Protective effect of vasostatin-1 plasmid-like nanoparticles on aortic aneurysm and its mechanism

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ABSTRACT

Vasostatin 1 (VS-1) plays an important role in the regulation of various tissue injury and repair processes, but its role in aortic aneurysm remains unclear. The plasmid-like nanoparticles containing the vasostatin-1 gene Pul-PGEA-pCas-sgVs-1 were constructed, and their guarantee, safety, hemolysis, and particle size were analyzed. Eighty-four eight-week-old male ApoE-mice were randomly divided into blank group (without any treatment), model group (Ang II aortic aneurysm model + tail injection of PBS), control group (modeling + tail injection of Pul-PGEA-pCas9), and experimental group (modeling + tail injection of Pul-PGEA-pCas-sgVs-1), with 21 rats in each group. The incidence, mortality, and maximum diameter of abdominal aortic aneurysm (AAA) and the contents of high sensitivity C-reactive protein (HS-CRP), soluble intercellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), and TNF-α in serum were compared in different groups of mice. The results showed that Pul-PGEA-pCas-sgVs-1 had good biosafety and transfection ability. The maximum diameter of abdominal aorta, incidence of abdominal aortic aneurysm, mortality, and the expression levels of HS-CRP, ICAM-1, VCAM-1, and TNF-α in the experimental group were lower than those in the model group (P < 0.05). These results indicated that the plasmid-like nanoparticles Pul-PGEA-pCas-sgVs-1 can inhibit the development of aorta by down-regulating the expression of inflammatory factors, which played a good protective role on the aorta.

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1. Introduction

Aortic aneurysm is the damage to the normal structure of the aortic wall caused by various reasons. Under blood flow pressure, the aortic wall locally or diffusely expands abnormally to more than 1.5 times the normal aortic diameter [1]. It often occurs in the ascending aorta, aortic arch, descending thoracic aorta, thoracic abdominal aorta, and abdominal aorta. Abdominal aortic aneurysm (AAA) refers to the aneurysm that occurs primarily in the abdominal aorta and is the most common chronic degenerative disease of the aorta. The incidence rate of AAA is about 3.9–11% for acute and critical macrovascular lesions with local or extensive abnormal dilatation of the aorta more than 1.5 times the normal diameter, or abdominal aortic diameter ≥30 mm. With the increase of age, the incidence gradually increases, the maximum diameter of the tumor gradually increases, and the risk of rupture also gradually increases. The emergency mortality rate is as high as over 90% [2]. The main pathological changes of AAA are a variety of inflammatory cells in the adventitia of the arterial wall, infiltration, formation of a large number of new blood vessels, and excessive degradation of elastic fibers in the middle of the wall [3]. Among them, the chronic inflammatory response in AAA aneurismal wall tissue is manifested by inflammatory cell infiltration and increased expression of inflammatory factors, which runs through the entire process of AAA formation, development, and rupture, and is the most central pathological change of AAA [4].

VS-1 is chromogranin A (CGA) released by the nervous, endocrine, and diffuse neuroendocrine systems of vertebrates and invertebrates under stress [5]. VS-1 polypeptide chains contain multiple domains with multifunctional and specific biological activities, which play an important role in tissue nonspecific homeostasis regulation during inflammation, injury, and repair of various tissues, including the cardiovascular system [6]. Some studies indicated that VS-1 can antagonize the increase of arterial endothelial permeability caused by inflammation, but the mechanism of VS-1 in aortic aneurysm is still unclear [7]. As an emerging gene editing tool, the CRISPR/Cas9 system based on the aggregation of regularly spaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9), which is with high efficiency and specificity, and has been widely used in molecular fields such as gene editing, correction of pathogenic genes, verification of functional genes, and treatment of genetic diseases [8]. However, the large size of CRISPR/Cas9 limits its development process due to its in vivo delivery [9]. Although a variety of CRISPR/Cas9 system delivery vectors have been applied in tumor diseases, their application in aortic aneurysm has been minimal.

In summary, it is speculated that VS-1 may play a certain role in the occurrence and development of abdominal aortic aneurysm. Based on the advantages and disadvantages of CRISPR/Cas9, in this study, plasmid-like VS-1 nanoparticles (Pul-PGEA-pCas-sgVs-1) were constructed by using Pullulan or cholesterol-functionalized PGEA nanoparticles as carriers to explore the mechanism of Pul-PGEA-pCas-sgVs-1 in aortic aneurysm and to provide a theoretical basis for gene therapy of abdominal aortic aneurysm.

