PhD,*‡* *Hyun-Wook Ryu, DDS, MSD,*‡* *Hwa-Jeong Lee, PhD,** *and Eun-Cheol Kim, DDS, PhD**

Abstract

Introduction: It was recently reported that heme oxygenase-1 (HO-1) activity is related to stem cell differentiation; however, the involvement of HO-1 in pulp cell growth and differentiation has not been well explored. The purpose of this study was to investigate the role of HO-1 in the growth and differentiation of human dental pulp cells (HDPCs). **Methods:** We evaluated cell growth by MTT assay, mineralization by alizarin red staining, and differentiation marker mRNA expression by reverse transcriptase polymerase chain reaction. **Results:** HO-1 induction by cobaltic protoporphyrin IX (CoPP) in HDPCs increased cell growth and mineralization and up-regulated the messenger RNA expression of such odontoblastic markers as alkaline phosphatase, osteopontin, bone sialoprotein, dentin matrix protein-1, and dentin sialophosphoprotein. Carbon monoxide scavenger, iron chelator, HO-1 inhibitor, and HO-1 small interfering RNA (siRNA) attenuated HDPC growth and differentiation. **Conclusions:** CoPP treatment results in dental pulp cell proliferation and odontoblast differentiation that appears partly mediated by HO-1. Our results suggest that odontoblastic differentiation and growth are positively regulated by HO-1 induction and negatively regulated by HO-1 inhibition. Thus, pharmacologic HO-1 induction might represent a potent therapeutic approach for pulp capping and the regeneration of HDPCs. *(J Endod 2010;36:1326–1331)*

Key Words

Differentiation, growth, heme oxygenase-1, human dental pulp cells

From the Departments of *Oral and Maxillofacial Pathology and ‡Conservative Dentistry, School of Dentistry, Wonkwang University, Iksan, South Korea; and †Department of Dental Hygiene, Cheongju University, Cheongju, South Korea.

This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A084458).

Address requests for reprints to Dr Eun-Cheol Kim, Department of Oral and Maxillofacial Pathology, Dental College, Wonkwang University, Sinyoungdong 344-2, Iksan City, Jeonbuk, 570-749, South Korea. E-mail address: eckwkop@wonkwang.ac.kr.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2010.04.011

Human dental pulp (HDP) healing and repair are the result of successive and interrelated processes, including the proliferation, chemotaxis, and differentiation of dental pulp cells into odontoblasts leading to reparative dentin formation (1). Thus, differentiated and undifferentiated cells within dental pulp may contribute to the dentinal regeneration process (2). Odontoblasts secrete several collagenous and noncollagenous proteins, including osteonectin, osteopontin (OPN), bone sialoprotein, dentin matrix protein-1 (DMP-1), and dentin sialophosphoprotein (DSPP) (3), which have been used as mineralization markers for the odontoblast-/osteoblast-like differentiation of HDP cells (HDPCs) (4). However, the molecular control mechanism underlying the effect of the inductive signal on odontoblastic growth and differentiation remains to be elucidated (5).

Heme oxygenase-1 (HO-1, heat shock protein 32) is the inducible isoform of the rate-limiting enzyme responsible for the breakdown of heme into carbon monoxide (CO), biliverdin, and free iron (6). Previously, we reported that the HO-1 pathway plays a key role in the adaptation of cells to stressful conditions and the recovery of HDPCs and periodontal ligament cells (PDLCs) from injurious events (7–12). Moreover, several studies have shown that HO-1 expression is related to adipogenesis in human mesenchymal stem cells (MSCs) (13), osteoblastic differentiation in PDLCs (7, 12), and neuronal differentiation in MSCs (14).

Cobaltic protoporphyrin IX (CoPP), or hemin, has been shown to strongly induce HO-1 expression both *in vivo* and *in vitro* (15). Hemin promotes proliferation and differentiation in endothelial progenitor cells (16) and erythroid differentiation in human myeloid leukemia cells (17). In addition, we previously showed that the induction of HO-1 by hemin enhanced the expression of such osteoblastic differentiation markers as OPN, osteonectin, OCN, and bone sialoprotein on PDLCs (7, 12).

