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Cerium dioxide nanoparticles modulate antioxidant defences and change vascular response in the human saphenous vein

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ABSTRACT

Nanoparticles have a promising future in biomedical applications and knowing whether they affect ex vivo vascular reactivity is a necessary step before their use in patients. In this study, we have evaluated the vascular effect of cerium dioxide nanoparticles (CeO_2NPs) on the human saphenous vein in response to relaxing and contractile agonists. In addition, we have measured the protein expression of key enzymes related to vascular homeostasis and oxidative stress.

We found that CeO_2NPs increased expression of both SOD isoforms, and the consequent reduction of superoxide anion would enhance the bioavailability of NO explaining the increased vascular sensitivity to sodium nitroprusside in the presence of CeO_2NPs. The NOX4 reduction induced by CeO_2NPs may lead to lower H_2O_2 synthesis associated with vasodilation through potassium channels explaining the lower vasodilation to bradykinin. In addition, we showed for the first time, that CeO_2NPs increase the expression of ACE2 in human saphenous vein, and it may be the cause of the reduced contraction to angiotensin II. Moreover, we ruled out that CeO_2NPs have effect on the protein expression of eNOS, sGC, BKca channels and angiotensin II receptors or modify the vascular response to noradrenaline, endothelin-1 and TXA_2 analogue.

In conclusion, CeO_2NPs show antioxidant properties, and together with their vascular effect, they could be postulated as adjuvants for the treatment of cardiovascular diseases.

1. Introduction

Nanoparticle-based therapeutic products are increasing because of their capacity to penetrate cell membranes, carry an active agent, or concentrate in the desired target tissue using directing agents anchored on the nanoparticle scaffold [1]. Among metal oxide nanoparticles, cerium dioxide nanoparticles (CeO_2NPs) are biocompatible in cells and tissues and have been used in some biomedical applications, mainly for their potential antioxidant effect [2]. Chemical reactions involving redox cycling between the Ce^{3+} and Ce^{4+} oxidation states and surface oxygen vacancies cause CeO_2NPs to decompose catalytically superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in abiotic systems. Therefore, CeO_2NPs can behave as superoxide dismutase (SOD) and catalase [3].

Oxidative stress is a phenomenon caused by excessive concentration of reactive oxygen species (ROS) and contributes to several pathological conditions including cardiovascular diseases [4]. In the cardiovascular system the endothelium plays an important role in controlling vascular tone through the release of relaxing factors, such as nitric oxide (NO), and contractile factors such as angiotensin II [5]. It is accepted that impaired ability of the endothelium to release NO and increased oxidative stress contribute to endothelial dysfunction [6,7]. At the vascular level, $O_2^{\cdot-}$, mainly synthesized by NADPH oxidase (NOX) [8], reacts with NO forming peroxynitrite ($ONOO^-$) [9]. This process decreases NO bioavailability leading to endothelial dysfunction. Defensive strategies exist in the vasculature to compensate for ROS excess, such as SOD or catalase among others [10]. However, at physiological

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concentrations, ROS also participate as a second messenger in physiological processes, being necessary for cell homeostasis [8,11,12], for instance H_2O_2 can produce vasodilation in some vessels [8]. NOX4, the most abundant isoform in the vascular system [13,14], releases H_2O_2 in preference to O_2^- [15]. Some studies have reported that NOX4 has a protective role against atherosclerosis, as H_2O_2 does not decrease NO bioavailability [16–21]. Hence, the interest in antioxidant substances that can minimise oxidative stress-induced cellular damage and at a time maintain balanced ROS levels.

One of the main concerns after coronary artery bypass surgery is the durability of the grafts. In fact, the main cause of coronary graft occlusion beyond the first year after surgery is atherosclerosis [22]. The formation of atherosclerosis plaque is strongly associated with endothelial dysfunction and oxidative stress [7], so the study of antioxidant agents at the vascular level is of great interest in the field of cardiovascular surgery and pharmacology. Given that CeO_2NPs exhibit antioxidant properties could be an interesting strategy to decrease ROS levels. Therefore, we hypothesized that they could modulate vascular oxidative stress by decreasing O_2^- synthesis, thus improving NO bioavailability and vascular homeostasis. However, in addition to their antioxidant properties, it is necessary to establish whether CeO_2NPs have vascular effects that may affect the therapeutic strategy. Despite the growing interest in nanoparticles in different medical applications, little is known about their effects on vascular reactivity. Hence, this study aimed to test the vascular effects of CeO_2NPs in the human saphenous vein from patients undergoing coronary surgery. We focused on both endothelium dependent and independent vasodilation, as well as the response to contractile agonists. We also analysed if CeO_2NPs affect protein expression of the main enzymes related to vascular homeostasis and oxidative stress.

2. Material and methods

2.1. Synthesis and characterization of cerium dioxide nanoparticles

CeO_2NPs were synthesized by slow hydrolysis of Ce^{3+} in an aqueous solution at controlled slightly basic pH as previously reported [23]. The colloidal dispersion of CeO_2NPs was aged by heating in a poly(ethylene terephthalate) vessel at 100 °C for 24h. The resulting yellow precipitate was filtered and dried under vacuum overnight. The CeO_2NPs size was determined by transmission electron microscopy (JEM-2100F, Peabody, MA, USA). The particle size analysis was calculated by using the ImageJ software (National Institute of Health, Bethesda, MD, USA). The presence of Ce^{3+} and Ce^{4+} ions was determined by X-ray photoelectron spectroscopy (XPS) using a SPECS spectrometer.