2. Materials and methods

2.1. Source and grouping of experimental animals

Eight-week-old male ApoE-mice weighing 20–22 g were selected, which were raised in a clean laboratory animal room at 25°C, with a relative humidity of about 55%, and under 12 hours of light. Free drinking and eating were allowed. All mice were bred adaptively for 2 weeks, and 84 mice were randomly divided into four groups, with 21 mice in each group. The blank group conducted no treatment. The aortic aneurysm model was established in the model group, and PBS was injected into the tail vein. The aortic aneurysm model was established in the control group, and Pul-PGEA-pCas9 was injected into the tail vein. The test group injected Pul-PGEA-pCas-sgVs-1 nanoparticles into the tail vein with the establishment of the aortic aneurysm model. All animal procedures in this experiment were approved by the Experimental Animal Management Committee, and the experimental methods were carried out in accordance with the approved guidelines.
2.2. Method of establishing aortic aneurysm model

The abdominal aortic aneurysm model of Ang II mice was established by referring to Sun et al. (2019) [10]. Mice in the model group and experimental group were fed a high-fat diet and drank normal water for four weeks at the age of six weeks. The mice were numbered and weighed. Ang II dosage was calculated according to 1,000 ng/kg/min, 25 µL/pump (Alzet company, type 2004). The prepared Ang II solution was injected into the capsule osmotic pump (Alzet, Type 2004). Anesthesia was performed by intraperitoneal injection of 10% chloral hydrate at 0.1 mL/100 mg body weight. After complete anesthesia, the mice were placed in prone position, their limbs and tail were fixed, the hair on the back was removed, and the skin of the scapular area on both sides was prepared for skin disinfection. Using a scalpel, a vertical incision of about 1.5 cm was cut from the left back of the mouse, about 1 cm from the midline. Forceps were used to separate the skin and muscle along the incision to the right, an activated capsule osmotic pump was inserted, and the skin was sutured layer by layer and disinfected again. The mice recovered and returned to the feeding chamber. The implantation cycle of the capsule osmotic pump was 28 days.

2.3. Measurement of maximum diameter of abdominal aortic aneurysm and HE staining of aorta in mice

The ultrasound examination was performed by an experienced ultrasound physician. Before examination, the mice were deprived of food and water for six hours, and the abdominal skin was prepared and weighed. Anesthesia was performed by intraperitoneal injection of 10% chloral hydrate at 0.1 mL/100 mg body weight. After complete anesthesia, the mice were placed supine on the examination table with the abdomen fully exposed, and ultrasonography was performed after application of the coupling agent. The transverse and longitudinal sections of abdominal aorta were measured, and the maximum diameter of abdominal aortic aneurysm was recorded.

At the end of the four-week experiment, the mice were anesthetized by intraperitoneal injection of 10% chloral hydrate at the weight of 0.1 mL/100 mg. Rapid thoracotomy and laparotomy were performed to fully expose the heart and abdominal aorta. The right atrial appendage was cut open, and a 10 mL syringe was connected with a scalp needle. The needle was punctured into the left ventricle, and the heart was lavaged with 20 mL PBS. Blunt dissection was performed on heart, ascending aorta, aortic arch, descending aorta, abdominal aorta to double iliac arteries, double renal arteries, and double kidneys. Part of abdominal aorta specimens were immersed in 4% paraformaldehyde and fixed for 24 h, which were then transferred to petri dishes with water. Under the microscope, the connective tissue around the heart, aorta, and kidney was carefully removed, the whole process of aorta was clearly exposed, and the maximum diameter of aorta was photographed and determined. Gradient alcohol dehydration and preparation of paraffin section were implemented. After the section dewaxing hydration, distilled water immersion was performed for 5 min. Hematoxylin was dropped, dyed for 3 min, washed with tap water for 2 min, differentiated with 1% alcohol hydrochloride for 5 times, and washed with tap water for 5 min. Eosin was added for 30s, followed by running water for 2 min, 95% alcohol for 1 min, and anhydrous ethanol for 5 min. After drying, the slices were sealed and observed under microscope.

2.4. Construction and screening of CRISPR/Cas9 system of targeting VS-1 gene

The MIT’s sgRNA online design program was adopted to design the sgRNA sequence for the VS-1 gene sequence. The CACCG sequence was added to the 5’end of the designed sgRNA sequence, and the AAAC sequence was added to the 3’end to obtain the corresponding upstream and downstream primers. They were annealed to form a double-stranded DNA with sticky ends, which was ligated to the linearized pX458 plasmid to obtain the recombinant plasmid Cas9-sgRNA. 5 × 10^5 mouse neuroma blast cells (N2a) were inoculated in a 6-well plate, and 2 mL 10% DMEM medium was added to each well. After 24 hours of...
culture, Lipo3000 was adopted to transfect the recombinant plasmid Cas9-sgRNA. After transfection for 24 h, the cells were collected to extract genomic DNA. The genomic DNA was amplified using VS-1 gene-specific primers VS-F: 5’-GGGGTACCATGCGCTCCGCCGCTGTCCT-3’ and VS-R: 5’-GAGCTCCTTCAGTAAATTCTGATGTCT-3’. After the amplified product was detected by 2% agarose gel electrophoresis, the target fragment was recovered by the gel, and the recovered product was verified by restriction digestion and sequencing.

