Vascular Pharmacology 115 (2019) 26–32

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Amorphous nano-selenium quantum dots improve endothelial dysfunction in rats and prevent atherosclerosis in mice through Na^+/H^+ exchanger 1 inhibition

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ARTICLE INFO

Keywords:
Na^+/H^+ exchanger 1
Endothelial dysfunction
Selenium
Atherosclerosis
Nanoparticles

ABSTRACT

Aim: Selenium, a trace element involved in important enzymatic activities inside the body, has protective effects against cardiovascular diseases including atherosclerosis. The safe dose of selenium in the organism is very narrow, limiting the supplementation of selenium in diet. The aim of this study is to explore whether selenium quantum dots (SeQDs) prevent atherosclerosis and to investigate the potential mechanisms.

Methods: An amorphous form of SeQDs (A-SeQDs) and a crystalline form of SeQDs (C-SeQDs) were prepared through self-redox decomposition of selenosulfate precursor. Endothelial dysfunction was induced by balloon injury plus high fat diet (HFD) in rats. Atherosclerotic model was established by feeding *Apoe*$^{-/-}$ mice with HFD.

Results: Administrations of A-SeQDs but not C-SeQDs dramatically improved endothelium-dependent relaxation, and accelerated would healing in primary endothelial cells isolated from rats, which was comprised by co-treatment of LiCl. Lentivirus-mediated knockdown of Na^+/H^+ exchanger 1 (NHE1) abolished LiCl-induced endothelial dysfunction in rats. In cultured endothelial cells, A-SeQDs, as well as cariporide, inhibited NHE1 activities, decreased intracellular pH value and Ca^{2+} concentration, and reduced calpain activity increased by ox-LDL. These protective effects of A-SeQDs were reversed by LiCl treatment in endothelial cells. In *Apoe*$^{-/-}$ mice feeding with HFD, A-SeQDs prevented endothelial dysfunction and reduced the size of atherosclerotic plaque in aortic arteries. Further, lentivirus-mediated NHE1 gene overexpression abolished the protective effects of A-SeQDs against endothelial dysfunction and atherosclerosis in *Apoe*$^{-/-}$ mice.

Conclusion: A-SeQDs prevents endothelial dysfunction and the growth of atherosclerotic plaque through NHE1 inhibition and subsequent inactivation of Ca^{2+}/calpain signaling. Clinically, the administration of A-SeQDs is an effective approach to treat atherosclerosis.

1. Introduction

Atherosclerosis is a progressive disease in artery that develops over decades, which is characterized by the fatty deposition in large- and medium-sized arteries [14,35]. In the initiation of atherosclerosis, endothelial cell dysfunction is appeared, leading to the increased transcytosis of oxidized low density lipoprotein (ox-LDL), and its subsequent deposition, retention and modification in the arterial intima [13]. Some new drugs targeting improvement of endothelial dysfunction are effective approaches to prevent atherosclerosis.

Selenium, as an essential element, plays an important trace mineral in the human body. It is a part of enzymes against free radicals in cells [17], which is firstly reported that selenium is reduced in the areas of atherosclerotic lesions [33]. Till now, many results support the concept

Abbreviations: NHE1, Na^+/H^+ exchanger 1; pHi, Intracellular pH value; $[Ca^{2+}]_i$, Intracellular Ca^{2+} concentration; eNOS, Endothelial Nitric Oxide Synthase; NO, Nitric Oxide; HUVECs, Human Umbilical Vein Endothelial Cells; Ach, Acetylcholine; SNP, Sodium Nitroprusside; DAF, Diaminofluorescein; HEPES, Hydroxyethyl Piperazine Ethanesulfonic Acid; BCECF, 2-carboxyethyl- 5(6)-carboxyfluorescein; A-SeQDs, Amorphous Form of Selenium Quantum Dots; C-SeQDs, Crystalline Form of Selenium Quantum Dots; Ox-LDL, Oxidized Low Density Lipoprotein

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https://doi.org/10.1016/j.vph.2019.01.005
Received 17 September 2018; Received in revised form 8 January 2019; Accepted 25 January 2019
Available online 26 January 2019

that optimal selenium intake can prevent atherosclerosis. The speciation of selenium is critical to the health outcomes of selenium supplementation [15]. The safe dose of selenium in the organism is very narrow, and it is easy to produce toxicity, which restricts the application of traditional selenium compounds. Therefore, the development of efficient and safe selenium form is crucial to the efficacy of selenium therapy.

Nanomaterials have been widely used in therapeutics, nano-imaging, and bio-sensing. Compared with the traditional selenium compounds, amorphous nano-selenium is expected to be a new selenium supplement and therapeutic drug because of its unique physical and chemical properties, and high bioavailability [16,22]. We have developed two nanoparticles of selenium quantum dots (SeQDs), namely amorphous SeQDs (A-SeQDs) and crystalline SeQDs (C-SeQDs), and found that A-SeQDs exhibited anti-proliferative effects on cancer cells in a polymorphs-dependent manner [26]. In this study, we aimed to examine the effects of both A-SeQDs and C-SeQDs in atherosclerosis, and found that A-SeQDs prevented endothelial dysfunction in rats and attenuated the growth of atherosclerotic plaque in mice by inactivating Na^+/H^+ exchanger 1 (NHE1).

