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Rapamycin/Paclitaxel이 사람과 흰쥐 평활근세포에서 Heme oxygenase-1의 발현에 미치는 영향

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Induction of Heme Oxygenase-1 Expression by Rapamycin and Paclitaxel in Smooth Muscle and Endothelial Cells of Human and Rat

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Running Title : Induction of HO-1 Expression by Rapamycin and Paclitaxel

Abstract

Background: Both rapamycin and paclitaxel are currently being used in drug-eluting stent to block the proliferation of vascular endothelial and smooth muscle cells. Heme oxygenase-1 (HO-1) has also known to be anti-proliferative against vascular cells. In this study, it focused on the role of HO-1 in cytoprotection and antiproliferation of paclitaxel. Methods: Proliferation of cells and metabolic activity of HO-1 were evaluated with the tetrazolium-based assay. HO-1 protein expression was shown by western blot analysis of vascular smooth muscle cells (VSMCs), or endothelial cells (ECs), using anti-HO-1 polyclonal antibody from untreated cells and cells exposed to rapamycin and paclitaxel. To determine whether the inhibition of paclitaxel on platelet-derived growth factor (PDGF)-dependent proliferation may be mediated by its induction of HO-1, we exposed cells to paclitaxel, PDGF and ZnPP, an inhibitor of HO activity. Survival rate was checked to find the anti-apoptotic effect of paclitaxel against tumor necrosis factor (TNF)-α-mediated apoptosis, in the presence or abscence of ZnPP. Result: Rapamycin induces HO-1 protein in rat VSMCs and human ECs. Exposure of VSMCs to paclitaxel for 12h resulted in a dose-dependent increase in HO-1 activity. Ten nM of paclitaxel led to a maximal expression of HO-1 protein at 12h after exposure. The addition of ZnPP abrogated the suppression effect of paclitaxel on PDGF-stimulated cell proliferation, and blocked paclitaxel-mediated suppression of TNF-α-mediated apoptosis. Hemoglobin, a scavenger of CO, abrogated the paclitaxel induced cytoprotection. Conclusion: Paclitaxel induced the expression of HO-1 gene in rat VSMCs and human ECs, in dose-dependent and time-dependent manner, like rapamycin.

Key word: rapamycin, paclitaxel, smooth muscle cell, endothelial cell, heme oxygenase-1

Introduction

Rapamycin (RAP), a lipophilic macrolide from fungus, was identified more than twenty years ago during antibiotic screening. Although lacking antibacterial activity, RAP shows potent anti-proliferative effects on growth factor-stimulated proliferations of T lymphocytes (T cells), endothelial cells (ECs), smooth muscle cells (SMCs), and fibroblasts,[1-4] and these effects of RAP have led to its use to prevent vascular neointimal proliferation and restenosis[5,6]. RAP inhibits T cell activation through the

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formation of pharmacologically active complexes with members of a family of intracellular receptors termed the FK506 binding proteins (FKBPs). The FKBP12-RAP complex interacts with the mammalian target of RAP (mTOR), and this leads to inhibition of both DNA and protein synthesis, resulting in the arrest from G_1 into the S phase of the cell cycle, thereby inhibiting T-cell proliferation.[7] The FKBP12-RAP complex also inhibits the proliferation of mesenchymal cells and ECs.[3]

Paclitaxel is one of the first effective anticancer agents with a novel mechanism of action to be developed in over a decade, and it has generated considerable enthusiasm in the oncology community because of its favorable response rate in patients with advanced metastatic ovarian, breast, and lung cancer.[8] The therapeutic mechanism of paclitaxel is thought to reside primarily in its unique effects on microtubules (tubulin polymers) against depolymerization.[9,11] Paclitaxel-treated cells develop large bundles in the M phase of the cell cycle.[11] Recently, paclitaxel is known to have distinct cell cycle-independent effects[12]. This drug is shown to provide a second signal for macrophage activation to tumoricidal activity via L-arginine-dependent nitric oxide synthesis.[13,14]

Heme oxygenase (HO) is the rate-limiting enzyme in the degradation of heme into biliverdin, carbon monoxide (CO), and free divalent iron (Fe^{2+}), and three distinct isoforms have been identified and cloned.[15,16] Among them, the HO-1 isoform is distributed ubiquitously and is strongly induced by a variety of physiologic and pathophysiologic stimuli, including heme, heavy metals, inflammatory cytokines, and nitric oxide.[16-18] Accumulating evidences show the pivotal importance of HO-1 expression in mediating anti-oxidant, anti-inflammatory, and anti-apoptotic effects.[19-21] Bilirubin generated by HO-1 is an anti-oxidant capable of scavenging peroxy radicals and inhibiting lipid peroxidation[22] The release of Fe^{2+} is known to mitigate any antioxidant actions of HO-1 gene expression and may explain rather narrow threshold of HO-1 overexpression to confer protection, because Fe^{2+}, like other transition metal ions, catalyzes the formation of ROS in the Harber-Weiss or Fenton reaction.[23,24] The cytoprotective effects of Fe^{2+} released by HO-1 have been explained by the fact that Fe^{2+} fosters gene expression of ferritin, a protein imparting additive cytoprotection against oxidative stress.[25] Furthermore, Fe^{2+} by itself has very recently been reported to have cytoprotective effect via NF-B activation.[26] Although intensively pursued, it still remains unclear how iron controls life and death of cell. Another product CO has properties similar to those of nitric oxide and alters the expression of inflammatory mediators, tumor necrosis factor, interleukin-1, and interleukin-10.[27-30] CO also has a protective role against vascular injury, because it inhibits vascular SMC proliferation.[29] Adenovirus-mediated HO-1 gene transfer inhibits balloon injury-induced neointimal formation, potentially through the generation of CO.[31] RAP and paclitaxel also protect against vascular injury by inhibiting VSMC proliferation.[32,33]

