Original Research

Paricalcitol protects against hydrogen peroxide–induced injury in endothelial cells through suppression of apoptosis

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Impact Statement

Much research has been done in recent years about vitamin D and its analogues. Paricalcitol is used to treat and prevent hyperparathyroidism in patients with chronic kidney disease (CKD). In this study, we discovered that paricalcitol has protective and therapeutic effects on oxidative stress–induced endothelial damage. Oxidative stress increases in patients with renal dysfunction and the use of paricalcitol may provide additional benefits in such patients by protecting endothelial cells from the effects of oxidative stress.

Abstract

The vascular endothelium is one of the main targets of oxidative stress which plays an important role in the pathophysiology of vascular damage. Recent studies show that vitamin D can positively regulate endothelial functions in various chronic diseases and in cases of increased oxidative stress. In our study, we investigated the restorative and protective potentials of paricalcitol which is frequently used in patients with chronic renal failure, a vitamin D analogue, in human umbilical vein endothelial cells (HUVEC) before and after H_2O_2-induced oxidative stress. Paricalcitol treatment after the oxidative stress induced by H_2O_2 increased cell viability in endothelial cells depending on the dose that was used. While paricalcitol (500 nM) decreased caspase-3 activity and mitochondrial membrane potential loss, it increased nitric oxide (NO) production and reduced glutathione (GSH) levels. Paricalcitol treatment before oxidative stress increased cell viability. It increased NO production and mitochondrial membrane potential while significantly reducing caspase-3 activity. While paricalcitol caused a significant inhibition of protein disulfide isomerase (PDI) reductase activity in healthy endothelial cells, it did not cause a significant change on the PDI reductase activity under oxidative stress conditions. Present study showed that paricalcitol has restorative and protective effects on endothelial cells against oxidative injury, but these effects occur at high concentrations of paricalcitol.

Keywords: Caspase-3, HUVEC, mitochondrial membrane potential, oxidative injury, paricalcitol, protein disulfide isomerase

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Introduction

Vascular endothelial cells play an important role as a barrier in the prevention of cardiovascular diseases. A number of active substances are secreted from the endothelial cells for the normal physiological functions of the cardiovascular system. These substances are effective on vascular wall tension, angiogenesis, blood flow, and inflammation. For these reasons, vascular endothelial health is very important. Some physical and chemical factors and chronic diseases may cause damage to the vascular endothelium.[1] Vasoprotective effects of vitamin D have been demonstrated. Vitamin D deficiency is a risk factor for endothelial dysfunction which is characterized by decreased nitric oxide (NO) bioavailability. Increased oxidative stress suppresses NO synthesis and consequently reduces NO bioavailability. Vitamin D shows anti-inflammatory activity and has been shown to play a role in regulating NO bioavailability by mediating the activity of endothelial NO synthase (eNOS) and increasing antioxidant capacity by affecting the activity of antioxidant enzymes.[2–4] Endothelial dysfunction can contribute to the development of cardiovascular complications in patients with chronic kidney disease (CKD). Calcitriol synthesis is decreased in chronic renal failure in direct response to decreased kidney function. The difference in calcitriol level results in decreased calcium absorption and increased parathyroid hormone (PTH) production. Selective vitamin D analogues and calcimimetics have been developed to suppress secondary hyperparathyroidism without increasing calcium and phosphate serum levels. Paricalcitol (19-nor-1,25-dihydroxyvitamin D2) is one such developed molecule and is a less calcemic and phosphatemic vitamin D analogue.[5] Clinical studies with paricalcitol showed that paricalcitol significantly suppresses PTH.[6] Inflammation is a strong indicator of increased morbidity and mortality in hemodialysis (HD) patients. Paricalcitol, a selective vitamin D receptor activator used in the prevention