2.2. Collection of samples and ethical approval

All experiments were performed in saphenous vein segments taken from 78 patients undergoing coronary bypass surgery (Table 1). The study was carried out following the ethical principles of the 1975 Declaration of Helsinki and the research was approved by the Clinical Research Ethics Committee at the *Hospital Clínico Universitario de Valencia*, Spain. Written informed consent was obtained from each patient included in the study.

2.3. Organ bath study

The experimental procedure for recording isometric force was previously described [24]. Briefly, two thin and rigid stainless-steel wires were introduced through vascular segment light. One of the wires was fixed to the wall while the other was attached to a tension transducer (Grass FT 03). Changes in isometric tension of the venous segments were recorded using the data acquisition system PowerLab/8e and Chart software, version 7 (ADInstruments, Bella Vista, Australia). Each segment was placed in a bath containing 4 ml of a Krebs-Henseleit solution bubbled with carbogen (95% O_2 and 5% CO_2) to obtain a pH of 7.3-7.4. The temperature was held at 37 °C. The optimal resting tension for the human saphenous vein was 3 g. Endothelium was considered functional if relaxation to a single concentration of bradykinin (10^{-7} M) in segments precontracted with noradrenaline (10^{-7} to $3x10^{-7}$ M) was ≥50%.

To examine the effects of CeO_2NPs, concentration-response curves to the different agonists were performed in the absence and presence of CeO_2NPs after 30 min of incubation.

Table 1
Patients' demographics, risk factors, analytics, and treatment.

Patients (n)	78
Age (mean, SD)	66.65 ± 1.31
Male gender (n, %)	70 (90%)
Risk factors	
Body mass index (mean, SD)	28.44 ± 0.85
Obesity (BMI >30)	13 (17%)
Hypertension (n, %)	60 (77%)
Diabetes mellitus (n, %)	42 (54%)
Smoking (n, %)	21 (27%)
Analytics	
Glycaemia	117.63 ± 7.71
HDLc	34.47 ± 2.24
LDLc	82.32 ± 8.73
Triglycerides	129.11 ± 14.32
Treatment (n, %)	60 (77%)
Beta-blockers (n, %)	13 (22%)
ARB (n, %)	18 (30%)
ACEI (n, %)	16 (27%)
Calcium channel blockers (n, %)	3 (5%)
Nitrovasodilators (n, %)	3 (5%)
α-1 adrenergic blockers (n, %)	5 (8%)
Diuretics (n, %)	16 (27%)
Statins (n, %)	8 (13%)
Antidiabetic agents (n, %)	36 (60%)
Insulin (n, %)	16 (27%)

SD, standard deviation; n: number of patients; BMI: body mass index; HDLc: high-density lipoprotein cholesterol; LDLc: low-density lipoprotein cholesterol; ACEI: angiotensin-converting enzyme inhibitors; ARB angiotensin II-receptor blockers.

2.4. Western blot analysis

Human saphenous vein samples for protein expression were incubated in the organ bath for 30 min in the absence and presence of CeO_2NPs (20 μg/ml), and stored in liquid nitrogen at -80°C until analysis. The Western blot technique was performed as described previously

Table 2
Primary antibodies used for Western blot analysis.

Antibody	Molecular weight (KDa)	Dilution	Supplier and reference
Polyclonal eNOS	133	1:250	Abcam #Ab5589
Polyclonal peNOS	140	1:250	Bioss #BS3447R
GCS-β-1 (G-3)	67	1:500	Santa Cruz #SC-514183
Anti-maxi Potassium channel alpha/SLO	115	1:500	Abcam #Ab3586
Polyclonal Cu/Zn-SOD (SOD1)	16	1:1000	Enzo Life Sciences #ADI-SOD-10
Monoclonal Mn-SOD (SOD2)	20	1:1000	Santa Cruz #SC-137254
Polyclonal NOX4	67	1:500	NOVUSBIO #NB110-58849SS
Polyclonal AGTR1	41	1:1000	Thermo Fisher Scientific #PA5-20812
Polyclonal AGTR2	41	1:1000	Thermo Fisher Scientific #PA5-20813
Polyclonal ACE2	120	1:1000	Thermo Fisher Scientific #MA5-31395

[24]. The primary antibodies (Table 2) were incubated overnight at 4°C. Monoclonal β-actin and α-tubulin (Sigma-Aldrich, Spain) were used as housekeeping proteins. The detection was performed with enhanced chemiluminescence (Amersham Biosciences, Barcelona, Spain) using the digital image system ImageQuant LAS 4000 (GE Healthcare, Little Chalfont, UK). Signals were quantified using ImageJ software (National Institute of Health, Bethesda, MD, USA).

2.5. Chemicals

All substances and reagents used in this study were purchased from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise specified. CeO_2NPs were dissolved in dimethyl sulfoxide (DMSO) and then diluted in purified water, keeping the final DMSO concentration below 0.5%. All drugs were dissolved in purified water except for U46619 and indomethacin which were dissolved in 100% ethanol, and subsequent dilutions were made in 0.9% NaCl.