2. Materials and methods

2.1. Preparation and characterization of SeQDs

As described previously [26], bovine serum albumin was added into the reaction system (pH = 6.0) after selenium powder was added into aqueous solution of sodium sulfite. For A-SeQDs, the reaction system was incubated at 20 °C for 12 h. For C-SeQDs, the reaction was performed at 80 °C for 24 h. After reaction, the dispersion was centrifuged, washed, and freeze-dried. The structures of SeQDs were characterized by HR-TEM (JEOL JEM-2100) and were shown in Online Fig. S1A.

A full description of materials and methods used, including materials and animals, preparation and characterization of SeQDs, protocols of animal studies, atherosclerotic lesion analysis, organ chamber, measurements of nitric oxide (NO), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), and blood glucose, human umbilical vein endothelial cells (HUVECs) and primary rat aortic endothelial cells cultures, cell migrations, evaluation of cell viability, endothelial NO synthase (eNOS) activity assay, measurement of intracellular pH (pH_i) value, measurement of NHE1 activity and mRNA, intracellular Ca^{2+} $[Ca^{2+}]_i$ concentration, calpain activity, and statistical analysis can be found in Online Supplements.

3. Results

3.1. A-SeQDs prevented endothelial dysfunction in rats

We firstly performed *in vivo* experiments to investigate whether SeQDs produces beneficial effects on endothelial functions in rats fed with high fat diet (HFD). Rats were treated with SeQDs for 2 weeks prior to HFD (Online Fig. S1B). Endothelial function was determined by measuring Ach-induced endothelium-dependent relaxation, which is an early marker in atherosclerosis [34]. As shown in Fig. 1A, induction of HFD plus balloon injury in rats (group b) markedly induced endothelial dysfunction in descending aortic arteries, compared to control rats (group a). Consistent with other reports [8], high selenium diet (group c) normalized Ach-induced endothelium-dependent relaxation. Importantly, administration of A-SeQDs (group e) but not C-SeQDs (group d) at dose of 0.1 mg/kg selenium dramatically reversed Ach-induced endothelium-dependent relaxation impaired by HFD, indicating that SeQDs prevents endothelial dysfunction in rats in a polymorphs-dependent manner. Further, the effect of A-SeQDs at this dose (0.1 mg/kg selenium) was stronger than high selenium diet (0.3 mg/kg selenium), implying that delivery of selenium through nanoparticle is much more effective than selenium supplementation through diet.

Both A-SeQDs and high selenium diet did not alter SNP-induced vessel relaxation (Fig. 1B), suggesting that the improvement of vascular bioactivity by A-SeQDs is due to the maintenance of endothelial function, but not to vascular smooth muscle. Interestingly, A-SeQDs had no effects on TC, TG, LDL, HDL and blood glucose (Online Table S1), implying that the protective effect of A-SeQDs is not related to improve the glucose and lipid metabolisms.

3.2. A-SeQDs increased the migration of primary endothelial cells from rats

Another phenotype of endothelial dysfunction is the impaired cell migration, which is crucial to the repair of injured endothelium [38]. Therefore, we investigated if A-SeQDs increased cell migrations in primary endothelial cells isolated from rats by performing scratch test. As shown in Fig. 1C and D, the migrations of endothelial cells from rats fed with HFD were totally damaged, while both A-SeQDs and high selenium diet increased the ability of endothelial cells to repair the scratch injury.

3.3. A-SeQDs alleviated the stenoses of carotid and retinal arteries in rats

Endothelial dysfunction contributes to restenosis caused by atherosclerotic plaque or endothelial injury [4]. To test this viewpoint, the diameter of carotid artery in rats was assayed by using ultrasound in Fig. 2A. Compared to control rats, the minimal diameter of carotid was significantly reduced (Fig. 2B). However, treatments of rats with A-SeQDs and high selenium diet dramatically increased the minimal diameter of carotid in atherosclerotic rats.

The effects of A-SeQDs on reducing the arterial stenosis were further confirmed by measuring the average diameter of retinal artery through fundus photography (Fig. 2C). As calculated in Fig. 2D, the average diameter of retinal artery in rats was significantly reduced, compared with control rats. Both A-SeQDs and high selenium diet administrations remarkably increased the average diameter of retinal artery in atherosclerotic rats.

3.4. Activation of NHE1 by LiCl abolished the effects of A-SeQDs on endothelial function in rats

It has been reported that inhibition of NHE1 produces many cardioprotective effects against endothelial dysfunction [5,36]. Thus, we hypothesized that A-SeQDs improves endothelial dysfunction through NHE1 inhibition. To test this concept, we treated rats with NHE1 activator LiCl [7] and examined whether LiCl affects the effects of A-SeQDs on endothelial dysfunction. As demonstrated, compared to rats treated with A-SeQDs alone, the effects of A-SeQDs on endothelial dysfunction (Fig. 1A–B), cell proliferations (Fig. 1C and D), the stenosis of carotid (Fig. 2A and B) and retinal arterials (Fig. 2C and D) were ablated by LiCl co-administration. These data indicate that NHE1 inhibition is required to A-SeQDs-prevented and endothelial dysfunction.