2.5. Pul-PGEA nanomaterial synthesis

Pul-PGEA nanomaterials were synthesized by referring to Roshanali et al. (2020) [11] and improved accordingly. 4 g large molecule pullulan was mixed with 2.4 g sodium acetate trihydrate, 1 mL acetic acid, and 70 mL water and stirred and dissolved at 40°C for 10 min. After 5 μL pullulan was added to react for 30 minutes, the temperature was raised to 120°C to obtain the nano-sized small molecule pullulan. 0.58 g small molecule pullulan and 153 mg 4-dimethylaminopyridine (4-dimethylaminopyridine, DMAP) were weighted and dissolved in 10 mL anhydrous dimethylformamide (DMF), which were then stirred for 1 hour. 150 μL 2-Bromoisobutyryl bromide (BIBB) drop-wise was slowly added on an ice water bath at 0°C and reacted for 24 h at 25°C. An excess of isopropyl ether was added, dialyzed and lyophilized to obtain Pul-Br. 154 mg Pul-Br and 200 μL glycidyl methacrylate (GMA) were dissolved in DMSO. After they were fully exhausted for 15 minutes, 40 μL pentamethyldiethylenetriamine (PMDETA) and 16 mg CuBr were added and reacted for 30 minutes under anaerobic conditions. Pul-PGMA was obtained after vacuum drying by adding excess methanol. Then, 100 mg Pul-PGMA was dissolved in 5 mL DMSO. 1 mL ethanolamine was added to exhaust gas for 5 min, and then, they were put in oil bath at 80°C for 30 min. They were cooled to 25°C and dialyzed and dried to obtain the Pul-PGEA nanomaterial.

2.6. Physical and chemical characterization and safety analysis of Pul-PGEA nanoparticles

Pul-PGEA nanoparticles with N/P ratios of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 were configured. After complexing with plasmid DNA for 30 minutes, they were put on a 0.8% agarose gel, and the blocking ability of Pul-PGEA on plasmid DNA was evaluated during electrophoresis. A laser particle size potentiometer was used to analyze the particle size and potential of Pul-PGEA nanoparticles under different N/P ratios. The atomic force microscope was used to observe the morphology of Pul-PGEA nanoparticles.

The anti-protein adsorption level is an important indicator for evaluating the safety of cationic gene delivery vectors [12]. Equal volume of nanoparticles was added to the 2 mg/mL BSA solution, and the supernatants of different products were collected by centrifugation after reacting for different times. The spectrophotometer was adopted to detect the absorbance of different concentrations of BSA protein at 590 nm. The hemolysis rate of RBCs is an important indicator for evaluating biocompatibility [13,14]. After RBCs cells were resuspended and cultured with 0.1 mg/mL, 0.5 mg/mL, and 1 mg/mL Pul-PGEA, the morphology of RBCs cells was observed under a microscope. The MTT method is one of the important indicators for evaluating the toxicity of gene delivery materials [15]. 104 BNC.CL2 cells were inoculated into a 96-well plate. After the cells were in the incubator for 24 h, Pul-PGEA-pCas-sgVS-1 nanoparticles under various N/P ratios were added. After 4 hours of incubation, 0.5 mg/mL MTT solution was added, and the absorbance at 570 nm was detected with a microplate reader. By referring to the method of Liu et al. (2015) [16], it could obtain RBCs, and 0.1 mg/mL PEI and 1 mg/mL Pul-PGEA were adopted to resuspend RBCs. After the cells were cultured for 4 hours, the spectrophotometer was adopted to measure the absorbance of different solution suspensions at 545 nm. The following equation was used to calculate the hemolysis rate, where $H_r$ was the hemolysis rate, $A_t$ was the absorbance of the
test sample, and $A_n$ and $A_p$ were the absorbance of the negative control and the positive control, respectively.

$$Hr = \frac{A_t - A_n}{A_p - A_n} \times 100$$ \hspace{1cm} (1)