2.6. Statistical analysis

Results are presented as mean ± standard error of the mean (SEM) for vascular reactivity results, and mean ± standard deviation (SD) for protein expression results. The sample size (n) is indicative of the number of patients. Vasoconstriction was expressed as a percentage of the response to KCl 60 mM. Vasodilation was expressed as a percentage of inhibition of noradrenaline-induced contraction. For each concentration-response curve, the maximum effect (E_{max}) and the negative log of concentration required to elicit 50% of the maximum effect (pEC_{50}) were calculated by nonlinear regression analysis. The statistical analyses were performed using Prism software (v8.3.0, GraphPad Software, CA, USA). The normality was tested using Shapiro-Wilk tests. Statistical comparisons for multiple group were performed by one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Synthesis of CeO_2NPs and its characteristics

CeO_2NPs were synthesized by hydrolysis under controlled pH of cerium nitrate. They exhibit a homogeneous morphology in TEM image with an average size of 5.4 ± 0.5 nm. The XPS analyses showed that CeO_2NPs had two oxidation states, Ce^{3+} and Ce^{4+}. The main peaks such as Ce^{4+} $3d_{3/2}$ and Ce^{4+} $3d_{5/2}$ related cerium 3d core spectrum are shown at binding energies of 916.46 and 898.325eV, respectively, indicating that cerium atoms are predominantly in the 4+ oxidation state.

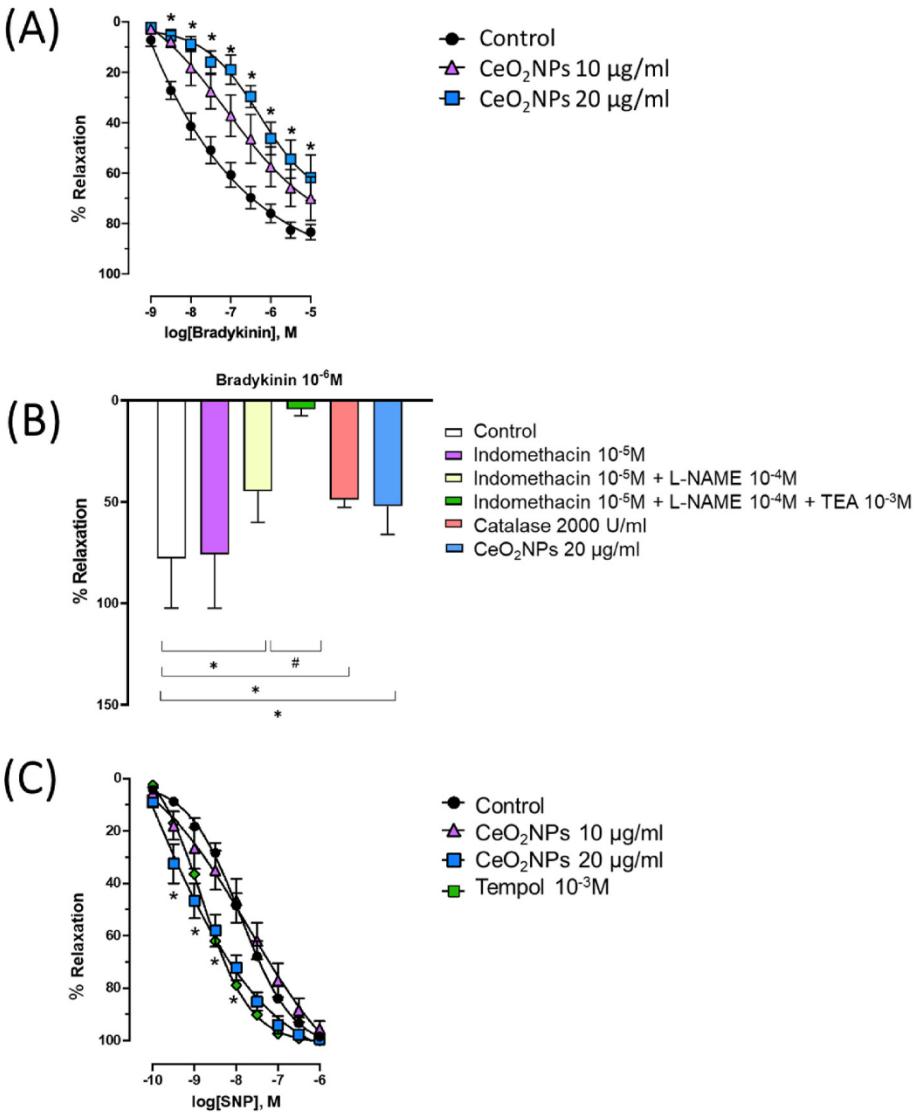

Fig. 1. Influence of CeO_2NPs on endothelium-dependent vasodilatation in human saphenous vein. (A) Concentration-response curves to bradykinin (10^{-9}-10^{-5} M) in the absence (control) and presence of CeO_2NPs (10–20 µg/ml). (B) Bar graph summarizing vasodilatation to bradykinin (10^{-6} M) in the absence (control) and presence of indomethacin (10^{-5} M), a non-selective COX inhibitor, as well as consecutive additions of L-NAME (10^{-4}M), to block nitric oxide synthase and tetraethylammonium (TEA, 10^{-3}M), a calcium-activated K^+ channels blocker. Catalase (2000 U/ml) and CeO_2NPs (20 µg/ml). (C) Concentration-response curves to sodium nitroprusside (SNP, 10^{-10}-10^{-6} M) in the absence (control) and presence of CeO_2NPs (10–20 µg/ml) and tempol (10^{-3}M). Data are shown as mean ± SEM. *P<0.05 vs control, #P<0.05 vs Indo + L-NAME. n = 5-8 patients.