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and treatment of secondary hyperparathyroidism, has been shown to have anti-inflammatory properties in experimental studies.[7] Decreased NO bioavailability can also be associated with inflammation. NO plays an important role in the pathogenesis of inflammation. While NO has an anti-inflammatory effect under normal physiological conditions, it can have a pro-inflammatory effect in pathological conditions.[8] Decreased NO bioavailability is observed in CKD, and this abnormality is accompanied by decreased expression or limited activation of eNOS associated with increased oxidative stress, which contributes to reduced vasodilator capacity. According to clinical experimental studies, the lack of substrate and cofactors required for NO synthesis, degradation of L-arginine by other metabolic pathways, and the presence of endogenous NO inhibitors have been suggested as the reason for the decrease in NO bioavailability. In addition, in the presence of oxidative stress, NO deficiency may occur because NO is inactivated by oxygen radicals and NOS becomes a superoxide producer.[2] There is clear evidence that oxidative stress occurs early on in the course of CKD and is exacerbated as the disease progresses.[9] NO produced by endothelial NOS controls vascular tone and platelet-endothelial cell interactions with endothelial-derived prostaglandins. Recent studies have shown that NO can partially achieve this effect through the inhibition of protein disulfide isomerase (PDI, $PDIa_1$). It has been shown that cell surface PDI can interact with NO and can inhibit its activity.[10] PDI is a thiol isomerase that catalyzes the formation and cleavage of thiol-disulfide bonds during protein folding in the endoplasmic reticulum (ER). PDI is primarily localized in the ER, but is found to a lesser extent in the nucleus, cytosol, and cell surface. PDI located in the plasma membrane regulates the function of cell surface molecules. Intravascular cell-derived PDI can play an important role in the initiation and progression of cardiovascular diseases, including thrombosis and vascular inflammation.[11]

In our study, the restorative and protective effects of paricalcitol against oxidative endothelial cell damage were investigated on apoptosis indicators, NO production, and PDI activity.

Materials and methods

Endothelial cell culture

HUVEC (ATCCO-CRL-1730O, human umbilical vascular endothelial cell line) cell line was used in the study. Antibiotic-free Eagle's MEM and RPMI 1640 media containing 10% fetal bovine serum and 100 mM/L glutamine were used for cell generation and experimental protocol. Production and proliferation of cells were performed in 25 cm^2 tissue culture flasks, and MTT measurement and analysis were performed in 48-well culture plates. In studies using 48-well culture plates, 2.5×10^4 cells/well were cultivated. The amount of medium in each well was 200 µL.

Determination of the H_2O_2 dose that causes damage to HUVEC cell lines

For the damage model in endothelial cells, the cells were incubated for 3 h with H_2O_2 at concentrations ranging from 10 to 200 µM.[3,4] The concentration of H_2O_2 to cause damage was determined. Briefly, 10, 25, 50, 100, and 200 µM H_2O_2 was added to the wells and the plate was incubated for 3 h in a cell culture incubator operating at 37°C, 95% humidity, and 5.0% CO_2. At the end of the specified times, the medium in the wells was removed and the wells were washed three times with sterile phosphate-buffered saline (PBS; pH 7.4). Two hundred microliters of fresh medium was added to the wells and MTT was measured to determine the % cell viability. After the incubations, the H_2O_2 concentration was determined by evaluating the MTT results.

Incubation with paricalcitol after H_2O_2 induced injury

By adding the optimum H_2O_2 concentration determined for the damage model, the microplate was incubated for 3 h in a cell culture incubator operating at 37°C, 95% humidity, and 5.0% CO_2. At the end of the period, the medium in the wells was discarded and the wells were washed three times with PBS (pH 7.4) and 200 µL of fresh medium was added. The plates were incubated for 24 h by adding paricalcitol and MTT was measured at the end of incubation. Thus, the damage-repairing effect of paricalcitol against the damage was investigated.

Incubation with paricalcitol before H_2O_2 induced injury

The specified amounts of paricalcitol were added to the microplates and the plates were incubated for 24 h in a cell culture incubator operating at 37°C, 95% humidity, and 5.0% CO_2. After 24 h, the medium was removed from the wells and the wells were washed three times with PBS (pH 7.4), 200 µL of fresh medium and H_2O_2 at the concentration determined for the damage model were added and the cells were incubated for 3 h. At the end of the period, MTT measurement was conducted. Thus, the protective effect of paricalcitol in cells against possible damage was investigated.