2.7. In vitro gene knockout potential and in vivo transfer efficiency of Pul-PGEA nanoparticles

BNC.CL2 cells were seeded into a 96-well plate at the density of 1 × 105 cells, cultured for 24 hours, and Pul-PGEA-pCas-sgVs-1 nanoparticles under various N/P ratios were added. After 4 hours of incubation, fresh 10% DMEM medium was changed and cultured for 24 hours. 70 μL cell lysate/well was added with PBS and reacted at 25°C for 30 minutes. The fluorescence intensity was detected by a luminometer, and the transfection efficiency was calculated. BNC.CL2 cells were inoculated with the cell density of 8 × 10^5 into a 6-well plate. After 24 h culture, when N/P = 1.5, Pul-PGEA and PEI complexed pCas-sgVs-1, respectively. After the complex was added to the cells and cultured for 12 hours, the cells were collected and the total RNA was extracted by the TRizol method, and the specific primers Cas9-F: 5’-ATGGACTATAAGGACCACGACGGAGAC-3’ and Cas9-R: 5’-CGGTGTTTGCCCAGCACCTTGAA-3’ were adopted to detect the mRNA level of Cas9.

pCas-sgVSCy5 was obtained by labeling pCas-sgVs-1 plasmid with fluorescent molecular probe Cy5. pCRISPR-Cy5, Pul-PGEA- pCas-sgVSCy5, and PEI- pCas-sgVSCy5 were injected into the tail vein of mice to analyze the biosafety of Pul-PGEA nanoparticles in vivo. 12 h after intravenous injection, mice were anesthetized by intraperitoneal injection of 10% chloral hydrate at a weight of 0.1 mL/100 mg, and then, the liver tissues of the mice were collected. A microscope was linked to a computer to take pictures of the liver tissue. Computer software was employed to measure the diameter of each part of the blood vessel and record the data. After imaging, liver tissues were embedded with OCT, and the nuclei of liver tissues were labeled with DAP1. Confocal microscopy was used to study the distribution of nanoparticles at the cellular level.

2.8. The content of inflammatory factors in serum detected by ELISA

Mice in the model group, control group, and experimental group were injected with equal volume of PBS, Pul-PGEA- pCas9, and Pul-PGEA-pCas-sgVs-1 through the tail vein, respectively. The injection dose was 20 μg/kg, and the total volume was 100μL. After 14 days of administration, blood was collected from the tail vein and serum was collected. The expression levels of vasostatin-1, high sensitivity C-reactive protein (HS-CRP), soluble intercellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), and TNF-α in serum were detected by ELISA.

2.9. Statistics

SPSS 19.0 was used for data statistics and analysis. Gene expression was expressed as mean plus or minus standard deviation ($\bar{X} \pm s$). Data differences between groups were analyzed by ANOVA, and $P < 0.05$ indicated that the differences were statistically significant.

3. Results

3.1. Characterization of physical and chemical properties of Pul-PGEA nanoparticles

Based on the current research results, it was speculated that VS-1 was correlated with the occurrence and development of abdominal aortic aneurysm. In order to verify this hypothesis, Pul-PGEA-pCas-sgVs-1 was constructed based on Pullulan and cholesterol functionalized PGEA nanoparticles. First, the characterization, biosafety and transfection performance of the nanoparticles in vivo and in vitro were analyzed. Then, the effect of Pul-PGEA-pCas-sgVs-1 on the expression of inflammatory factors in aortic aneurysm was studied in mice to preliminarily verify the correlation between VS-1 and abdominal aortic aneurysm.

The results of the agarose gel experiment on the blocking ability of PEI and Pul-PGEA against pCas-sgVs-1 under various N/P ratios were shown in Figure 1(a). With the increase of N/P, the electrophoretic bands of PEI and Pul-PGEA became brighter, and the bands of pCas-sgVS-1 plasmid became weaker.
and even disappeared. When N/P = 1:1 in PEI, it could effectively block the migration of pCas-sgVs-1 plasmid. When N/P = 1.5:1 in Pul-PGEA, it could effectively block the migration of pCas-sgVs-1 plasmid. The diameter detection results of Pul-PGEA-pCas-sgVs-1 nanoparticles under various N/P ratios by laser particle size analyzer were shown in Figure 1 (b). Compared with PEI-pCas-sgVs-1, Pul-PGEA-pCas-sgVs-1 nanoparticles formed by Pul-PGEA complexed with pCas-sgVs-1 were more uniform in size. As the N/P increased, the particle size of Pul-PGEA-pCas-sgVs-1 nanoparticles tended to be a stable state, with the particle size between 100 nm-200 nm. When N/P = 1.5:1, the size of Pul-PGEA-pCas-sgVs-1 nanoparticles was 150 nm.

The measurement results of the surface potential of nanoparticles under various N/P ratios were shown in Figure 2 (a). As N/P increased, the potential on the surface of the nanoparticles tended to rise. The surface potential of PEI-pCas-sgVs-1 nanoparticles was lower than that of Pul-PGEA-pCas-sgVs-1 nanoparticles.