Moreover, the Ce^{3+} $3d_{3/2}$ and Ce^{3+} $3d_{5/2}$ peaks are assigned at 900.77 and 882.19, respectively, confirming a population of cerium atoms in 3+ oxidation state as a consequence of oxygen vacancies (Supplementary Fig. 1).

3.2. Influence of CeO_2NPs on endothelium-dependent vasodilatation

We performed concentration-response curves to bradykinin (10^{-9}-10^{-5} M) in the absence and presence of CeO_2NPs showing that CeO_2NPs (10–20 μg/ml) significantly right-shifted bradykinin curve and Emax decreased at 20 μg/ml (from 83.40 ± 2.94% to 61.77 ± 8.94%, p = 0.026) (Fig. 1A). To further determine which endothelial relaxing factors were involved in bradykinin-induced vasodilation, we used following inhibitors: indomethacin 10^{-5} M (inhibitor of cyclooxygenase, COX), Nω-nitro-L-arginine (L-NAME, 10^{-4} M, inhibitor of nitric oxide synthase, NOS), and tetraethylammonium chloride (TEA, 10^{-3}M), a blocker of Ca^{2+}-activated K^+ channels. Vasodilation to bradykinin (10^{-6} M) was significantly reduced in the presence of indomethacin plus L-NAME (from 77.79 ± 8.18% to 44.64 ± 6.28%, p = 0.0117). The addition of TEA further decreased the relaxant response to bradykinin (from 44.64 ± 6.28% to 4.26 ± 1.12%, p<0.0001). Moreover, catalase (2000 U/ml) also decreased the vasodilation to bradykinin, in a similar way as CeO_2NPs (20 μg/ml) did (Fig. 1B).

In another set of experiments, we demonstrated that H_2O_2 caused vasodilation in human saphenous vein and TEA (10^{-3}M) reversed this effect (Supplementary Fig. 2). These results indicated that bradykinin-induced vasodilation in the human saphenous vein was related to NO and Ca^{2+}-activated K^+ channels activated by H_2O_2. Therefore, the impaired relaxation induced by CeO_2NPs could be attributed to one or both of these two mechanisms.

3.3. Influence of CeO_2NPs in the response to NO donor

CeO_2NPs (20 μg/ml) significantly left-shifted the concentration-response curves to sodium nitroprusside (SNP, 10^{-10}-10^{-6} M) (from 8.53 ± 0.12 to 7.91 ± 0.06, p = 0.0004) (Fig. 1C). It is possible that CeO_2NPs enhanced NO bioavailability by reducing oxidative stress, since reduction of O_2^- levels with tempol (10^{-3} M), mirrored the effect of CeO_2NPs (Fig. 1C).

3.4. Effect of CeO_2NPs on vasoconstriction

CeO_2NPs (20 μg/ml) did not modify the contractile response to noradrenaline (NA, 10^{-9}-10^{-4} M), endothelin-1 (ET-1, 10^{-11}-10^{-6} M) and the thromboxane A_2 analogue (U-46619, 10^{-10} -10^{-6} M) (Fig. 2A–C). In contrast, the presence of CeO_2NPs significantly reduced angiotensin II (Ang II, 10^{-10} -10^{-6} M)-induced vasoconstriction (Emax value decreased from 94.81% ± 7.16%–63.63% ± 6.40%, p = 0.0011) (Fig. 2D). To assess whether O_2^- was released in response to angiotensin II, concentration-response curves were performed in the presence of tempol (10^{-3} M), finding no changes (Fig. 2D).

3.5. Protein expression of eNOS, peNOS, BKca, sGC, SOD1, SOD2, NOX4, AT1R, AT2R, and ACE2

We investigated whether the presence of CeO_2NPs (20 μg/ml) inhibited the expression or activation of eNOS by measuring the protein expression of eNOS and peNOS (eNOS Ser1177 phosphorylation). We did not find significant changes (Fig. 3A–C). Furthermore, protein expression of BKca channels and sGC remains unaltered in the presence of CeO_2NPs (20 μg/ml) (Fig. 3D and E). To evaluate the effects of