In this study, the authors examined the possibility that the cytoprotective activities of RAP and paclitaxel may include the induction of HO-1. Human and rat ECs and VSMCs were evaluated for HO-1 expression in response to RAP and paclitaxel and whether it mediates the anti-proliferative and cytoprotective activities of RAP and paclitaxel.

Materials and Methods

1. Reagents

RAP, platelet-derived growth factor (PDGF), and tricarbonyldichloro-ruthenium dimer (RuCO) were purchased from Sigma-Aldrich Co.. Zinc protoporhyrin (ZnPP) was from Porphyrin Products (Logan Co., UT), and the Cell Proliferation Kit II (2,3-bis[2-4-nitro-5-sulfophenyl]2H-tetrazolium-5-carboxanilide, XTT) was obtained from Boehringer Mannheim Co.. Anti-HO-1 and b-actin Abs were purchased from Santa Cruz Co.. Unless indicated otherwise, all other chemicals were obtained from Sigma Chemical Co..

2. Cell culture

Rat VSMCs were obtained as previously described[34] and human ECs were purchased from Clonetics Co.. The cells were grown in endothelial growth medium (EGM-MV, Clonetics Co.) plus 10% FBS at 37℃ in a humidified atmosphere of 95% air and 5% CO_2.

3. Western blot analysis for HO-1

Western blot analysis was performed as follows. Briefly, cells were harvested, washed twice with ice-cold phosphate-buffered saline (PBS), and resuspended in 20 mM Tris-HCl buffer (pH 7.4) containing a protease inhibitor mixture (0.1mM phenylmethylsulfonyl fluoride, 5 mg/mL aprotinin, 5 μg/mL pepstatin A, and 1 μg/mL chymostatin). Protein concentration was determined with the Lowry protein assay kit (P5626, Sigma Co.). An equal volume of 2× SDS-sample buffer was added, and the samples were boiled for 5 min. Samples (40 μg) were subjected to electrophoresis in a 12% SDS-polyacrylamide gel for 2 h at 20mA and then transferred to nitrocellulose. The membranes were incubated for 1 h in 5% (wt/vol) dried milk protein in PBS containing 0.05% (vol/vol) Tween-20. The membranes were washed in PBS containing 0.05% (vol/vol) Tween-20 and incubated for 1 h in the presence of goat polyclonal antibody against mouse HO-1 (Santa Cruz CA). The membranes were washed extensively and then incubated for 1 h with anti-goat IgG conjugated to HRP (1:4,000). The membranes were washed extensively again and the protein bands were visualized using chemiluminescent reagents according to the manufacturer's instructions (Supersignal Substrate; Pierce).

4. Assay for HO activity

HO enzyme activity was measured by previously described method.[35] Cells were homogenized in 0.5mL ice-cold 0.25M sucrose solution containing 50mM phosphate buffer (pH7.4). Homogenates were centrifuged at 200*g* for 10min. The supernatants were centrifuged at 9000*g* for 20min, and these supernatants were further centrifuged at 105000*g* for 60min. The microsomal pellet was then resuspended in 50mM phosphate buffer (pH7.4). The reaction mixture (200L) containing 0.2mM hemin, 0.25-0.50mg/mL of retinal microsomal proteins, 0.5mg/mL rat liver cytosol, 0.2mM $MgCl_2$, 2mM glucose-6-phosphate, 1U/mL glucose-6-phosphate dehydrogenase, 1mm NADPH and 50mM potassium phosphate buffer (pH7.4) was incubated at 37℃ for 2h. The reaction was stopped with 0.6mL of chloroform and after extraction the chloroform layer was spectrophotometrically measured. Bilirubin formation was calculated from the difference in absorption between 464 and 530nm.

5. Cell Proliferation

Proliferation/metabolic activity was evaluated with the tetrazolium-based assay XTT (Boehringer Mannheim) according to manufacturer's instructions. Cells were incubated in 96-well plates in cell culture media with or without PDGF (50ng/mL), rapamycin (10mM), or ZnPP (10mM). XTT at a concentration of 0.3mg/mL was added to each well for 8 h at 37℃, and the absorbance of the samples was measured between 450 and 500nm with a references wavelength >650 nm.

6. Cell Viability

For the determination of the cell viability, 50mg/mL of 3-[4.5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to 1 ml of cell suspension (1×10^6cells/ml in 24-well plates) for 4 h, and the formazan formed was dissolved in acidic 2-propanol; optical density were measure d using an assay reader at 590 nm. The optical density of formazan formed in control (untreated) cells was taken as 100% of viability.

7. Statistical analysis

Differences in the data among the groups were analyzed by the Student's *t* test, and all values were expressed as mean ±S.D. The differences between groups were considered to be significant at $P < 0.01$.