Figure 1. Comparison of blocking ability and particle size of PEI and Pul-PGEA under various N/P ratios. (a: PEI and Pul-PGEA blocking ability under various N/P ratios; b: comparison of particle sizes of PEI and Pul-PGEA under different N/P ratios)

Figure 2. Analysis of the surface potential and AFM diagram of nanoparticles under various N/P ratios. (a: contrast of the surface potential of PEI and Pul-PGEA nanoparticles under various N/P ratios; b: AFM images of PEI and Pul-PGEA nanoparticles under various N/P ratios. a: AFM image of PEI-pCas-sgVs-1 nanoparticles when N/P = 1:1; b: AFM image of Pul-PGEA-pCas-sgVs-1 nanoparticles when N/P = 1:1; c: AFM image of PEI-pCas-sgVs-1 nanoparticles when N/P = 1.5:1; d: AFM image of Pul-PGEA-pCas-sgVs-1 nanoparticles when N/P = 1.5:1)
3.2. In vitro safety of Pul-PGEA nanoparticles

The detection results of the anti-protein adsorption capacity of Pul-PGEA nanomaterial were shown in Figure 3(a). After Pul-PGEA was added to BSA and reacted for 1 h, there was still 45.72% free BSA. However, when PEI was added to BSA at 0 h, significant adsorption occurred, with only 21.36% of the free BSA. The protein resistance of Pul-PGEA complex plasmid was notably higher than PEI, and the

![Figure 3. Comparison of adsorption capacity and hemolysis rate of nanoparticles.](image)

(a: protein adsorption capacity of PEI and Pul-PGEA nanoparticles under various N/P ratios; b: comparison of hemolysis rate of PEI and Pul-PGEA nanoparticles at different concentrations)

![Figure 4. Morphology and cytotoxicity analysis of RBCs treated with two kinds of nanoparticles when the N/P ratio was 1.5:1.](image)

(a: contrast of morphology of RBCs treated with two kinds of nanoparticles; b: cytotoxicity analysis of two kinds of nanoparticles. Inset a: morphology of RBCs treated with PBS; inset b: morphology of RBCs treated with Pul-PGEA-pCas-sgVs-1; inset c: morphology of RBCs treated with PEI-pCas-sgVs-1.)
difference was notable ($P < 0.05$). Further statistical results of the hemolysis rate at different doses of PEI and Pul-PGEA were shown in Figure 3(b). When the concentration was 0.1 mg/mL, the hemolysis rate of PEI reached 16.25%. With the increasing concentration of Pul-PGEA, the hemolysis rate of Pul-PGEA changed a little. When the concentration reached the maximum of 1 mg/mL, the hemolysis rate was only 2.07%. Figure 4(a) showed that the morphology of RBCs treated with different solvents was observed. Compared with PBS, the morphology of RBCs treated by PEI was obviously changed, while the morphology of cells treated with Pul-PGEA was almost not notably different from PBS. The toxicity of Pul-PGEA on liver cells BNL.CL2 was detected by MTT colorimetric assay, as shown in Figure 4(b). As N/P increased, the cell survival rate of both nanoparticles became lower and lower. Under various N/P ratios, the survival rate of Pul-PGEA-pCas-sgVs-1 was notably higher in contrast to PEI-pCas-sgVs-1 nanoparticles. When N/P = 1.5:1, the survival rate of Pul-PGEA-pCas-sgVs-1 was 71.14%, which was obviously higher than 50.86% of the gold standard PEI-complexed nanoparticles.

### 3.3. Analysis of transfection ability of Pul-PGEA nanoparticles

The transfection ability of Pul-PGEA to BNL.CL2 cells was shown in Figure 5. With the increase of N/P, the transfection efficiency of Pul-PGEA and PEI to BNL.

![Figure 5](image_url)

**Figure 5.** Nanoparticle transfection ability and GFP fluorescence. (a: comparison of the transfection ability of two kinds of nanoparticles under various N/P ratios; b: GFP fluorescence images of the two nanoparticles.)
CL2 cells showed the trend of increase first and then decrease. In addition, the transfection efficiency of Pul-PGEA was notably higher than PEI under the same N/P ratio ($P < 0.05$). Fluorescence microscopy analysis of the plasmid delivery potential of Pul-PGEA and PEI nanoparticles was shown in Figure 5(b). When N/P = 1.5:1, the expression of GFP in Pul-PGEA cells was higher than that of PEI. The quantitative analysis of the potential of flow cytometry to deliver plasmids of nanoparticles was shown in Figure 6. The positive cell rate of Pul-PGEA was 21.29%, the positive cell rate of PEI-treated cells was 7.93%, and the positive cell rate of Pul-PGEA was obviously higher than PEI ($P < 0.05$).