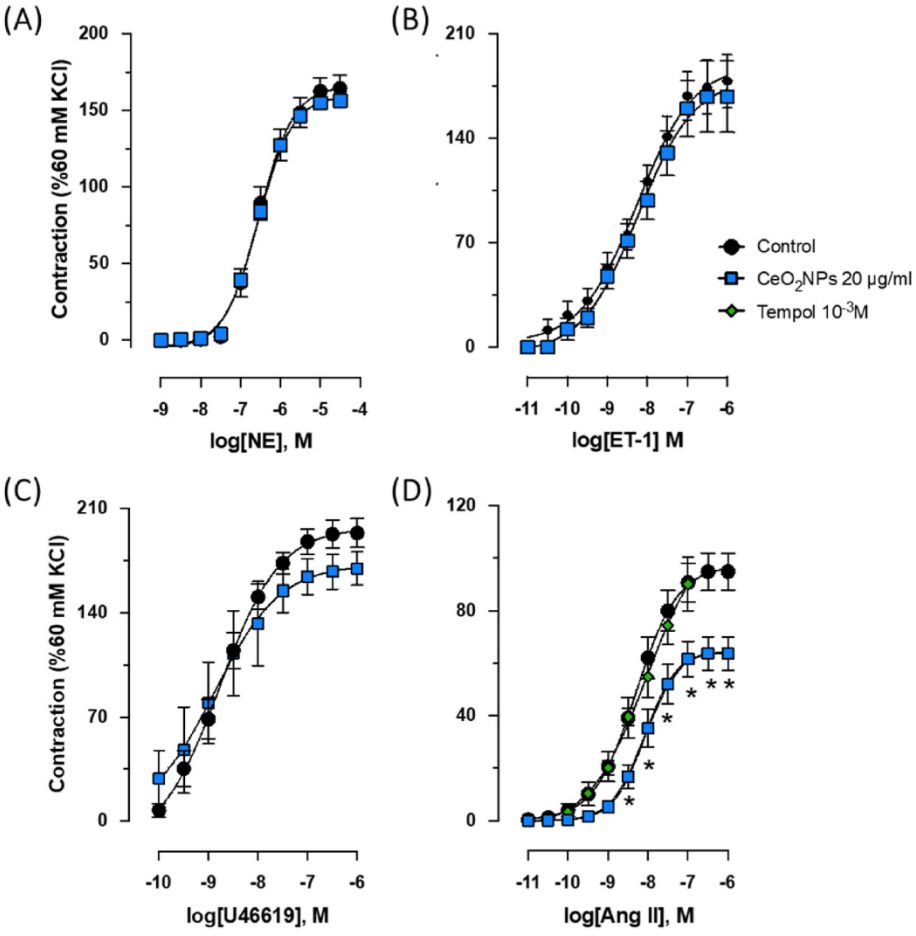

Fig. 2. Effects of CeO_2NPs on vasoconstriction in human saphenous vein. (A) Concentration-response curves to noradrenaline (NE) (10^{-9} -10^{-4} M), (B) endothelin-1 (ET-1) (10^{-11}-10^{-6} M) and (C) U46619 (10^{-10}-10^{-6} M) in the absence (control) and presence of CeO_2NPs (20 μg/ml). (D) Concentration-response curves to angiotensin II (Ang II, 10^{-11}-10^{-6} M) in the absence (control) and presence of CeO_2NPs (20 μg/ml) and tempol (10^{-3}M). Data are shown as mean ± SEM. *P<0.05 vs control. n = 10-14 patients.