Determination of PDI activity

PDI activity was measured using a commercial kit (ENZO, Cat. No: ENZ-51024-KP002). In summary, PDI in the presence of DTT causes insulin to reduce disulfide bonds and form insulin aggregates. These aggregates bind to the fluorogenic PDI detection reagent in the test kit, causing a fluorescent red color. Fluorescence intensity was measured in a microplate reader (Glomax Multi Detection System, Promega, Ex/Em: 500/603 nm). Results were expressed as RFU/mg protein.

Determination of GSH levels

GSH levels were determined using a fluorometric kit (Abcam, Cat No: ab138881). The non-fluorescent dye gives a strong green fluorescent signal after reacting directly with GSH. This fluorescent signal was measured on a fluorometer (Ex/Em: 490/520 nm control) and the results were expressed as nmol/mg protein using GSH standard curve.

Determination of NO levels

The half-life of NO in biological fluids is short. Since it is rapidly converted to nitrite and nitrate, NO levels can be

determined from these metabolites. A commercial kit (Abcam, Cat No: ab65328) was used for the measurement of NO metabolites. In summary, nitrate is reduced to nitrite with nitrate reductase and NADPH and then reagent 1 and reagent 2 are added to determine nitrite levels. The absorbance of samples is measured at 540 nm using a microplate reader. Deionized water was used at all stages for NO measurements and the results were expressed as NO μmol/10^6 cells.

Determination of caspase-3 activity

Caspase 3 activity was measured using a commercial kit (Merck Millipore, Cat No: APT165). The pNA is released as a result of the cleavage of the specific substrate peptide, Asp-Glu-Val-Asp (DEVD) labeled with *p*-nitroaniline (pNA) by the caspase-3 enzyme. The color intensity that emerged with the pNA release was evaluated spectrophotometrically at 405 nm. Results were expressed as μmol/mg protein.

Determination of mitochondrial membrane polarization

Mitochondrial membrane polarization was measured using the cationic dye 5,5,6,6′-tetrachloro-1,1′,3,3′ tetraethylbenzimidazoylcarbocyanine iodide (JC-1; Cayman, USA) in a fluorescent microplate reader. In living cells, JC-1 accumulates as J-aggregates in the mitochondria resulting in red fluorescence. The intensity of red fluorescence is proportional to the membrane potential. In cells with low mitochondrial membrane potential, JC-1 exists in its green fluorescent monomeric form. The samples were mixed with 2.5 μL of JC-1 at room temperature for 10 min and then centrifuged at 400*g* at room temperature (22°C–25°C) for 5 min. The cells were washed twice and suspended in 100 μL assay buffer. The fluorescence intensity of the samples was measured using two filters (green excitation: 485 nm/emission: 535 nm, red excitation: 535 nm/emission: 590 nm). The results are expressed as J-aggregates RFU/monomer RFU ratio.

Protein determination

The protein contents of the samples were determined using a NanoDrop UV-Vis spectrophotometer (Epoch™ Take3 Plate, Biotek) according to the Warburg–Christian (absorption at 280/260 nm UV) method.

Statistical analysis

The Graphpad Prism 5 (Graphpad Software, San Diego, USA) was used for statistical analysis. The IC_{50} value was calculated using the nonlinear regression analysis. The data of multiple groups were analyzed by one-way analysis of variance (ANOVA), and the Tukey post hoc test was used for calculations of statistical significance. The *t*-test was used to compare the paired groups where $P < 0.05$ was considered significant.

Results

H_2O_2-induced cell injury

Oxidative damage to endothelial cells was induced by incubating the cells with H_2O_2 at concentrations ranging from 0 to 200 μM for 3 h. Cell viability (%) was evaluated using MTT assay and the results are given in Figure 1. The 50% viability rate (IC_{50}) was calculated as 94.74 ± 11.09 μM. Considering this result, the H_2O_2 concentration to be used to create H_2O_2 damage was determined as 100 μM.