3.4. In vitro gene knockout potential analysis of Pul-PGEA nanoparticles

The results of the in vitro gene knockout potential of Pul-PGEA nanoparticles are shown in Figure 7. The Pul-PGEA-mediated Cas9 expression level was notably higher in contrast to PEI with extremely significant difference ($P = 0.022 < 0.05$). Enzyme digestion assay verified the gene editing ability of the Pul-PGEA-mediated CRISPR-Cas9 system, as shown in Figure 7(b). Compared with the empty plasmid Pul-PGEA-pCas9, Pul-PGEA-pCas-sgVs-1 showed a specific fragment of VS-1 gene after digestion.

3.5. Efficiency of Pul-PGEA nanoparticles to deliver the CRISPR-Cas9 system in vivo

The enrichment of nanoparticles in the liver was observed by in vivo imaging, as shown in Figure 8. After 12 hours of injection of nanoparticles through the tail vein, limited amounts of nanoparticles were enriched in the liver tissue. Compared with PEI, Pul-PGEA treated nanoparticles had higher fluorescence intensity in the liver.
Furthermore, the nuclei of liver tissue sections were labeled with DAPI, and then, confocal microscopic imaging was performed. Figure 8(b) illustrated that the imaging results of pCRISPR-Cy5 and Pul-PGEA-pCas-sgVSCy5 were similar.

3.6 Identification of animal model of abdominal aortic aneurysm

The rat abdominal aortic aneurysm model was established by the Ang II perfusion method. Ultrasonography was performed on the 1st, 3rd, 7th, 14th, and 28th days after surgery to detect the change of abdominal aortic diameter. Samples were taken 28 days after the maximum operation to verify whether the aneurysm model was successfully constructed. Postoperative ultrasonic examination results of different groups were shown in Figure 9(a, b). The diameter of abdominal aorta in the model group increased with time after osmotic pump implantation. The wall of the aorta in the upper quadrant of the kidney of mice was not clearly demarcated from the surrounding tissue. In the blank group, the diameter of abdominal aorta did not change significantly within 28 days after surgery, the surface of abdominal aorta was smooth without obvious exudation, and the boundary between abdominal aorta and surrounding tissues was clear.

The maximum diameter of abdominal aorta of mice in the blank group, and the model group was compared and analyzed on the 1st, 3rd, 7th, 14th, and 28th day after surgery Figure 9(c). The maximum diameter of the abdominal aorta in the model group increased significantly from the 7th day after operation, while the maximum diameter of the abdominal aorta in the control group did not change significantly during the observation period. The maximum diameter of abdominal aorta in model group and control group was (1.51 ± 0.16) mm and (0.96 ± 0.10) mm, respectively, on day 7 after surgery. The maximum diameter of abdominal aorta in the model group was larger than that in the control group, and the difference was statistically significant \( (P=0.037 < 0.05) \). The maximum diameter of abdominal aorta in the model group was significantly different from that in the control group on the 14th day after operation \( (P<0.01) \). The maximum diameters of abdominal aorta in the model group and control group were (1.03 ± 0.11) mm and (3.37 ± 0.35) mm, respectively, on the 28th day after operation. The maximum diameter of abdominal aorta in the model group was significantly different from that in the control group on the 28th day after surgery \( (P<0.001) \). Compared with the blank group, HE staining results in the model group showed that three layers of arterial wall were disorganized, the lumen was significantly enlarged, and the outer membrane was infiltrated by multiple inflammatory cells [Figure 9(d, e)].

3.7 Comparison of AAA incidence rate and maximum diameter of AAA in different groups

To systematically evaluate the role and mechanism of VS-1 in abdominal aortic aneurysm, the incidence and mortality of abdominal aortic aneurysm and the maximum diameter of abdominal aorta in different groups of mice were compared and analyzed (Figure 10). To systematically evaluate the role and mechanism of VS-1 in abdominal aortic aneurysm, comparative analysis was performed on the
incidence and mortality of abdominal aortic aneurysm and the maximum diameter of abdominal aorta in different groups of mice (Figure 11). The maximum diameter of abdominal aorta of mice in the blank group was (1.04 ± 0.25) mm, and that of mice in the model group was (2.39 ± 0.22) mm. The incidence of abdominal aortic aneurysm was 85.71%, and the mortality rate of mice during

![Figure 9](image_url)