Results

1. RAP induces HO-1 protein expression in rat VSMCs and human ECs

The effects of RAP treatment on HO-1 protein expression were examined in rat VSMCs and human ECs by Western blot analysis. As shown in Fig 1A and B, exposure to RAP (10 μM) increased HO-1 protein levels in rat VSMCs and human ECs in a time-dependent manner.

The peak induction was observed at 24 h in rat VSMCs and human ECs. 10 mM of RAP, which markedly increased HO protein expression, did not affect the cell viability compared with untreated cells (Fig. 2). However, treatment of rat VSMCs and human ECs with RAP (100 μ M) reduced the viability of the cells to about 65% and 56% of control ($P<0.05$; Fig. 2A and B).

2. Effects of paclitaxel on HO activity and HO-1 protein expression in rat VSMCs and human ECs

To determine whether paclitaxel could affect HO activity and HO-1 protein expression in rat VSMCs and human ECs, we treated the cells for 12 h with various concentrations of paclitaxel. The effects of various concentrations of paclitaxel (0.1-50nM) on HO activity and HO-1 protein expression in rat VSMCs are shown in Fig. 2. Exposure of VSMCs to paclitaxel for 12 h resulted in a dose-dependent increase in HO-1 activity (Fig. 3A). The increase was significantly different from control (untreated cells, $P<0.01$), with a maximal enzymatic activity at 10 nM of paclitaxel. Western blot analysis revealed that enhanced HO activities by paclitaxel treatments were directly correlated with HO-1 protein levels (Fig. 3B). A time dependent induction of HO-1 in response to 10nM of paclitaxel is shown in Fig. 3C. This dose of paclitaxel led to a maximal expression of HO-1 protein at 12 h after exposure. HO-1 protein level showed marking from maximum after 24 h incubation (Fig. 3C).

Fig. 4A shows a HO activity of human ECs exposure to increasing concentrations of paclitaxel, 0.01 to 10nM for 12 h. 10 nM of paclitaxel, which markedly increased HO activity. This effect was correlated with dose-dependent increases in HO-1 protein expressions, as shown by Western blot analysis (Fig. 4B). Using 10nM of paclitaxel, the HO protein expression was evident as early as 6 h, and reached a maximum at 12 h after treatment of the cells with paclitaxel (Fig. 4C).

In rat VSMCs, 100 nM of paclitaxel did not affect cell viability (Fig. 5A). However, at the higher concentration of 500nM, cell death was evident. In human ECs, 100nM paclitaxel did affect the cell viability compared with untreated cells but 10nM of paclitaxel did not (Fig. 5B).

3. Effects of paclitaxel on the proliferation of rat VSMCs

HO-1 or one of its products, CO, has been shown to suppress vascular SMC proliferation in response to an injury.[29] Among the similar previous studies, we found that paclitaxel inhibited PDGF-dependent rat vascular SMC proliferation (Fig. 6).

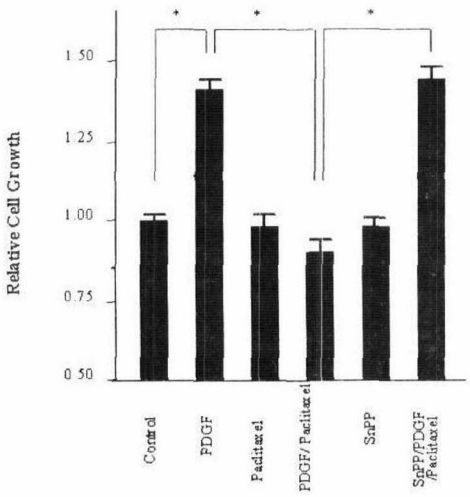

Fig. 6 Effect of paclitaxel on the proliferation of rat VSMCs. The cells were incubated with or without PDGF (50ng/mL), paclitaxel (10nM), or ZnPP (10mM). Cell proliferation was assessed using the tetrazolium-based XTT assay. Each bar represents the mean SD of three independent experiments. *; $P < 0.01$

To determine whether the inhibition of paclitaxel on PDGF-dependent proliferation may be mediated by its

induction of HO-1, we exposed cells to paclitaxel, PDGF, and ZnPP, an inhibitor of HO activity. The addition of ZnPP abrogated the suppressive effects of paclitaxel on PDGF-stimulated cell proliferation (Fig. 6). These results suggest that paclitaxel-mediated increases in HO-1 protein expression contribute to the antiproliferative effects of paclitaxel.

4. HO expression by paclitaxel protects human ECs from TNF-α-mediated apoptosis

We examined the effects of paclitaxel pretreatment on TNF-α-mediated apoptosis in human ECs. The human ECs were pretreated 12 h with 10nM of paclitaxel, and then exposed to 100nM of TNF-α for 24 h. Paclitaxel was found to decrease TNF-α-mediated apoptosis (Fig. 7). Exposure of the cells for 24 h to 100nM of TNF-α caused about 62% decrease in cell viability (P<0.05; Fig. 7). However, pretreatment of the cells with paclitaxel recovered the viability of the cells to about 82% of control (P<0.05; Fig. 7). The involvement of HO-1 in the cytoprotective effect of paclitaxel was confirmed using an inhibitor of HO activity, ZnPP, which at the concentration of 10mM significantly blocked paclitaxel-mediated suppression of TNF-α-mediated apoptosis (Fig. 7).