3.5. Lentivirus-mediated gene knockdown of NHE1 abolished LiCl-induced endothelial dysfunction in rats

As an NHE1 activator [7], LiCL does not specifically affect NHE1 but has many effects on cell metabolism [25]. To determine if LiCl *via* NHE1 activation produces protective effects on endothelial dysfunction in rats, we downregulated NHE1 gene expression by injecting rats with lentivirus expressing NHE1 shRNA followed by LiCl administration (Online Fig. S1C). As shown in Fig. 3A, lentivirus-mediated gene knockdown dramatically inhibited NHE1 mRNA levels in aortic arteries, indicating the high efficiency of lentivirus-mediated gene downregulation *in vivo*. LiCl administration remarkably impaired Ach-induced endothelium-dependent relaxation in rats injected with lentivirus harboring scramble shRNA but not in rats expressing NHE1 shRNA (Fig. 3B). Both LiCl administration and NHE1 shRNA did not

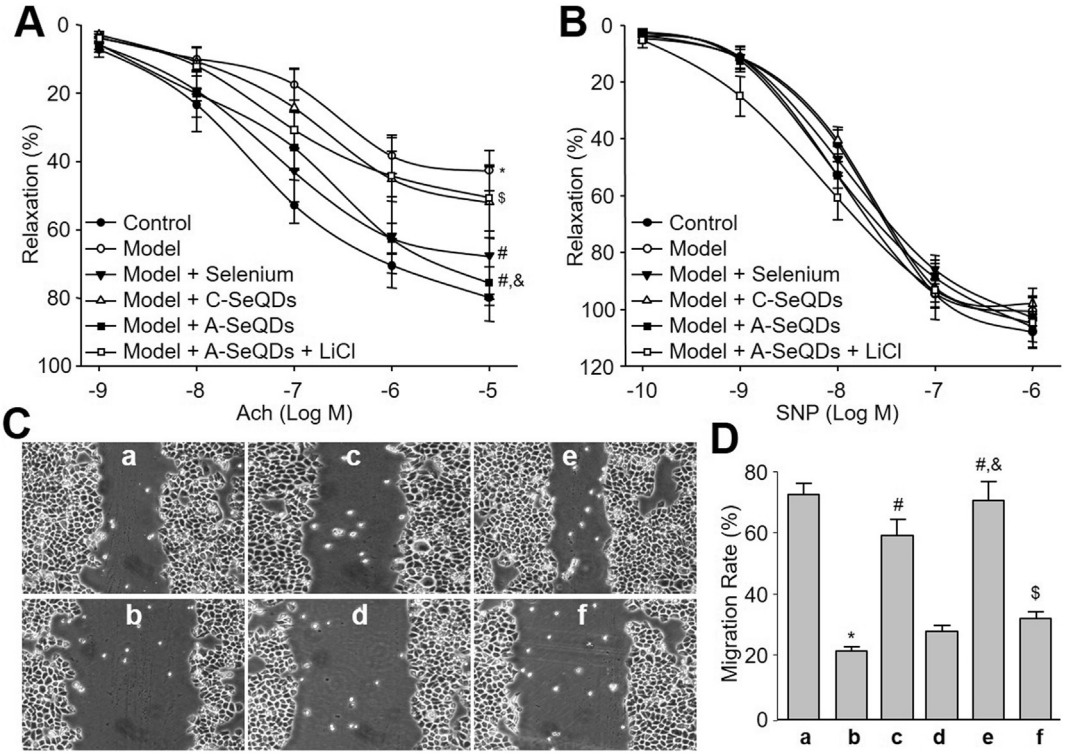

Fig. 1. A-SeQDs prevented endothelial dysfunction and accelerated wound healing in endothelial cells isolated from rats. The protocol of animal experiment was shown in Online Fig. S1B. (A and B) At the end of experiments, rats were sacrificed under anesthesia. Aortic rings isolated from descending aorta artery were subjected to assay (A) endothelium-dependent relaxation induced by Ach and (B) endothelium-independent relaxation induced by SNP in organ chamber. (C) At the end of experiments, primary endothelial cells isolated from rats in group a to f were subjected to determine wound healing 3 days after scratch test. (D) Quantitative analyses of endothelial cell migrations were performed. a, Control; b, Model; c, Model + Selenium; d, Model + C-SeQDs; e, Model + A-SeQDs; f, Model + A-SeQDs + LiCl. All data were expressed as mean ± SEM. 10–15 rats in each group. $^{*}P < .05$ *VS* Control (a), $^{\#}P < .05$ *VS* Model (b). $^{\&}P < .05$ *VS* Model + Selenium (c). $^{\$}P < .05$ *VS* Model + A-SeQDs (e).

alter SNP-induced vessel relaxation (Fig. 3C). Taking these data together, it suggest that LiCl produces detrimental effects on endothelial dysfunction through NHE1 activation in endothelial cells.