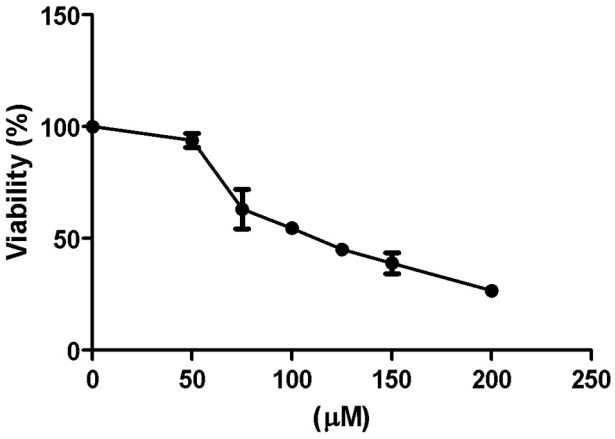

Figure 1. The effect of H_2O_2 on cell viability in endothelial cells.

Effect of paricalcitol on cell viability

Endothelial cells were incubated with paricalcitol after or before H_2O_2 injury. The effect of paricalcitol on cell damage was determined by % cell viability values using the MTT test (Figure 2). The oxidative stress damage induced by H_2O_2 has significantly reduced viability of cells when compared to the control group ($P < 0.001$). Paricalcitol treatment after H_2O_2 injury caused a significant increase in viability at 400, 500, and 1000 nM concentrations when compared to the H_2O_2-induced (100 μM) group ($P < 0.01$ and $P < 0.001$, respectively), while no significant difference was observed at 100, 200, and 300 nM concentrations. Paricalcitol treatment before H_2O_2 injury caused a significant increase in viability at 500 and 1000 nM concentrations when compared to the group treated with only H_2O_2 ($P < 0.001$), while no significant difference was observed at 100, 200, 300, and 400 nM concentrations of paricalcitol.

PDI activity

PDI activity was measured fluorometrically after 24 h of incubation with paricalcitol (500 nM), 16F16 (Methyl 2-(2-chloroacetyl)-1-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate, 2-(2-Chloroacetyl)-2,3,4,9-tetrahydro-1-methyl-1H-pyrido[3,4-b]indole-1-carboxylic acid methyl ester, specific PDI inhibitor, Sigma-Aldrich, SML0021) at 50 μM concentration. As shown in Figure 3, paricalcitol and 16F16 caused a significant reduction in PDI activity when compared to the control ($P < 0.001$). Oxidative damage induced by H_2O_2 caused suppression of PDI activity. Although PDI activity decreased with the treatment of paricalcitol and 16F16 after oxidative damage, this decrease was not found statistically significant. PDI activity did not change with paricalcitol and 16F16 treatments before oxidative damage.