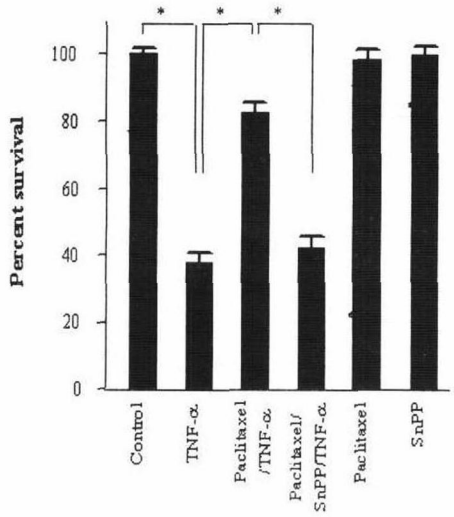

Fig. 7 i-apoptotic effect of paclitaxel against TNF-a- ediated apoptosis. Human ECs were treated with 10nM of paclitaxel for 12 h in the presence or absence of 10mM of ZnPP. After this pretreatment, cells were incubated for 24 h with TNF-a (100ng.mL). Cell viability was assessed using the MTT assay.Each bar represents the mean SD of three independent experiments. *; P < 0.01

These data show that the cytoprotective effect of paclitaxel might be due to the induction of HO-1.

5. Endogenous CO mediates the antiapoptotic effect of HO-1 on human ECs

Since HO-1 enzymatic activity is need for its antiapoptotic effect, this suggests that this antiapoptotic effect is mediated through one or more end products of heme catabolism by HO-1, i.e., CO, Fe^{2+}, and bilirubin. To determine whether the inhibition of paclitaxel on TNF-α-mediated apoptosis may be mediated by CO, we exposed cells to paclitaxel, PDGF, and Hb, a scavenger of CO. As shown in Fig. 8, we found that Hb (40mg/mL) abrogated the paclitaxel-induced cytoprotection. These results suggest that CO might mediate the inhibitory effect of paclitaxel-induced HO-1 on TNF-α-mediated apoptosis.

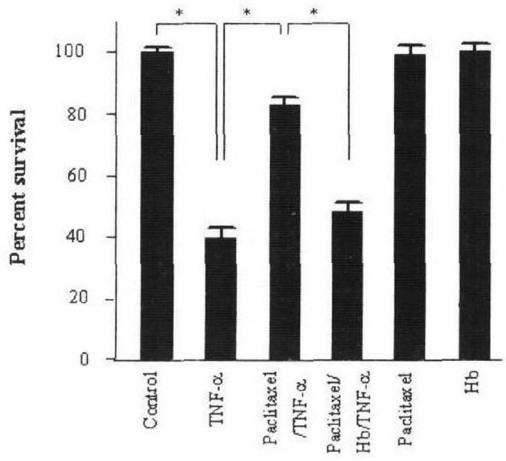

Fig. 8 Anti-apoptotic effect of HO-1 is mediated via CO. Human ECs were treated with 10 nM of paclitaxel for 12 h in the presence or absence of 40 mg/mL of Hb. After this pretreatment, cells were incubated for 24 h with TNF-a (100 ng.mL). Cell viability was assessed using the MTT assay.Each bar represents the mean SD of three independent experiments. *; P < 0.01

Discussion

Coronary arteriosclerosis and accompanying vessel stenosis can lead to myocardial ischemia and, without intervention, to death.[36] A common treatment for this condition is percutaneous transluminal coronary angioplasty, but a large percentage (30-70%) of the vessels subjected to this procedure restenose. In such instances, coronary artery restenosis is frequently the result of intimal hyperplasia, a process during which SMC in the medial layer of the vessel wall undergo what has been termed phenotypic modulation and migrate into the vessel intima, where they begin to proliferate.[37] Recently, it is reported that the precursor cells originated from bone marrow migrate to the lesion.[38] Polypeptide growth factors secreted by platelets or by injured endothelial cells are believed to provide the mitogenic and chemotactic signals that drive SMC phenotypic modulation during this process[39] Accordingly, researchers wish to identify compounds that block SMC proliferation and thus may be used clinically to control intimal hyperplasia and restenosis.

In an attempt to reduce the rate of restenosis, coronary stent was devised, but restenosis is still a serious problem. Recently, chemicals that can block SMC proliferation are being tried to coat the stent (drug-eluting stent) that releases the chemical for prolonged periods.[40] Among many chemicals, RAP and paclitaxel are most frequently used as coating chemicals without knowing the action mechanisms.[41]

The role of HO in heme and heme-iron metabolism is well established. The chemical diversity of HO-1 inducers (heme, ultraviolet irradiation, sodium arsenite, hyperoxia, inflammatory cytokines, glutathione depleting agents, nitric oxide, catalposide) in conjunction with the properties of the catalytic reaction products,[16,42] however, argues for a broader physiological role for HO in cellular and systemic functions. Consistent with this hypothesis, HO-1 has been shown to have anti-oxidant, anti-inflammatory and anti-apoptotic properties.[19-21]

In this study, the authors tried to know the action mechanism of RAP and paclitaxel which are used in the drug-eluting stent to reduce the restenosis. Specifically, the authors examined if paclitaxel could induce HO-1 expression in VSMCs and ECs like RAP which was recently reported to induce HO-1 in SMCs and ECs. As shown in Fig. 1, 3 and 4, paclitaxel as well as RAP induce HO-1 gene in rat VSMCs and human ECs. The induction of HO-1 gene by RAP in rat VSMCs and human ECs is corresponded with the results of Visner et al[43] (Fig. 1).