However, no information is available regarding the effects of HO-1 induction by CoPP on the odontogenic potential of HDPCs. To elucidate the role of HO-1 in HDPCs, we investigated the effects of CoPP (an HO-1 inducer), tin protoporphyrin (SnPP, an HO-1 inhibitor), HO-1 siRNA, and HO-1 metabolites on the growth and differentiation of HDPCs.

Materials and Methods

Reagents

Dulbecco modified Eagle medium, fetal bovine serum, and the other tissue culture reagents were obtained from Gibco BRL (Grand Island, NY). CoPP, SnPP, and hemin were obtained from Porphyrin Products (Logan, UT). Hemoglobin (Hb) and desferrioxamine (DFO) were purchased from Sigma (St Louis, MO).

Cell Culture

We used the HDPCs lines immortalized by transfection with the telomerase catalytic subunit hTERT gene (18). Cells were cultured in Dulbecco modified Eagle medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere of 5 % CO_2 at 37°C. For mineralization experiments, cells were cultured in osteogenic media (OM) including 50 μg/mL ascorbic acid, 10^{-7} mol/L dexamethasone, and 10 mmol/L β-glycerophosphate as described previously (19).

TABLE 1. RT-PCR Primers Sequence

Gene	Sequence (5′ -3′)	Size (bp)
Heme oxygenase-1 (HO-1)	Forward: AAGATTGCCCAGAAAGCCCTGG Reverse: AACTGTCGCCACCAGAAAGCTGAG	399
Alkaline phosphatase (ALP)	Forward: ACGTGGCTAAGAATGTCATC Reverse: CTGGTAGGCGATGTCCTTA	475
Osteopontin (OPN)	Forward: CCAAGTAAGTCCAACGAAAG Reverse: GGTGATGTCCTCGTCTGTA	347
Dentin-matrix protein-1 (DMP-1)	Forward: CAGGAGCACAGGAAAAGGAG Reverse: CTGGTGGTATCTTCCCCCAGGAG	213
Dentin sialophosphoprotein (DSPP)	Forward: CAGGAGCACAGGAAAAGGAG Reverse: CTGATTTGCTGCTGTCTGAC	488
	GAPDH Forward: CGGAGTCAACGGATTTGGTCGTAT Reverse: AGCCTTCTCCA TGGTGGTGAAGAC	306

Cell Viability Assay

After treatment of HDPCs with CoPP or other drugs, 50 μL 3-(4,5-dimethylthiazol-2-yl)-2.5 diphenyltetrazolin bromide (MTT, 2 mg/mL) was added to each well, and then the samples were incubated for 4 hours and centrifuged (200 g for 10 minutes). After aspiration of supernatant, the cells were lysed and solubilized by the addition of 50 μL dimethyl sulfoxide (DMSO). The absorbance of each sample was analyzed at 540 nm using the microtiter plate reader. Cytotoxicity (%) was calculated relatively to the control.

Semiquantitative Reverse Transcriptase Polymerase Chain Reaction

Total RNA was extracted from the cells by using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Then, 1 μg RNA was reverse transcribed for first-strand complementary DNA synthesis (Gibco BRL, Rockville, MD). The cDNA was amplified in a final volume of 20 μL containing 2.5 mmol/L magnesium dicholoride, 1.25 U Ex Taq polymerase (Bioneer, Daejeon, Korea), and 1 μmol/L specific primers. The sequences of the specific primers used in this study are detailed in Table 1. Reverse transcriptase polymerase chain reaction products were electrophoresed on 1.5 % agarose gel with 0.5 mg/mL ethidium bromide. Bands were detected by ultraviolet illumination of ethidium bromide–stained gels.