3.6. A-SeQDs inhibited NHE1 activation, decreased pHi value, and inactivated Ca^{2+}/calpain signaling in HUVECs treated with ox-LDL

NHE1 activation decreases pHi value, leading to intracellular Ca^{2+} overload and calpain activation [27]. We next investigated that the effects of A-SeQDs on NHE1 activity, pHi value, Ca^{2+} concentration and calpain activity in cultured HUVECs. Ox-LDL was used to mirror the micro-environment of endothelial cells in atherosclerosis [11]. Treatment of HUVECs with ox-LDL dramatically activated NHE1 (Fig. 4A), induced intracellular alkalization (Fig. 4B) and Ca^{2+} overload (Fig. 4C), and increased calpain activity (Fig. 4D). As expected, all these effects of ox-LDL were reversed by treatment of A-SeQDs in cells.

3.7. A-SeQDs via NHE1 inhibition downregulated Ca^{2+}/calpain signaling in HUVECs treated with ox-LDL

To determine if the Ca^{2+}/calpain axis inactivated by A-SeQDs is NHE1 dependent, HUVECs were treated with NHE1 specific inhibitor cariporide alone or A-SeQDs plus LiCl. As shown in Fig. 4A–D, cariporide mirrored the effects of A-SeQDs in NHE1 activity, pHi value, Ca^{2+} concentration and calpain activity in HUVECs treated with ox-LDL. Furthermore, co-incubation of cells with LiCl abrogated these effects produced by A-SeQDs. These data suggest that A-SeQDs *via* inhibition of NHE1 inactivated Ca^{2+}/calpain signaling in endothelial cells.

3.8. A-SeQDs increased eNOS activity and NO production, and improved cell viabilities in endothelial cells, which was NHE1 dependent

As reported previously [23], calpain-mediated loss of heart shock protein 90 from the eNOS complex results in decreased eNOS activity and NO release in pulmonary artery endothelial cells. Therefore, we examined the effects of A-SeQDs in eNOS activity, NO production, and cell viabilities in endothelial cells. As represented in Fig. 5A–C, ox-LDL decreased eNOS activity and NO production, and damaged cell viabilities in HUVECs. As a sequence, these detrimental effects induced by ox-LDL were bypassed by cariporide and A-SeQDs. Further, co-incubation of cells with LiCl comprised these beneficial effects produced by A-SeQDs in cultured cells treated with ox-LDL.

3.9. Lentivirus-mediated NHE-1 gene overexpression abolished the effects of A-SeQDs on endothelial dysfunction in $Apoe^{-/-}$ mice

We finally performed *in vivo* experiments to investigate whether NHE1 inhibition is crucial to the action of A-SeQDs produces to prevent endothelial dysfunction and atherosclerosis. To this end, $Apoe^{-/-}$ mice, which are well-recognized model for studying atherosclerosis [28], were injected with lentivirus expressing NHE1 cDNA followed by treatment of A-SeQDs for 4 weeks prior to feeding mice with HFD for another 8 weeks (Online Fig. S1D). The protein level of NHE was assayed by IFC in Fig. 6A. The lentiviral NHE1 overexpression was confirmed by measuring mRNA in Fig. 6B. Expectedly, A-SeQDs remarkably reversed serum NO levels (Fig. 6C) and improved Ach-induced endothelium-dependent relaxation in descending aortas isolated from $Apoe^{-/-}$ mice (Fig. 6D). SNP-induced endothelium-independent relaxation was not altered by A-SeQDs (Fig. 6E).