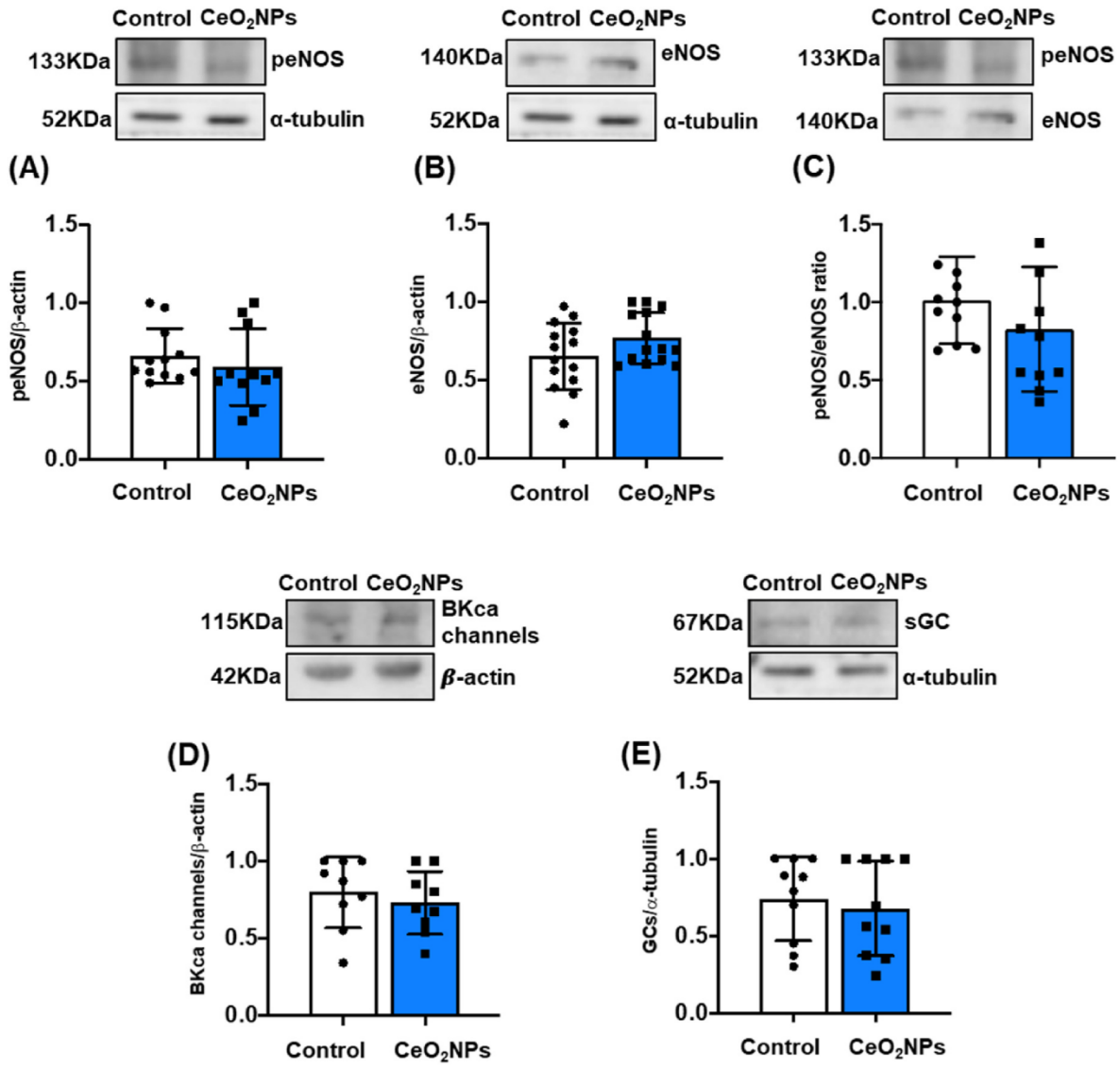

Fig. 3. Effects of CeO_2NPs on peNOS, eNOS, BKca channels and sGC protein expression in the **human saphenous vein.** (A–E) Representative Western blot for total and phosphorylated proteins in the absence (control) and presence of CeO_2NPs (20 μg/ml). β-actin and α-tubulin were used as internal control and the signal intensity was plotted as the ratio of the target protein to β-actin or α-tubulin as indicated. Data are shown as mean ± SD. n = 5-10 patients.

CeO_2NPs on oxidative stress, we measured the protein expression of the main enzymes related to vascular oxidative stress in human saphenous vein samples incubated with CeO_2NPs (20 μg/ml). Our results showed that CeO_2NPs significantly increased the expression of antioxidant enzymes SOD1 ($p = 0.0308$ vs. control) and SOD2 ($p = 0.0238$ vs. control) and reduced the expression of the prooxidant enzyme NOX4 ($p = 0.0004$ vs. control) (Fig. 4). We tested whether CeO_2NPs inhibited the expression of the angiotensin II receptors. In this case, the protein expression of both angiotensin II receptors (ATR1 and ATR2) was not modified by CeO_2NPs (20 μg/ml) (Fig. 5A and B). We also measured ACE2 protein expression level and found that CeO_2NPs (20 μg/ml) significantly increased it ($p = 0.0327$ vs. control) (Fig. 5C).

4. Discussion

CeO_2NPs could be used as drug carriers because they can penetrate cell membranes and have a large surface area to include an active agent. In this respect, it would be interesting if their effects were minimal at the vascular level. In this work we demonstrate that, the vascular responses to noradrenaline, U-46619, or ET-1 were not altered in the presence of CeO_2NPs. However, our results showed that CeO_2NPs have effects and change vascular response aimed at improving NO bioavailability and decreasing response to angiotensin II and bradykinin. These effects seem to be related with their antioxidant effect by increasing the protein expression of SOD1, SOD2 and decreasing NOX4. Incubation with CeO_2NPs also overexpressed ACE2, involved in angiotensin II degradation.

To test the mechanisms implicated in the decreased response to bradykinin in the presence of CeO_2NPs, we analysed whether CeO_2NPs modify eNOS-NO-sGC pathway and Ca^{2+}-activated K^+ channels. CeO_2NPs did not modify the protein expression of eNOS, peNOS (phosphorylation of eNOS on Ser1177, the active form of eNOS [25]) or sGC. Since cerium nanoparticles interact with phosphate esters of various biomolecules such as ATP [26], it seemed plausible that CeO_2NPs could modify peNOS expression. However, these findings show that the diminished vasodilation response to bradykinin in the presence of CeO_2NPs is not caused by modification of eNOS-NO-sGC pathway.

Then, we analysed the protein expression of BKca channels in the presence of CeO_2NPs. The opening of BKca channels leads to smooth muscle cell hyperpolarisation and this mechanism is also involved in bradykinin-induced relaxation in the human saphenous vein, as our results indicated by the blockade with TEA. Some nanoparticles have