Alizarin Red Stain

After 2 weeks of differentiation induction, cells were rinsed with phosphate buffered saline, air dried, and fixed in ice-cold 95% ethanol for 30 minutes at −20°C. Subsequently, the cells and the matrix were

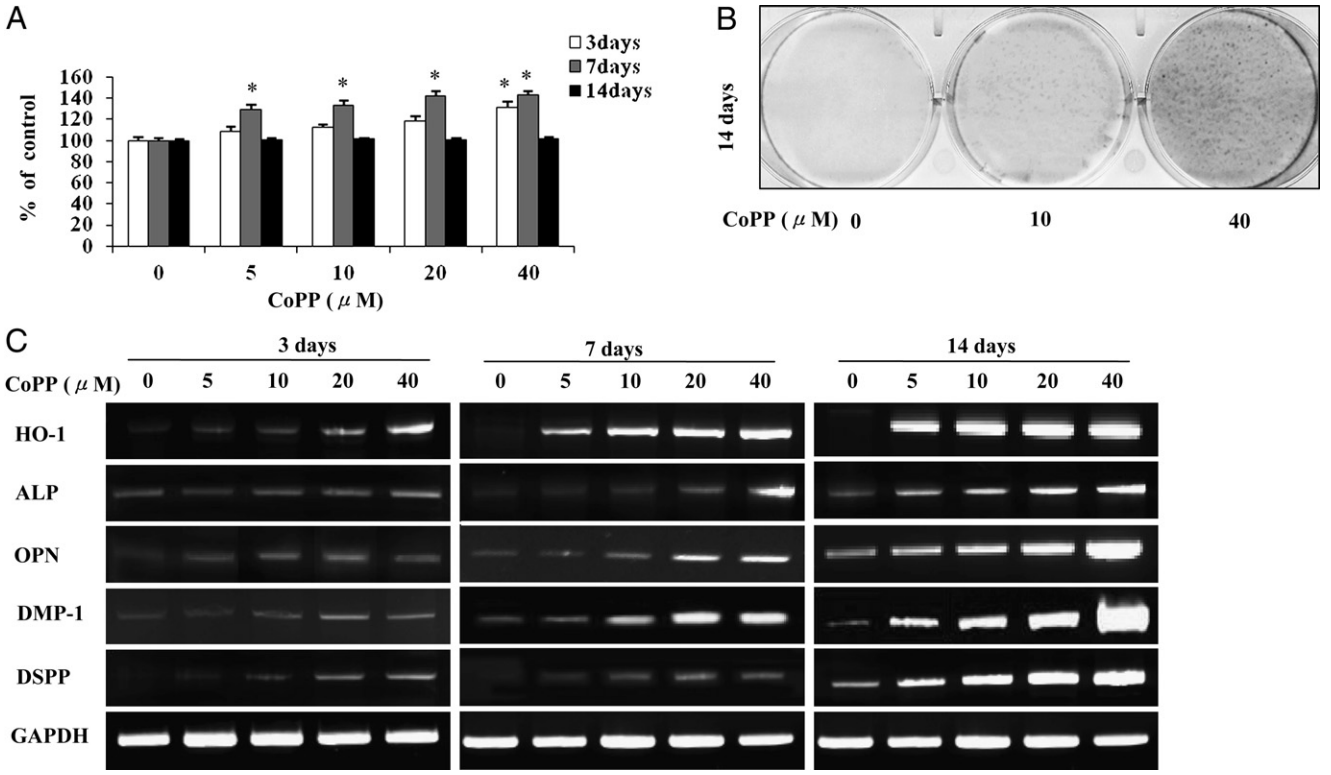

Figure 1. The effects of CoPP on (*A*) growth, (*B*) mineralized nodule formation, and (*C*) odontoblastic differentiation in HDPCs. Cells were cultured with CoPP at concentrations ranging from 0 to 40 μmol/L for 3, 7, and 14 days in OM with 10 mmol/L β-glycerophosphate, 10^{-7} mol/L dexamethasone, and 50 μg/ml L-ascorbic acid. (*A*) Cell viability was evaluated by using an MTT assay. (*B*) Mineralization was analyzed by alizarin red staining. (*C*) The mRNA expression of HO-1 and such odontoblastic differentiation markers as DMP-1, OPN, and DSPP were assessed by RT-PCR analysis. These data are representative of three independent experiments. ∗Statistically significant difference as compared with the control, $p < 0.05$.