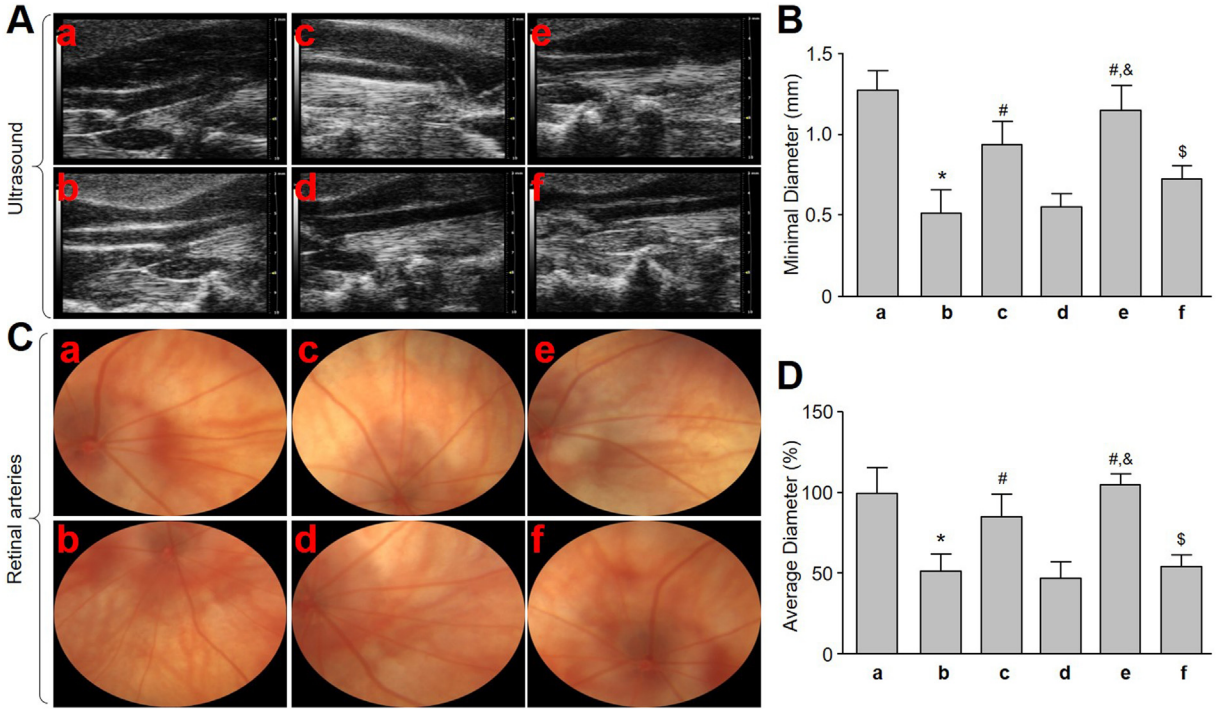

Fig. 2. A-SeQDs alleviated the stenosis of coronary and retinal arteries in rats. The protocol of animal experiment was shown in Online Fig. S1B. (A and B) At the end of experiment, the diameter of carotid artery was determined by ultrasound and the representative pictures were shown in A. Quantitative analyses of the minimal diameter of carotid artery diameter were performed in B. (C and D) The diameter of retinal artery was determined by ophthalmoscopy in C and quantitative analyses of retinal artery diameter in average were performed in D. a, Control; b, Model; c, Model + Selenium; d, Model + C-SeQDs; e, Model + A-SeQDs; f, Model + A-SeQDs + LiCl. All data were expressed as mean ± SEM. 10–15 rats in each group. $^{*}P < .05$ *VS* Control (a), $^{\#}P < .05$ *VS* Model (b). $^{\&}P < .05$ *VS* Model + Selenium (c). $^{\$}P < .05$ *VS* Model + A-SeQDs (e).

Administration of A-SeQDs had no effects on TC, TG, LDL, HDL and blood glucose in *Apoe*$^{-/-}$ mice (Online Table S2).

3.10. A-SeQDs via NHE1 inhibition attenuated the formation of aortic atherosclerotic plaque in *Apoe*$^{-/-}$ mice

The atherosclerotic plaques in whole aortas and aortic roots were determined by Oil Red staining and HE staining. As indicated in Online Fig. S2A and S2B, the size of atherosclerotic plaque in the whole aorta was reduced remarkably by A-SeQDs in vector-infected *Apoe*$^{-/-}$ mice. Similarly, A-SeQDs significantly decreased the size of atherosclerotic plaque in aortic root in *Apoe*$^{-/-}$ mice expressing blank vector (Online Fig. S2C and S2D). Of note, A-SeQDs did not decrease the sizes of atherosclerotic plaque in the whole aortas and in the aortic roots in *Apoe*$^{-/-}$ mice infected with lentivirus containing NHE1 cDNA, compared with vector-infected *Apoe*$^{-/-}$ mice, demonstrating that NHE1 downregulation is required for A-SeQDs-induced suppression on atherosclerotic plaque growth.

4. Discussion

In this study, we firstly developed a novel drug of A-SeQDs to prevent endothelial dysfunction and atherosclerosis. Our results demonstrated that A-SeQDs but not C-SeQDs inhibited NHE1 activity to inactivate Ca^{2+}/calpain signaling and upregulate eNOS functions in endothelial cells. *In vivo* gene manipulation of lentivirus-mediated NHE1 overexpression abolished A-SeQDs-increased eNOS activity and improvement of endothelial dysfunction in *Apoe*$^{-/-}$ mice. To the best of our knowledge, this study is firstly to report that amorphous selenium *via* inhibition of NHE1 to prevent endothelial dysfunction and