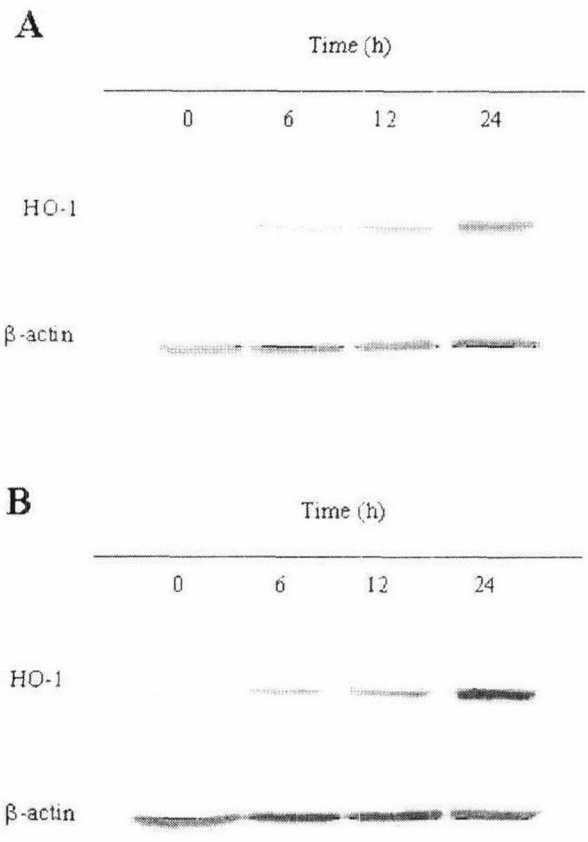

Fig. 1 RAP induces HO-1 protein expression in rat VSMCs and human ECs. Western blot analysis of VSMCs (A) or ECs (B) using an anti-HO-1 polyclonal antibody from untrated cells and cells exposed to RAP for 12 h at various concentrations indicated in the figures. Data are representative of three independent experiments.

However, the authors firstly demonstrated that paclitaxel

induced the expressions of HO-1 gene in the rat VSMCs and human ECs in dose-dependent and time-dependent manners (Fig. 3 and 4 respectively).

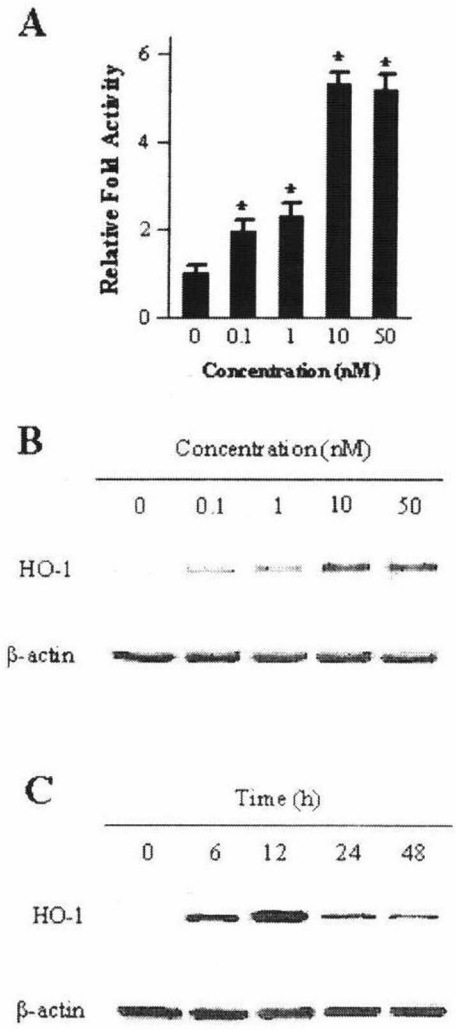

Fig. 3 Effect of Paclitaxel on HO activity and HO-1 protein expression in rat VSMCs. HO-1 enzyme activity (A) and HO protein expression (B) were measured in rat VSMCs at 12 h after treatment of the cells with various concentrations of paclitaxel. The cells were treated with 10nM of paclitaxel, and HO-1 protein expression (C) was measured at various time points indicated in the figures. Western blot analysis was performed using specific antibodies for HO-1 and b-actin. HO enzyme activity was determined via measuring bilirubin formation as described in materials and methods. Each bar represents the mean SD of three independent experiments. *; $P < 0.01$