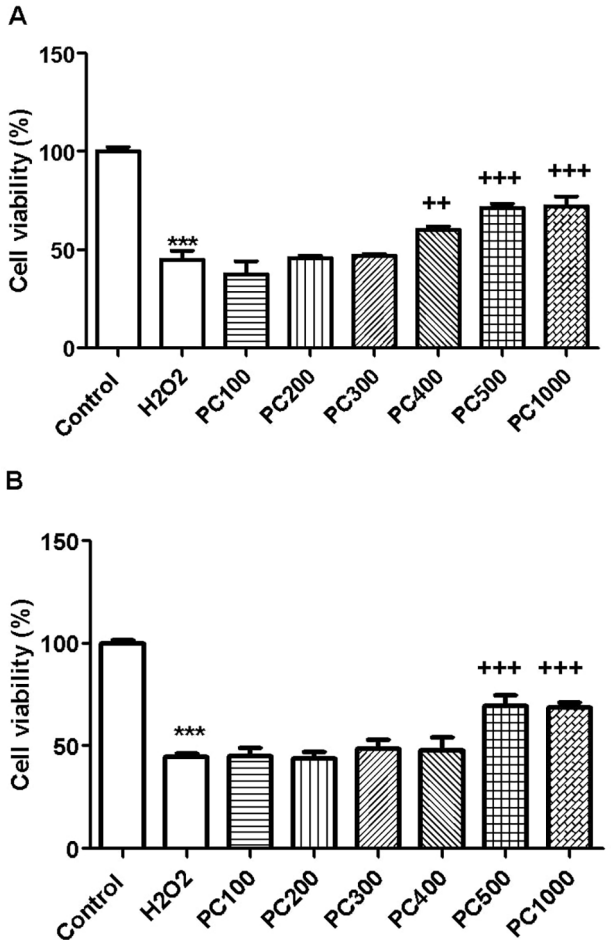

Figure 2. Effect of paricalcitol on cell viability in endothelial cells where oxidative damage was induced by H_2O_2: (A) paricalcitol treatment after H_2O_2 injury and (B) paricalcitol treatment before H_2O_2 injury. PC: paricalcitol. $^{***}P < 0.001$ versus control group; $^{++}P < 0.01$, $^{+++}P < 0.001$ versus H_2O_2 group.

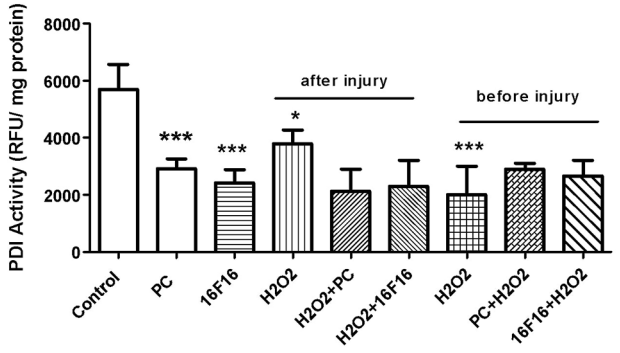

Figure 3. The effect of paricalcitol on PDI activity. PC: paricalcitol. $^{***}P < 0.001$, $^{*}P < 0.05$ versus control group.

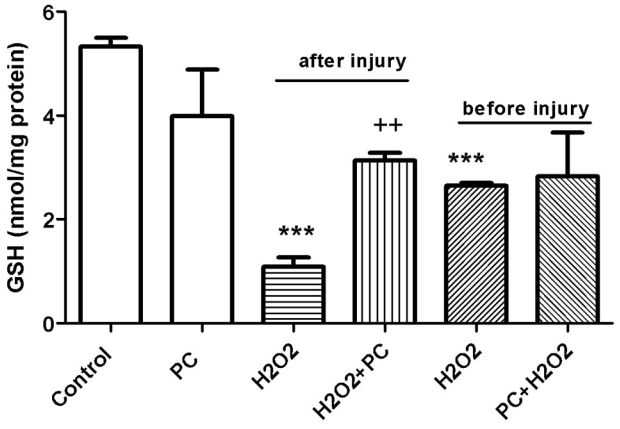

Figure 4. GSH levels. PC: paricalcitol. $^{***}P < 0.001$ versus control group; $^{++}P < 0.01$ versus H_2O_2 group.

The effect of paricalcitol on GSH levels in endothelial cells

After the injury of endothelial cells with H_2O_2, GSH levels decreased significantly when compared to the control group ($P < 0.001$, Figure 4). While paricalcitol (500 nM) treatment after incubation with H_2O_2 led to a significant increase in GSH levels in endothelial cells prior to oxidative damage when compared to the H_2O_2-induced group ($P < 0.01$), no significant increase was observed in GSH levels with paricalcitol treatment before oxidative damage when compared to the H_2O_2-induced group.

Effect of paricalcitol on NO levels in endothelial cells

After oxidative damage induced by H_2O_2 in the endothelial cells, NO production was significantly reduced by paricalcitol when compared to the control group ($P < 0.001$, Figure 5). Paricalcitol caused a decrease in NO levels in healthy cells ($P < 0.001$). The treatment of paricalcitol after incubation with H_2O_2 significantly increased NO levels compared to the H_2O_2-induced group ($P < 0.01$). Paricalcitol treatment before oxidative damage also increased NO levels in the endothelial cells ($P < 0.05$).