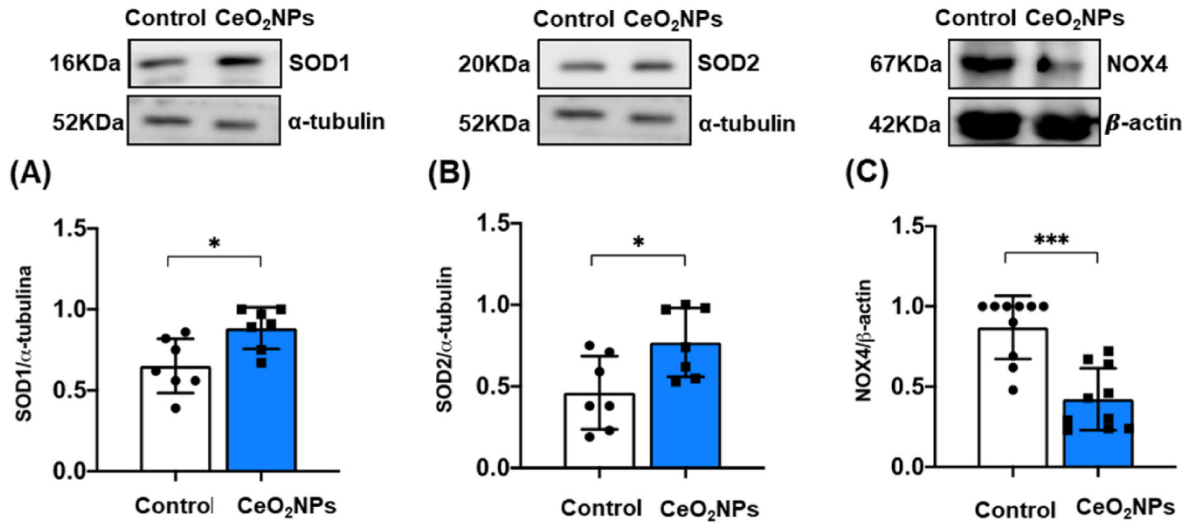

Fig. 4. Effects of CeO_2NPs on SOD1, SOD2, and NOX4 protein expression in the **human saphenous vein.** (A–C) Representative Western blot for total proteins in the absence (control) and presence of CeO_2NPs (20 μg/ml). β-actin and α-tubulin were used as internal control and the signal intensity was plotted as the ratio of the target protein to β-actin or α-tubulin as indicated. Data are shown as mean ± SD. *P < 0.05 vs control, ***P < 0.001 vs control. n = 8 patients.

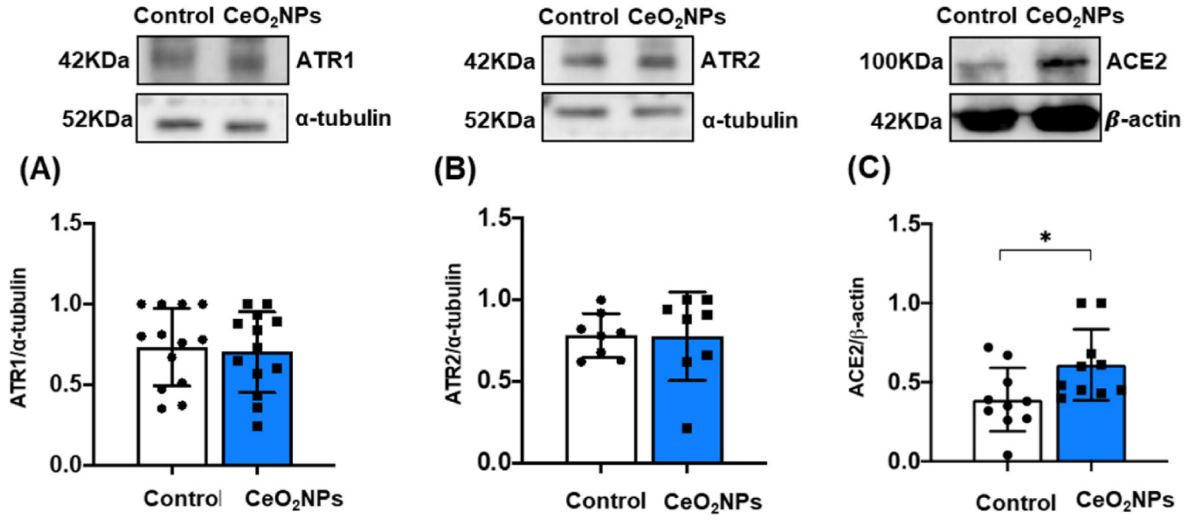

Fig. 5. Effects of CeO_2NPs on ATR1, ATR2, and ACE2 protein expression in the **human saphenous vein.** (A–C) Representative Western blot for total proteins in the absence (control) and presence of CeO_2NPs (20 μg/ml). β-actin and α-tubulin were used as internal control and the signal intensity was plotted as the ratio of the target protein to β-actin or α-tubulin as indicated. Data are shown as mean ± SD. *P < 0.05 vs control. n = 9 patients.

been shown to interact with ion channels in vitro, for instance, Melnyk et al. concluded that C60-fullerene inhibited BKca channels in pulmonary artery smooth muscle cells [27]. Although we did not find differences in the BKca channels protein expression in the presence of CeO_2NPs, these results did not rule out that CeO_2NPs were affecting BKca channels activation. Endothelium-derived hyperpolarisation is a mechanism that is related to calcium-activated potassium channels and may or may not involve a factor released from the endothelium [28], such as potassium ion [29,30] or H_2O_2 [31,32]. Vascular NOX4 produces more H_2O_2 than O_2^-, giving it a vascular protective role [8,15,33]. Our results showed that incubation with CeO_2NPs decreased the expression of NOX4, therefore, H_2O_2 production would be decreased. If H_2O_2 were the factor released by the endothelium in response to bradykinin that activates BKca channels, it could explain the decreased bradykinin-relaxing effect in the presence of CeO_2NPs. Our results with catalase confirming this mechanism, that according to other authors also happens in the human mammary artery, where H_2O_2 induces relaxation through hyperpolarisation of the vascular smooth muscle by activation of K^+ channels [34].