**Figure 9.** Ultrasonography of abdominal aorta of mice in the blank group and the model group on day 28 after surgery. (a: ultrasonography of abdominal aorta of mice in the blank group on day 28; b: ultrasonography of abdominal aorta of mice in the model group on day 28 after surgery; c: comparison of the maximum diameter of abdominal aorta between the blank group and model group at different time periods; d: HE staining of abdominal aorta of mice in the blank group; E: HE staining of abdominal aorta in the model group) (¢ represents a statistical difference compared with the blank group, \( P < 0.05 \); ** indicated highly significant difference compared with blank group, \( P < 0.05 \); and *** represents a highly significant difference compared with the blank group, \( P < 0.001 \)).

blank group was (1.04 ± 0.25) mm, and that of mice in the model group was (2.39 ± 0.22) mm. The incidence of abdominal aortic aneurysm was 85.71%, and the mortality rate of mice during

![Figure 10](image_url)

**Figure 10.** Comparison of AAA incidence rate and maximum diameter of AAA in different groups. (a: comparison of AAA incidence and mortality among different groups; b: comparison of maximum diameters of AAA in different groups) (¢ represents a statistical difference compared with the blank group, \( P < 0.05 \); ** indicated highly significant difference compared with blank group, \( P < 0.05 \); and *** represents a highly significant difference compared with the blank group, \( P < 0.001 \)).
modeling was 9.52%. In the control group, the maximum diameter of abdominal aorta was (2.43 ± 0.36) mm, and the incidence of abdominal aortic aneurysm was 90.47%. The mortality rate of mice during modeling was 14.28%. The maximum diameter of abdominal aorta in the experimental group was (1.32 ± 0.19) mm, the incidence of abdominal aortic aneurysm was 28.57%, and the mortality rate of mice during modeling was 0. Autopsy results showed that all the mice died of rupture of aortic dissection aneurysm. The maximum diameter of abdominal aorta, incidence, and mortality of abdominal aortic aneurysm in the model group and control group were significantly higher than those in the control group (P < 0.01). The maximum diameter of abdominal aorta, incidence, and mortality of abdominal aortic aneurysm in the experimental group were significantly lower than those in the model group (P < 0.05).

3.8. Analysis of expression levels of VS-1, hs-CRP, sICAM-1, sVCAM-1, and TNF-a in serum

The expression levels of VS-1, hs-CRP, ICAM-1, VCAM-1, and TNF-a in the serum of all groups were analyzed, as illustrated in Figure 11. The expression of VS-1 in the model group and control group was notably lower in contrast to the blank group (P = 0.017 < 0.05). The expression of VS-1 in the test group was notably different from the control group (P > 0.05). The expression levels of hs-CRP, ICAM-1, VCAM-1, and TNF-a in the model group and control group were notably lower in contrast to the blank group (P = 0.026 < 0.05). The expression of hs-CRP, ICAM-1, VCAM-1, and TNF-a in the test group was obviously different from that of the control group (P = 0.033 < 0.05).