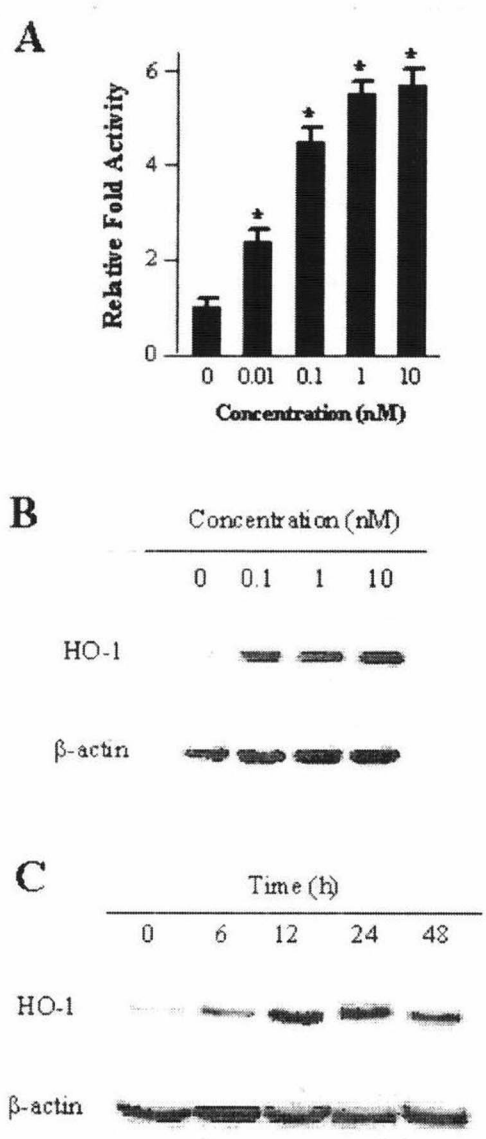

Fig. 4 Effect of Paclitaxel on HO activity and HO-1 protein expression in human ECs. HO-1 enzyme activity (A) and HO protein expression (B) were measured in human ECs at 12 h after treatment of the cells with various concentrations of paclitaxel. The cells were treated with 10nM of paclitaxel, and HO-1 protein expression (C) was measured at various time points indicated in the figures. Western blot analysis was performed using specific antibodies for HO-1 and b-actin. HO enzyme activity was determined via measuring bilirubin formation as described in materials and methods. Each bar represents the mean SD of three independent experiments. *; $P < 0.01$

Moreover, the HO-1 activities exactly correlated with the

expressions of HO-1 protein. Both RAP and paclitaxel showed no cytotoxicities in the ranges of doses used in this study (Fig. 2 and Fig. 5).

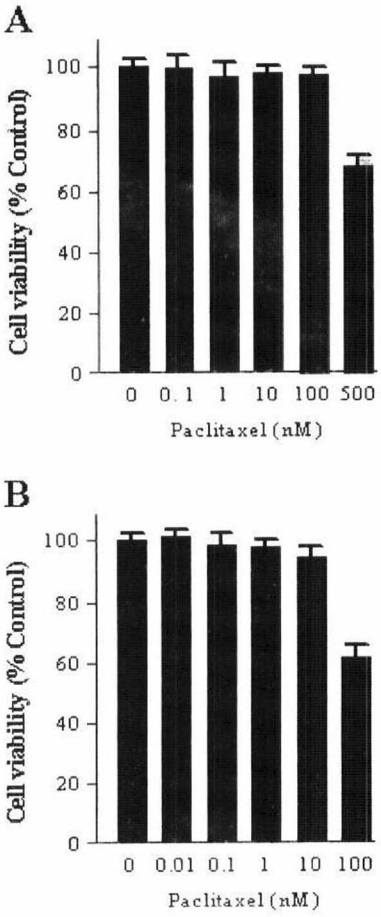

Fig. 2 Cytotoxicity by RAP in rat VSMCs and human ECs. Rat VSMCs (A) and human ECs (B) were treated with various concentrations of RAP and then incubated for 24h. After this treatment, cells were washed and viability was assessed using the MTT assay. Each bar represents the mean SD of three independent experiments.

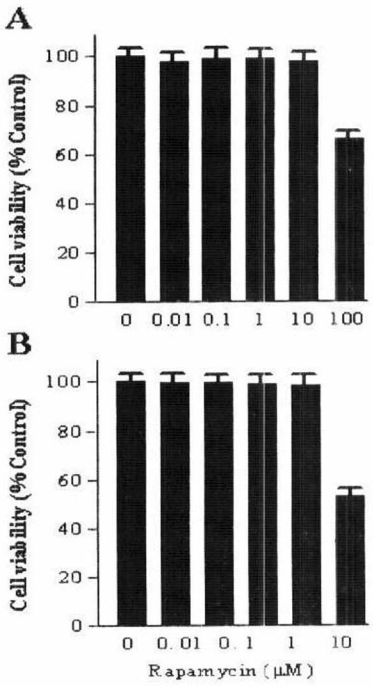

Fig. 5 Cytotoxicity by paclitaxel in rat VSMCs and human ECs. Rat VSMCs (A) and human ECs (B) were treated with various concentrations of paclitaxel and then incubated for 24 h. After this treatment, cells were washed and viability was assessed using the MTT assay. Each bar represents the mean SD of three independent experiments.

The neointimal formation and hyperplasia shown in the lesion of restenosis is known to be composed of smooth muscle cell proliferation.[44] In this study the authors addressed, if PDGF induced proliferation of VSMC could be inhibited by paclitaxel, the inhibition of VSMC proliferation might be mediated by HO-1. As shown in Fig. 6, paclitaxel inhibited the PDGF-induced increased proliferation of VSMC and the inhibition was blocked by the use of HO-1 inhibitor ZnPP, which demonstrates paclitaxel inhibits the VSMC proliferation by the induction of HO-1.