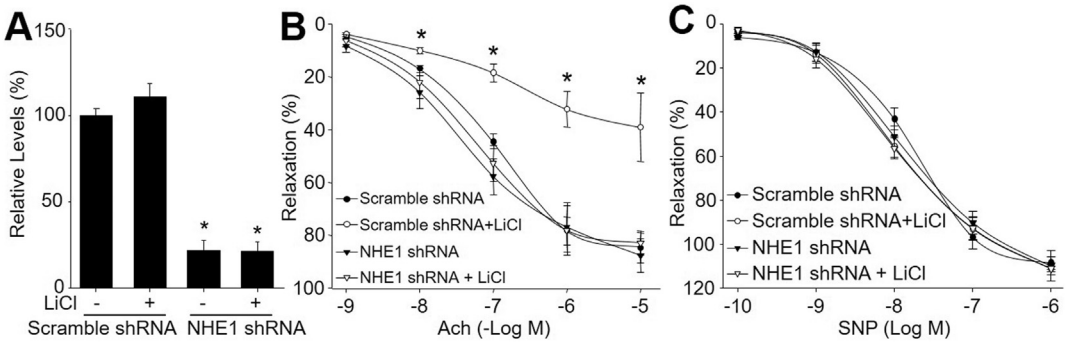

Fig. 3. Lentivirus-mediated knockdown of NHE1 abolished LiCl-induced endothelial dysfunction in rats. The protocol of animal experiment was shown in Online Fig. S1C. (A) The mRNA levels of NHE1 in aortic tissues from lentivirus-infected rats were determined by RT-PCR. (B and C) At the end of experiments, rats were sacrificed under anesthesia. Aortic rings isolated from descending aorta artery were subjected to assay (B) endothelium-dependent relaxation induced by Ach and (C) endothelium-independent relaxation induced by SNP in organ chamber. N is 10–15 in each group. $^{*}P < .05$ *vs.* Scramble shRNA alone.

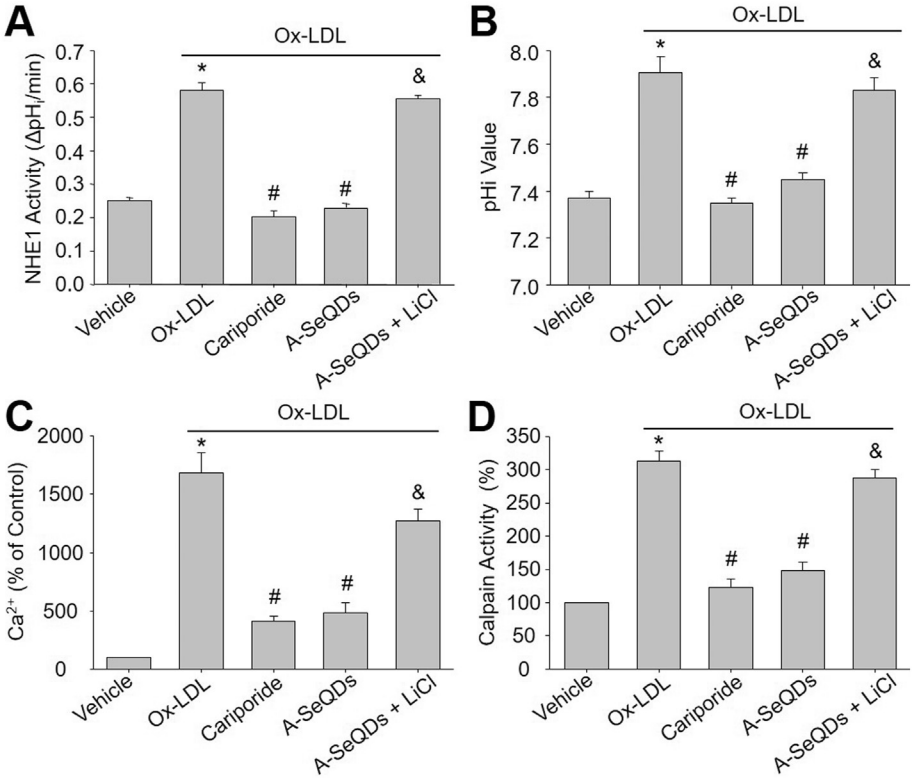

Fig. 4. A-SeQDs inhibited ox-LDL-induced NHE1 activation, decreased pHi value, and reduced $[Ca^{2+}]_i$ and calpain activity in cultured HUVECs. Cultured HUVECs were incubated with ox-LDL (100 μg/ml) for 6 h in presence of cariporide, A-SeQDs, and A-SeQDs plus LiCl. (A) NHE1 activity was determined by NH_4Cl pulse method. (B) pHi value was assayed by BCECF fluorescence. (C) Ca_i^{2+} concentration was detected by Fluo-4 fluorescence. (D) Calpain activity was assayed by fluorogenic peptide. Data are expressed by mean ± SEM. N is 3 in each group. $^{*}P < .05$ *vs* vehicle. $^{\#}P < .05$ *vs* ox-LDL alone. $^{\&}P < .05$ *vs* ox-LDL plus A-SeQDs.

atherosclerosis *in vivo*.

Selenium, an essential trace element, is incorporated into selenoproteins that play a crucial role in human health and disease [8]. Evidence from animal studies shows consistent results that selenium and selenoproteins might prevent experimental atherosclerosis, which can be explained by inhibiting oxidative stress, modulating inflammation, lowering serum lipids, suppressing endothelial dysfunction, and protecting vascular cells against apoptosis and calcification [15,18,19]. Methylated selenium is a good choice for selenium supplementation to correct a deficiency with lower toxicities, compared to selenite. In the absence of a route for adventitious incorporation into proteins, it may be a good choice for future clinical trials of selenium supplementation in disease prevention [32]. In this study, by using nanomaterials, we developed two kinds of nano-selenium particles with different structure and size and observed that A-SeQDs but not C-SeQDs prevented endothelial dysfunction and attenuated the growth of atherosclerotic plaque in rats and mice.