Effect of paricalcitol on mitochondrial membrane potential

As seen in Figure 6, oxidative damage induced by H_2O_2 significantly reduced mitochondrial membrane potential when compared to the control group ($P < 0.001$). Paricalcitol did not change mitochondrial membrane potential levels in healthy cells. The treatment of paricalcitol after incubation with H_2O_2 significantly increased mitochondrial membrane potential compared to the H_2O_2-induced group ($P < 0.05$). Mitochondrial membrane potential was lower in the endothelial cells with paricalcitol treatment before oxidative damage than in the H_2O_2-induced group ($P < 0.05$).

Effect of paricalcitol on caspase-3 activity

Treatment of endothelial cells with paricalcitol before and after injury caused a significant decrease in caspase-3 activity compared to the H_2O_2-induced groups ($P < 0.01$ and $P < 0.001$ respectively). Paricalcitol did not cause any change in caspase-3 activity in undamaged cells (Figure 7).

Discussion

Oxidative stress can cause dysfunction in endothelial cells and endothelial dysfunction together with oxidative stress are the main pathophysiological mechanisms of many diseases such as hypertension, atherosclerosis, dyslipidemia,

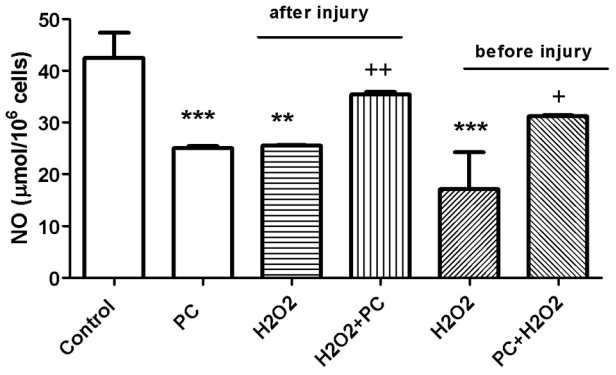

Figure 5. NO levels. PC: paricalcitol. $^{***}P < 0.001$, $^{**}P < 0.01$ versus control group; $^{++}P < 0.01$, $^{+}P < 0.05$ versus H_2O_2 group.

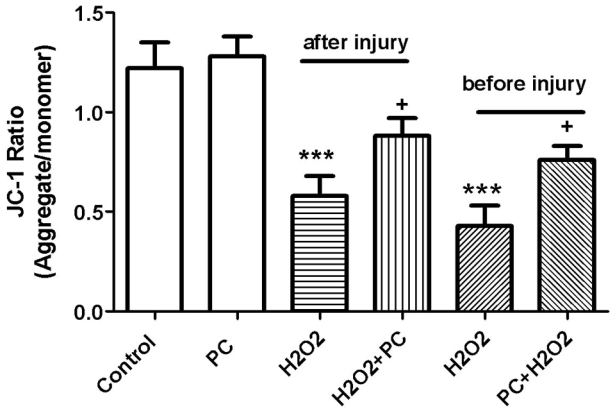

Figure 6. Mitochondrial membrane potential. PC: paricalcitol. $^{***}P < 0.001$ versus control group; $^{+}P < 0.05$ versus H_2O_2 group.

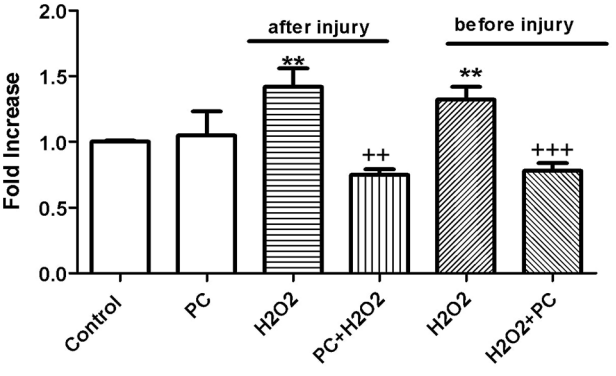

Figure 7. Caspase-3 activity. $^{**}P < 0.01$ versus control; $^{+++}P < 0.001$, $^{++}P < 0.01$ versus H_2O_2 group.