4. Discussion

Most cationic gene carriers can be complexed with DNA to form nanoparticles, which can prevent nucleic acid degradation by affecting nucleases [17]. Studies have pointed out that the size and surface potential of nanoparticles formed by the complexation of cationic carriers and DNA have a significant correlation with the uptake capacity of cells [18,19]. CRISPR/Cas9 is a relatively large-sized plasmid, which optimizes the size, potential, and surface morphology of nanoparticles formed by complexing with cationic gene carriers [20]. The complexing capacity of the Pul-PGEA cationic gene carrier to pCas-sgVs-1 was analyzed via agarose gel block test, nanoparticle size distribution,
zeta surface potential, and AFM images. The results showed that PEI and Pul-PGEA could effectively block the migration of pCas-sgVs-1 plasmid when N/P = 1:1 and 1.5:1, respectively, which may be related to the charge density of PEI and Pul-PGEA. The positive charge density on the PEI chain was higher [21] and could be complexed with a large amount of negatively charged pCas-sgVs-1. The size and surface potential of nanoparticles were related to their transfection ability and uptake in cells. Compared with PEI-pCas-sgVs-1, the Pul-PGEA-pCas-sgVs-1 nanoparticles formed by Pul-PGEA complexed with pCas-sgVs-1 were more uniform in size. The results of AFM detection showed that the particle size of PEI-pCas-sgVs-1 and Pul-PGEA-pCas-sgVs-1 nanoparticles was both about 100 nm. The size of the nanoparticles of the two was relatively uniform, which was inconsistent with the detection result of the laser particle size analyzer. The possible reason for the analysis was that the object detected by the AFM image was dry nanoparticles, while the laser particle size analyzer detected nanoparticles in liquid state. When charged nanoparticles enter the body, they will cause protein modification processes such as aggregation or degradation of proteins in the body [22]. BSA is one of the proteins with the highest content in serum [23], and it is often selected to evaluate the anti-protein adsorption ability of materials [24]. The adsorption capacity of Pul-PGEA and PEI on BSA protein was discussed, and it was found that the anti-protein ability of Pul-PGEA complex plasmid nanoparticles was notably stronger than PEI (P < 0.05). The main pathological changes in AAA were chronic inflammatory reaction of vascular wall, rupture and degradation of elastic fibers in the middle layer of artery, and the formation of a large number of new vessels in the outer membrane of artery. Chronic inflammation of the vascular wall is the most important pathological change of abdominal aortic aneurysm and runs throughout its pathogenesis, manifested as multiple inflammatory cell infiltration in the outer membrane of the artery [29]. On one hand, these inflammatory cells can secrete various inflammatory factors such as interleukin-1 β, monocyte macrophage chemokine-1, and tumor necrosis factor. These inflammatory factors can damage the vascular wall and chemotax more inflammatory cells to gather in the outer membrane of blood vessels, and the positive feedback aggravates the inflammatory response [30]. In this research, the Ang II aortic aneurysm model was used to investigate the effect of Pul-PGEA-pCas-sgVs-1 nanoparticles on AAA. The Ang II aortic aneurysm model does not require anatomical exposure of the abdominal aorta, nor does it require local mechanical and chemical stimulation, which is consistent with the characteristics of abdominal aortic aneurysm formation [33]. Therefore, in this study, Ang II perfusion was used to establish the aortic aneurysm model, and the success of the model was verified. The results showed that the maximum diameter of abdominal
aorta was significantly different between the model group and the control group on day 28 after surgery (P<0.001). Compared with the blank group, HE staining results in the model group showed three layers of arterial wall disorder, obvious enlargement of lumen, and infiltration of inflammatory cells in the outer membrane. These results indicated that the abdominal aorta model was successfully constructed. Current research results show that the expressions of hs-CRP, ICAM-1, VCAM-1, and TNF-a in abdominal aortic aneurysm tissues are significantly higher than those in the normal abdominal aorta [34]. The results showed that the maximum diameter of abdominal aorta, the incidence, and mortality of abdominal aortic aneurysm in the experimental group were significantly lower than those in the model group (P<0.05). These results indicated that the incidence, maximum diameter, and mortality of abdominal aortic aneurysm in mice were significantly reduced after transfection with Pul-PGEA-pCas-sgVs-1 nanoparticles, and the expression of inflammatory factors in corresponding cells was also decreased. The reason may be related to the inhibition of inflammation induced by the VS-1 gene [35]. In summary, Pul-PGEA-pCas-sgVs-1 nanoparticles can inhibit the occurrence and development of abdominal aortic aneurysm. Pul-PGEA-pCas-sgVs-1 nanoparticles can be used as a therapeutic direction for abdominal aortic aneurysm.

5. Conclusion
The Pul-PGEA cationic gene vector was complexed with pCas-sgVs-1 to form Pul-PGEA-pCas-sgVs-1 nanoparticles. The biological characteristics of the nanoparticles were studied, and the relationship between Pul-PGEA-pCas-sgVs-1 and the inflammatory factors related to the occurrence of aortic aneurysms was further studied. It was found that the nanoparticle with high biosafety and high transfection efficiency was successfully constructed, which could effectively increase the expression of VS-1 in aortic aneurysms, while reducing the expression of hs-CRP, ICAM-1, VCAM-1, and TNF-a. However, there are still some shortcomings in this work. The expression levels of inflammatory factors hs-CRP, ICAM-1, VCAM-1, and TNF-a in mice after the transfection of Pul-PGEA-pCas-sgVs-1 nanoparticles were only analyzed, and the specific pathways involved in these factors were not discussed. In the future, based on the current research results, the related pathways and mechanisms of the protective effect of Pul-PGEA-pCas-sgVs-1 nanoparticles on abdominal aortic aneurysm will be further analyzed, so as to provide a complete theoretical basis for the treatment and prognosis of abdominal aortic aneurysm. In conclusion, Pul-PGEA-pCas-sgVs-1 nanoparticles can inhibit the development of abdominal aortic aneurysm by decreasing the expression levels of hs-CRP, ICAM-1, VCAM-1, and TNF-a, which provides a new idea for gene therapy of abdominal aortic aneurysm.

Highlights
Pul-PGEA-pCas-sgVs-1 had transfection in vitro and in vivo. Pul-PGEA-pCas-sgVs-1 reduced the incidence of aortic aneurysm. Pul-PGEA-pCas-sgVs-1 inhibited aorta progression for protection.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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Data availability statement
All data, models, and code generated or used during the study appear in the submitte.

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