NHE1 is expressed ubiquitously in the plasma membrane of mammalian cells and exchanges intracellular H^+ for extracellular Na^+ to regulate pHi value and the concentration of $[Na^+]_i$ [21]. The activation of NHE1 increases intracellular $[Na^+]_i$ that leads to Ca^{2+} overload through the Na^+/Ca^{2+} exchanger, which is assumed to be the crucial factor in diabetic complications [10,20,30,31]. Previous study has also demonstrated that inhibition of NHE1 by cariporide inhibited hyperglycemia-induced inactivation of eNOS through disassociation of hsp90 with eNOS *via* Ca^{2+}-dependent calpain [27]. In this study, we uncovered a new mechanism that selenium therapy improves endothelial dysfunction by inhibiting NHE1 in endothelial cells. Further, A-SeQDs induced intracellular acidosis to inactivate Ca^{2+}/calpain signaling, resulting in NO production derived from eNOS. In this way, A-SeQDs prevents endothelial dysfunction and atherosclerosis as following evidence. Lentivirus-mediated NHE1 overexpression completely

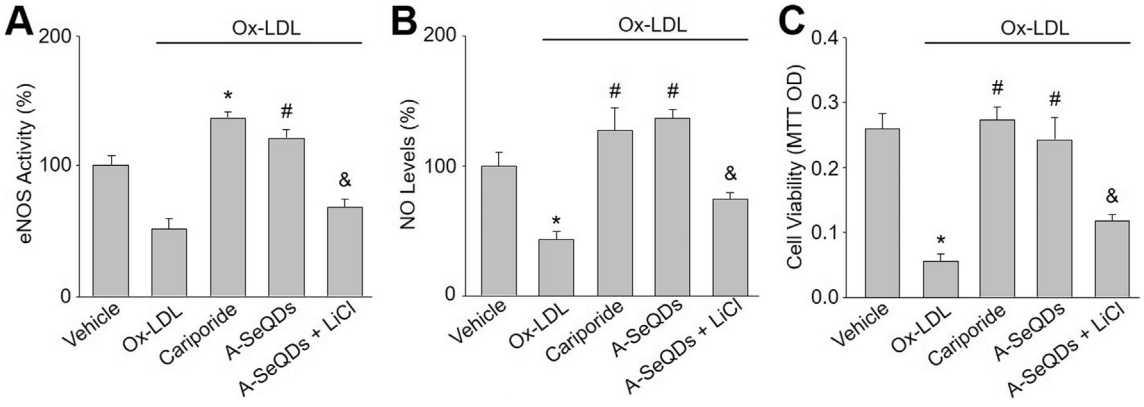

Fig. 5. A-SeQDs increased eNOS activity and NO production, and improved cell viability in cultured HUVECs. Cultured HUVECs were incubated with ox-LDL (100 μg/ml) for 6 h in presence of cariporide, A-SeQDs, and A-SeQDs plus LiCl. Cells were subjected to detect (A) eNOS activity by L-[^{3}H]-citrulline production from L-[^{3}H]-arginine, (B) NO production by DAF fluorescence, and (C) cell viability by MTT. Data are expressed by mean ± SEM. N is 3 in each group. $^{*}P < .05$ *vs* vehicle. $^{\#}P < .05$ *vs* ox-LDL alone. $^{\&}P < .05$ *vs* ox-LDL plus A-SeQDs.