As shown in Fig. 7, paclitaxel inhibited TNF-α-induced apoptosis of ECs, which was also reversed by ZnPP, representing the involvement of HO-1. Finally, the authors attempted to pin-point which one among HO-1 reaction-products is responsible for the cytoprotective action of paclitaxel. As shown in Fig. 8, CO scavenger Hb

reversed the cytoprotective effect of paclitaxel on ECs suggesting CO from HO-1 induced by paclitaxel is responsible for the protection of ECs from cytotoxicity.

Conclusion

The authors demonstrated that HO-1 is differentially expressed in human ECs and rat VSMCs by RAP and paclitaxel and that the antiproliferative and cytoprotective effects may be mediated by CO production via the HO-1 induction. These studies identify potentially unique antiproliferative and cytoprotective effects of RAP and paclitaxel on vascular cells, the induction of HO-1, which could explain the mechanisms of most frequently using drugs as coating chemicals for stent.

References

1. Sehgal SN: Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. Clin Biochem 31:335-340, 1998
2. Marx SO, Jayaraman T, Go LO, Marks AR: Rapamycin-FKBP inhibits cell cycle regulators of proliferation in vascular smooth muscle cells. Circ Res 76:412-417, 1995
3. Cao W, Mohacsi P, Shorthouse R, Pratt R, Morris RE: Effects of rapamycin on growth factor-stimulated vascular smooth muscle cell DNA synthesis. Inhibition of basic fibroblast growth factor and platelet-derived growth factor action and antagonism of rapamycin by FK506. Transplantation 59:390-395, 1995
4. Mohacsi PJ, Tuller D,. Hulliger B, Wijngaard PL: Different inhibitory effects of immunosuppressive drugs on human and rat aortic smooth muscle and endothelial cell proliferation stimulated by platelet-derived growth factor or endothelial cell growth factor. J Heart Lung Transplant 16:484-492, 1997
5. Suzuki T, Kopia G, Hayashi S, Bailey LR, Llanos G, Wilensky R, Klugherz BD, Papandreou G, Narayan P, Leon MB, Yeung AC, Tio F, Tsao PS, Falotico RA, Carter J: Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. Circulation 104:1188-1193, 2001
6. Burke SE, Lubbers NL, Chen YW, Hsieh GC, Mollison KW, Luly JR, Wegner CD: Neointimal formation after balloon-induced vascular injury in Yucatan minipigs is reduced by oral rapamycin. J Cardiovasc Pharmacol 33:829-835, 1999
7. Terada N, Lucas JJ, Szepesi A, Franklin RA, Domenico J, Gelfand EW:. Rapamycin blocks cell cycle progression of activated T cells prior to events characteristic of the middle to late G1 phase of the cycle. J Cell Physiol 154:7-15, 1993
8. Rowinsky EK, Onetto N, Canetta RM, Arbuck SG: Taxol: the first of the taxanes, an important new class of antitumor agents. Semin Oncol 19:646-662, 1992
9. Manfredi JJ, Parness J, Horwitz SB: Taxol binds to cellular microtubules. J Cell Biol 94: 688-696, 1982
10. Rao S, Horwitz SB, Ringel I: Direct photoaffinity labeling of tubulin with taxol. J Natl Cancer Inst 84:785-788, 1992
11. Rowinsky EK, Donehower RC, Jones RJ, Tucker RW: Microtubule changes and cytotoxicity in leukemic cell lines treated with taxol. Cancer Res 48:4093-4100, 1988
12. Ding AH, Porteu F, Sanchez E, Nathan CF:. Shared actions of endotoxin and taxol on TNF receptors and TNF release. Science 248:370-372, 1990
13. Moncada S, Palmer RM, Higgs EA: Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109-142, 1991
14. Lowenstein CJ, Snyder SH: Nitric oxide, a novel biologic messenger. Cell 70:705-707, 1992
15. Tenhunen R, Marver HS, Schmid R: Microsomal heme oxygenase. Characterization of the enzyme. J Bio Chem 244:6388-6394, 1969
16. Maines MD: The heme oxygenase system: a regulator of second messenger gases. Annu Rev Pharmacol Toxicol 37:517-554, 1997
17. Durante W, Kroll MH, Christodoulides N, Peyton KJ, Schafer AI: Nitric oxide induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle cells. Circ Res 80:557-564, 1997
18. Yet SF, Pellacani A, Patterson C, Tan L, Folta SC, Foster L, Lee WS, Hsieh CM, Perrella MA: Induction of heme oxygenase-1 expression in vascular smooth muscle cells. A link to endotoxic shock. J Biol Chem 272:4295-4301, 1997
19. Otterbein LE, Bach FH, Alam J, Soares M, Lu HT, Wysk M, Davis RJ, Flavell RA, Choi AM: Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nat Med 6:422-428, 2000
20. Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, Soares P: Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. J Exp Med 192:1015-1026, 2000
21. Poss KD, Tonegawa S: Reduced stress defense in heme oxygenase 1-deficient cells. Proc Natl Acad Sci USA 94:10925-10930, 1997
22. Stocker R, McDonagh AF, Glazer AN, Ames BN: Antioxidant activities of bile pigments: biliverdin and bilirubin. Methods Enzymol 186:301-309, 1990
23. Kroemer G, Dallaporta B, Resche-Rigon M: The mitochondrial death/life regulator in apoptosis and necrosis. Annu Rev Physiol 60:619-642, 1998