On the other hand, CeO_2NPs increased the vasodilator response to sodium nitroprusside, a NO donor that activates the sGC-cGMP pathway. As mentioned above, no changes in sGC in the presence of nanoparticles were found, so the increased vasodilator response to sodium nitroprusside is not secondary to an increase in sGC expression. Since our results showed that CeO_2NPs increased protein expression of both SOD isoforms, we hypothesized that the mechanism by which CeO_2NPs increased the vasodilatory effect of the exogenous NO donor could be by decreasing O_2^- levels and thus increasing the bioavailability of exogenously administered NO. To confirm this hypothesis, we incubated the human saphenous vein with tempol, a SOD mimetic, observing that there was an increase in the vasodilator response to sodium nitroprusside. Moreover, the concentration-response curve obtained overlaps with that obtained when incubated with CeO_2NPs. This allows us to conclude that the antioxidant mechanism is probably the reason why CeO_2NPs increase the vasodilator response to sodium nitroprusside.

On the other hand, CeO_2NPs decreased the vasoconstrictor response

to angiotensin II. This effect was not attributed to the ability of nanoparticles to modify AT receptors, since their expression did not vary in the presence of CeO_2NPs. Moreover, the vasoconstrictor effect of angiotensin II and its contribution to endothelial dysfunction has been strongly related to the production of O_2^- [35–37]. As our results showed, CeO_2NPs exhibit beneficial properties by increasing the expression of SOD1 and SOD2, so it seems likely that the decrease in the contractile effect of this agonist in the presence of the nanoparticles may be related to their O_2^- neutralising power. To test this hypothesis, concentration curves to angiotensin II in the presence of tempol were performed without finding differences, concluding that angiotensin II vasoconstriction in this vascular bed is not mediated by O_2^-. Our results are in line with those observed by Schiijt et al. [35].

Finally, angiotensin II is degraded by angiotensin-converting enzyme 2 (ACE2) [38]. Two forms of ACE2 have been described, the full-length ACE2, which is a transmembrane protein widely present in organs, including the endothelium of blood vessels; the other form is the soluble one present in blood plasma, without the membrane anchor [39]. ACE2 plays a key role in the cardiovascular and renal homeostasis, providing protection by acting as a suppressor of angiotensin II [40]. In fact, selective disruption of ACE2 in mice has been shown to cause a severe defect in cardiac contractility and increased levels of angiotensin II [41]. Therefore, ACE2 activators, as well as AT2 receptor agonists, have been investigated in recent years to treat hypertension [42]. Consequently, the increase in ACE2 protein expression levels in the presence of CeO_2NPs observed in our experiments could explain the reduced contractile response to angiotensin II. Hence, CeO_2NPs could have a positive impact on the treatment of cardiovascular diseases, such as hypertension. On the other hand, ACE2 also degrades the active metabolite of bradykinin (des-Arg -BK) [43,44] which activates the B1 bradykinin receptor, highly expressed in inflammatory conditions [45,46]. Activation of both receptors B2 and B1 by bradykinin and its active metabolite causes massive vascular permeability and increased levels of inflammatory cytokines implicated in the cytokine storm observed in several infections [47], such as SARS-CoV-2 infection [48]. Therefore, because of ACE2 depletion, angiotensin II and des-Arg-bradykinin levels would increase. Although our study uses an ex vivo approach with consequent limitations, it indicates that CeO_2NPs could reverse these effects and together with their antioxidant properties could be postulated as adjuvants for the treatment of cardiovascular diseases.

Finally, it is important to note that in order to transfer the key observations of CeO_2NPs effects to humans, other aspects must be taken into account such as toxicity or distribution. In this sense, to consider the use of CeO_2NPs as therapeutic agents, we must be aware of the possible cytotoxic effects that they may cause by themselves or by interfering with cellular homeostasis. In particular, metal oxides are toxic per se, and this cytotoxicity has proven useful in certain cases such as cancer treatment [49]. On the other hand, the liver is one of the passive targets of CeO_2NPs, remaining for a long time after intravenous injection, so this site of action has been crucial for testing the toxicological effects of CeO_2NPs, which remain contradictory [50]. In any case, it has been concluded that CeO_2NPs at standard therapeutic doses do not usually show toxicity, but at high doses (hundreds of mg/kg body weight) they show toxicity in rodents [50,51]. However, the use of a coating can reduce their toxicity and by modifying certain properties such as size, shape, composition and surface electrical charge, can improve their activity [51]. Therefore, there is still work to be done before CeO_2NPs can reach patients.

5. Conclusions

CeO_2NPs increase expression of both SOD isoforms, and the consequent reduction of O_2^- would increase the bioavailability of NO. Moreover, CeO_2NPs increase the expression of ACE2 in human saphenous vein, and it may be the cause of the lower contractile effect of angiotensin II in the presence of these nanoparticles. These findings are potentially beneficial, as the hallmark of cardiovascular disease is endothelial dysfunction associated with a decrease in NO and a tendency to vasoconstriction.

On the other hand, the decrease in NOX4 expression induced by CeO_2NPs could led to a lower H_2O_2 production, associated with vasodilation induced by bradykinin. Although at first, we might think that the nanoparticles have a negative effect by reducing the vascular response to bradykinin, this potent endothelium-dependent vasodilator, is also a contractile agonist in nonvascular smooth muscle, and is related to edema, inflammation and the pain mechanism. Therefore, in this line, the fact that CeO_2NPs decreased the response to bradykinin could have beneficial effects in terms of inflammation or pain.

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Declarations of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradbiomed.2022.11.012.

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