diabetes, cardiovascular disease, renal failure, and ischemia-reperfusion injury. ROS can modulate cellular function, receptor signaling, and immune responses under physiological conditions, but at high concentrations ROS can cause proliferation and migration of vascular smooth muscle and inflammatory cells, stimulation of endothelial cell apoptosis, activation of transcription factors such as NFkB and AP1, and overexpression of inflammatory cytokines and adhesion molecules (ICAM-1, VCAM-1, E-selectin).[12] It is known that oxidative stress increases in the early stages of CKD, progresses over time with kidney failure, and is exacerbated by the HD process.[13]

H_2O_2 is frequently used to create an oxidative stress model in cell culture studies. However, it has been observed that different concentrations cause damage on endothelial cells with different incubation times.[3,4] For this reason, optimization studies were carried out to determine the appropriate amount and duration of incubation with H_2O_2 by experimenting with different H_2O_2 concentrations and incubation times. The IC_{50} value for H_2O_2 damage was determined as $97 \pm 11.09\,\mu M$ and the incubation time was determined as 3 h in our study conditions.

Vitamin D has beneficial effects on cardiovascular function and homeostasis of different tissues such as skeletal muscle, vascular smooth muscle, myocardium, and endothelium. Therefore, it has been suggested that vitamin D deficiency is a potential mediator of many non-skeletal pathologies, including cardiovascular diseases. Vitamin D receptor (VDR) is found in many cells and exerts pleiotropic effects. The active form of vitamin D, which regulates many physiological processes, can be synthesized by VDR-expressing endothelial cells. The beneficial effects of vitamin D on the vascular wall are well documented in the literature.[2,14] However, studies on the effect of paricalcitol, a vitamin D analogue, on endothelial/vascular cells are limited. Recent clinical data suggest that vitamin D analogues provide a survival benefit for CKD patients independent of PTH and calcium.[15,16] In our study, we examined the effect of paricalcitol on oxidative damage and apoptosis in vascular endothelial cells.

In an *in vitro* endothelial cell culture study, it was reported that $200\,\mu M$ H_2O_2 causes a decrease in the viability of cells, and preincubation with vitamin D increases the viability of these cells.[4]

Our findings show that administration of paricalcitol after H_2O_2-induced cell damage increases cell viability. At the same time, it was observed that the application of paricalcitol in undamaged cells can also protect against subsequent damage and significantly increase cell viability. However, these effects can occur at high concentrations. GSH is an important endogenous antioxidant for cellular redox homeostasis and antioxidant defense. It was previously reported that the decrease of GSH synthesis in endothelial cells may cause renal fibrosis by decreasing the phosphorylation of eNOS and increasing ROS levels in basal and stimulated cells.[17] In a clinical study conducted in HD patients, it was reported that plasma GSH levels were lower than normal in these patients and significant increase was observed in GSH levels after 3 months of paricalcitol administration.[18] Preclinical studies show that low GSH levels cause a decrease in vitamin D regulatory gene levels.[19] Our study was carried out at the cellular level. Consistent with the above findings, we observed that paricalcitol caused significant increases in GSH levels in endothelial cells. However, when hydrogen peroxide damage is created after preincubation with paricalcitol, GSH levels were not significantly restored by paricalcitol.

NO plays an important role in maintaining various vascular hemostasis balance and normal endothelial function, such as modulation of vascular dilatation, regulation of cell growth, and protection of the vessel from the harmful effects

of platelets and cells circulating in the blood.[2,19] Endothelial nitric oxide synthase (eNOS) is a critical regulator of vascular homeostasis by producing NO from L-arginine. Impairment of NO synthesis and release from vascular endothelium can cause serious problems. For this reason, numerous treatment avenues are being investigated to reverse endothelial dysfunction by increasing NO release from the endothelium.[2,20] In a study *conducted* by Polidoro and colleagues,[3] it was shown that vitamin D reduces H_2O_2-induced stress in HUVEC endothelial cells, decreases superoxide production and apoptotic cells, or increases cell viability. They suggested that this antioxidant effect of vitamin D is provided by MEKs/ERKs/SIRT-1. According to their findings, vitamin D up-regulated SIRT-1 and reverted the SIRT-1 down-regulation induced by H_2O_2. Furthermore, in another study,[4] it was suggested that vitamin D protects mitochondrial function and cell viability by inhibiting superoxide anion formation, maintaining mitochondrial function and cell viability, activating ERK and Akt, and inducing NO production in oxidative stress conditions. Due to these effects, they suggested that vitamin D is able to reduce the apoptosis-related gene expression.