24. Meneghini R: Iron homeostasis, oxidative stress, and DNA damage. Free Rad Biol Med 23: 783-792, 1997
25. Vile GF, Basu-Modak S, Waltner C, Tyrrell RM: Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. Proc Natl Acad Sci USA 91: 2607-2610, 1994
26. Xiong S, She H, Takeuchi H, Han B, Engelhardt JF, Barton CH, Zandi E, Giulivi C, Tsukamoto H: Signaling role of intracellular iron in NF-kB activation. J Biol Chem 278: 17646-17654, 2003
27. Lee TS, Chau LY: Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nature Med 8:240-246, 2002
28. Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, Soares MP: Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. J Exp Med 192:1015-1025, 2000
29. Duckers HJ, Boehm M, True AL, Yet SF, San H, Park JL, Webb RC, Lee ME, Nabel GJ, Nabel EG: Heme oxygenase-1 protects against vascular constriction and proliferation. Nature Med 7:693-698, 2001
30. Choi BM, Pae HO, Kim YM, Chung HT: Nitric oxide-mediated cytoprotection of hepatocytes from glucose deprivation-induced cytotoxicity: Involvement of heme oxygenase-1. Hepatology 37:810-823, 2003
31. Liu XM, Chapman GB, Wang H, Durante W: Adenovirus-mediated heme oxygenase-1 gene expression stimulates apoptosis in vascular smooth muscle cells. Circulation 105: 79-84, 2002
32. Gallo R, Padurean A, Jayaraman T, Marx S, Roque M, Adelman S, Chesebro J, Fallon J, Fuster V, Marks A,.Badimon JJ: Inhibition of intimal thickening after balloon angioplasty in porcine coronary arteries by targeting regulators of the cell cycle. Circulation 99: 2164-2170, 1999
33. Lanza GM, Yu X, Winter PM, Abendschein DR, Karukstis KK, Scott MJ, Chinen LK, Fuhrhop RW, Scherrer DE, Wickline SA: Targeted antiproliferative drug delivery to vascular smooth muscle cells with a magnetic resonance imaging nanoparticle contrast agent: implications for rational therapy of restenosis. Circulation 106:2842-2847, 2002
34. Visner GA, Staples ED, Chesrown SE, Block ER, Zander DS, Nick HS: Isolation and maintenance of human pulmonary artery endothelial cells in culture isolated from transplant donors. Am J Physiol 267:406-413, 1994
35. Kutty RK, Maines MD: Oxidation of heme with derivatives by purified heme oxygenase. Evidence for the presence of one molecular species of heme oxygenase in the rat liver. J Bio Chem 257:9944-9952, 1982
36. Gielen S, Schuler G, Hambrecht R: Exercise Training in Coronary Artery Disease and Coronary Vasomotion. Circulation 103:e1-e6, 2001
37. Kosokabe T, Okumura K, Sone T, Kondo J, Tsuboi H, Mukawa H, Tomida T, Suzuki T, Kamiya H, Matsui H, Hayakawa T: Relation of a common methylenetetrahydrofolate reductase mutation and plasma homocysteine with intimal hyperplasia after coronary stenting. Circulation 103:2048-2054, 2001
38. Caplice NM, Bunch TJ, Stalboerger PG, Wang S, Simper D, Miller DV, Russell SJ, Litzow MR, Edwards WD: Smooth muscle cells in human coronary atherosclerosis can originate from cells administered at marrow transplantation. Proc Natl Acad Sci USA 100: 4754-4759, 2003
39. Holm T, Damas JK, Holven K, Nordoy I, Brosstad FR, Ueland T, Wahre T, Kjekshus J, Froland SS, Eiken HG, Solum NO, Gullestad L, Nenseter M, Aukrust P: CXC-chemokines in coronary artery disease: possible pathogenic role of interactions between oxidized low-density lipoprotein, platelets and peripheral blood mononuclear cells. J Thromb Haemost 1:257-262, 2003
40 Palmaz JC: New advances in endovascular technology. Tex Heart Inst J 24:156-159, 1997
41. Oberhoff M, Herdeg C, Baumbach A, Karsch KR: Stent-based antirestenotic coatings (sirolimus/paclitaxel). Catheter. Cardiovasc Interv 55:404-408, 2002
42. Moon MK, Choi BM, Oh GS, Pae HO, Kim JD, Oh H, Oh CS, Kim DH, Rho YD, Shin MK, Lee HS, Chung HT: Catalposide protects Neuro 2A cells from hydrogen peroxide-induced cytotoxicity via the expression of heme oxygenase-1. Toxicol Lett 145: 46-54, 2003
43. Visner GA, Lu F, Zhou H, Liu J, Kazemfar K, Agarwal A: Rapamycin induces heme oxygenase-1 in human pulmonary vascular cells; Implications in the antiproliferative response to rapamycin. Circulation 107:911-916, 2003
44. Chen D, Krasinski K, Sylvester A, Chen J, Nisen PD, Andres V: Downregulation of cyclin-dependent kinase 2 activity and cyclin A promoter activity in vascular smooth muscle cells by p27(KIP1), an inhibitor of neointima formation in the rat carotid artery. J. Clin Invest 99:2334-2341, 1997