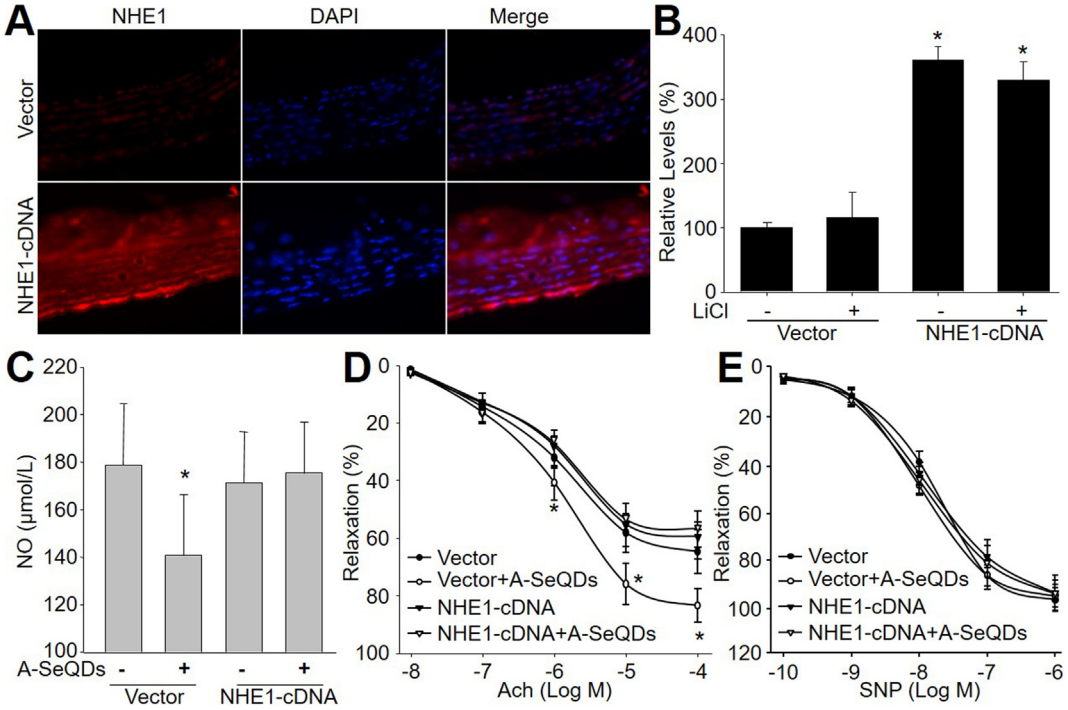

Fig. 6. A-SeQDs *via* NHE-1 inhibition prevented endothelial dysfunction in *Apoe*$^{-/-}$ mice fed with HFD. The protocol of animal experiment was shown in Online Fig. S1D. (A) Protein levels of NHE1 in aortic tissues from *Apoe*$^{-/-}$ mice were assayed by IHC. (B) The mRNA levels of NHE1 in aortic tissues from *Apoe*$^{-/-}$ mice were determined by RT-PCR. (C) At the end of experiments, blood was collected to assay serum NO levels. (D) Endothelium-dependent relaxation induced by Ach and (E) endothelium-independent relaxation induced by SNP were detected by organ chamber. N is 10–15 in each group. $^{*}P < .05$ *vs.* Vector alone.

abolished A-SeQDs-inhibited atherosclerosis in *Apoe*$^{-/-}$ mice feeding with HFD.

Because lentiviral NHE1 overexpresssion blocked the beneficial effects of A-SeQDs on endothelial dysfunction and atherosclerotic disease in *Apoe*$^{-/-}$ mice (Fig. 6D, Online Fig. S2A-S2C), we would expect that A-SeQDs may produce a protective effect on atherosclerosis development or progression as endothelial dysfunction is not only an initiation but also an contributor to the stability of atherosclerotic plaque and rupture to induce myocardial infraction [24]. This needs further investigations.

An issue is how A-SeQDs inhibits NHE1 in endothelial cells. We speculated multiple signaling pathways, such as Akt, AMP-activated protein kinase, prostacyclin synthase, microRNAs including miR-133 and miR-199, as the important regulators of endothelial cell functions, may contribute to endothelial dysfunction because these pathways are dysregulated in cardiovascular diseases [3,9,11,12,27,29,37].

A limitation of this study is that LiCl is used as an NHE1 activator, while it does not specifically affect NHE1 but has many effects on cell metabolism [25]. Thus, it is hard to speculate that cariporide mimics the effects of the quantum dots, which were driven by an effect on NHE1. To determine whether LiCl *via* NHE1 activation produces protective effects on endothelial dysfunction in rats, we downregulated gene expression of NHE1 by injecting rats with lentivirus expressing NHE1 shRNA followed by LiCl administration. As shown in Fig. 3B, we observed that NHE1 shRNA blocked the detrimental effects of LiCl on endothelial function in rats, indicating the effect of LiCl on endothelial dysfunction is NHE1 dependent. Moreover, the effects of A-SeQDs on endothelial dysfunction in *Apoe*$^{-/-}$ mice were abolished by lentivirus-mediated NHE1 overexpression (Fig. 6D). These data suggest that, as far as endothelial dysfunction, inhibition of NHE1 is, at least, the common pathway shared by cariporide and A-SeQDs.

In summary, the present study supports a novel drug of A-SeQDs developed by us, which inhibits NHE1 activity to inactivate Ca^{2+}/calpain signaling to prevent endothelial dysfunction (Online Fig. S2E). As a result, A-SeQDs attenuated the growth of atherosclerotic plaque *in vivo*. The finding that A-SeQDs prevent endothelial dysfunction may have broad applications since endothelial dysfunction is a common character at the beginning and in the progress in a number of vascular diseases including atherosclerosis [2,6], hypertension and diabetes [1].

Author contributions

M.L.Z. conducted the experiments and analyzed the data. G.W., H.W., Y.M.G., P.S., J.X., and S.X.W. partially performed some experiments. P.L. and L.Y. convinced the whole project and revised the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China (81874312, 81770493, 21771058, 21571053, U1704168, 81673423, and 81570723), the 111 project (D17007) and Henan Center for Outstanding Overseas Scientists (GZS2018003). S.X.W. is a distinguished professor of Henan province, a recipient of Zhong-Yuan-Qian-Ren Program of Henan province (194200510005), and the Tai-Hang Professional Scholarship of Xinxiang Medical University (505067).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vph.2019.01.005.

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