The antioxidant potential of vitamin D was directly proportional to its concentration and was maximum at the highest concentration (100 nmol/L) used. This study did not investigate the effect of paricalcitol on NO release in endothelial cells. In our study, paricalcitol caused an increase in NO production in endothelial cells damaged by hydrogen peroxide. However, NO levels decreased in healthy cells with paricalcitol treatment. Therefore, the increase of NO levels may be associated with increased cell viability with paricalcitol in damaged cells. In a clinical study, intravenous paricalcitol administration was shown to reduce oxidative stress and nitrite levels in plasma in HD patients. This study was not at the cellular level, and paricalcitol was used for a long term.[18] Endothelial cell apoptosis can increase smooth muscle cell proliferation, blood coagulation, and leukocyte infiltration into the endothelium, leading to endothelial dysfunction.[21]

In our study, we measured caspase-3 activity as a marker of endothelial cell apoptosis. Caspases are important mediators of programmed cell death and are a good predictor of apoptosis. Caspase-3 is known as an executioner caspase in apoptosis. It is synthesized as a proenzyme and is activated by stimulation of the caspase cascade during apoptosis. It is a good apoptotic indicator as it is located in the common part of the intrinsic and extrinsic apoptotic pathway.[22] Mitochondrial membrane potential was also examined in our study. The mitochondria of healthy cells maintain an electrochemical gradient ($\Delta\psi$) across their inner membrane created by the pumping of protons from the matrix into the intermembrane space. Mitochondrial depolarization and loss of electrochemical gradient ($\Delta\psi$) are closely associated with apoptosis and cell death.[23] It has been shown that vitamin D protects against the depolarization of mitochondrial potential and increase of caspase-3 activity during oxidative stress induced by administration of hydrogen peroxide.[4] Our findings showed that, like vitamin D, paricalcitol (pre or post-incubation) increases endothelial cell viability by suppressing the increase in caspase-3 activity and loss of mitochondrial membrane potential in endothelial cells under oxidative stress conditions.

Considering the role of PDI in protein folding, inhibition of PDI enzymatic activity may sensitize cells to apoptosis.[24,25] However, the increase of ER stress may cause the release of the enzyme from the ER and PDI may play a proapoptotic role. Likewise, when PDI accumulates at threshold levels in response to misfolded proteins with extensive DNA damage, it can stimulate apoptotic cell death pathways. It has been reported that PDI induces Bak-dependent but non-Bax-dependent mitochondrial outer membrane permeability *in vitro*, possibly by triggering Bak oligomerization on mitochondria. More evidence of the proapoptotic activities of PDI members has emerged. In a study in human endothelial cells, it has been shown that reduction of PDIa3 expression inhibited hyperoxia or tunicamycin-induced apoptosis by blocking caspase-3 activation.[26] In another study, it has been shown that increased PDI expression in HL1 cells protects against hypoxia-induced apoptosis.[25] Presumably, this issue is unclear due to the different subcellular localization and multiple functions of PDI. In our study, although paricalcitol inhibited PDI reductase activity in healthy cells, we could not clearly observe this effect under oxidative stress conditions. Our study is a preliminary study. The suppression of the increase in caspase-3 activity and the loss of mitochondrial membrane potential with paricalcitol may also be due to PDI inhibition or other mechanisms. Further studies are needed to understand the role of PDI in this regard.

In conclusion, under oxidative stress conditions, paricalcitol has therapeutic and protective effects on endothelial cells. The use of paricalcitol may provide additional benefit in patients with renal dysfunction by protecting endothelial cells from the effects of oxidative stress.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, and review of the manuscript; MMK: Investigation and writing. TŞ: Investigation and writing. ÖÇ: Formal analysis and investigation. AŞ: Supervision, writing – review & editing.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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