Research Article

Effect of Low-Pressurized Perfusion with Different Concentration of Elastase on the Aneurysm Formation Rate in the Abdominal Aortic Aneurysm Model in Rabbits

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Establishing an animal model of abdominal aortic aneurysm (AAA) is the key to study the pathogenesis and the pathophysiological features of AAAs. We investigated the effects of low-pressurized perfusion with different concentrations of elastase on aneurysm formation rate in the AAA model. Fifty male New Zealand white rabbits were randomly divided into A, B, C, D, and E groups. 10 μL of normal saline was perfused into the abdominal aorta in group A and 1 U/mL, 10 U/mL, 100 U/mL, or 200 U/mL of elastase was, respectively, perfused for the other four groups. All the animals were perfused for 7 min. Doppler ultrasound examinations of the abdominal aorta were performed before surgery and on day 14 after surgery. The rabbits were sacrificed and the perfused segment of the abdominal aorta was observed visually and after staining. The aneurysm formation rate of group A, group B, group C, group D, and group E was, respectively, 0%, 0%, 33.3%, 102.5–146.8%, and 241.5–255.2%. The survival rate of five groups was 90%, 90%, 90%, 90%, and 40%, respectively. So, we concluded that low-pressurized perfusion with 100 U/mL of elastase can effectively establish AAAs in rabbits with a high aneurysm formation rate.

1. Introduction

Abdominal aortic aneurysm (AAA) is a common and severe cardiovascular disease, with an incidence of 5–8% in males older than 60 years of age [1]. However, once the AAA ruptures, the mortality rate can reach 80–90% [2]. The pathogenesis of AAAs is still unclear. By establishing an animal model of AAA, we can study the pathogenesis and the pathophysiological features of AAAs and determine measures for early prevention and treatment. Several small animal models have been developed to assist in understanding the mechanisms of AAA pathogenesis. Anidjar et al. [3] first introduced an elastase-induced AAA, one of the most commonly used models in rats. Periarterial elastase incubation is also used to create an aneurysm in the carotid [4–7] and aortic arteries [8–10] in small animals. Currently, elastase perfusion is the most commonly used method for animal models of AAA. However, as shown by the existing literature, different production methods of abdominal aortic aneurysm model, the elastase concentrations vary greatly [11–14]. To evaluate the effect of elastase concentrations, we established an AAA model in rabbits by perfusing different concentrations of elastase. The aneurysm formation rate and dilation of the abdominal aorta were measured. Thus, the optimal concentration of elastase for inducing an AAA was determined.

2. Materials and Methods

2.1. Materials. Fifty healthy male New Zealand white rabbits (Beijing Haidian Xingwan Animal Breeding Farm, License no. SCXK (Beijing) 2006-0006), weighing approximately 2.50 ± 0.20 kg, were used. The rabbits were allowed free access to water and food and were reared in separate cages with an ambient temperature of 23–25°C. The housing environment was dry with good ventilation, adequate lighting, and easy drainage. The cage was 60 cm in width and 50 cm in height, and one animal was fed per cage. The
feed formulation included alfalfa powder, wheat bran, flour, corn, soybean meal, fish meal, bone meal, yeast powder, salt, and fish liver oil. Clean tap water was provided as drinking water. Pancreatopeptidase E (elastase at pH 8.8, salt, and fish liver oil). Clean tap water was provided as drinking water. Pancreatopeptidase E (elastase at pH 8.8, salt, and fish liver oil. Clean tap water was provided as drinking water. Pancreatopeptidase E (elastase at pH 8.8, salt, and fish liver oil.

...Numerical values are presented as mean ± standard deviation. Differences were considered statistically significant at \( P < 0.05 \).
Table 1: Diameter changes measured by Doppler ultrasound.

| Group  | Maximum aneurysm diameter before surgery (mm) | Maximum aneurysm diameter after surgery (mm) | Dilation rate (%) | Aneurysm formation rate (%) | Survival rate |
|--------|---------------------------------------------|---------------------------------------------|------------------|-----------------------------|---------------|
| Group A | 2.32 ± 0.03                                 | 2.32 ± 0.03                                 | 0                | 0/9 (0)                     | 90            |
| Group B | 2.36 ± 0.05                                 | 2.95 ± 0.06                                 | 24.9 ± 2.5       | 0/9 (0)                     | 90            |
| Group C | 2.39 ± 0.03                                 | 3.43 ± 0.17                                 | 43.9 ± 7.69      | 3/9 (33.3)                  | 90            |
| Group D | 2.36 ± 0.04                                 | 5.32 ± 0.34                                 | 125.4 ± 16.1*    | 9/9 (100)*                  | 90            |
| Group E | 2.33 ± 0.03                                 | 8.11 ± 0.11                                 | 248.2 ± 5.6      | 4/4 (100)                   | 40*           |

*P < 0.05, compared to groups A, B, and C; *P < 0.05, compared to groups A, B, C, and D.

3. Results

Each group of A, B, C, and D had a rabbit died during the surgical procedures (one rabbit died due to aortic dissection during operation and other three died of vascular rupture during operation). Fourteen days after operation, nine rabbits survived in each group of A, B, C, and D, resulting in a survival rate of 90%. No rabbit died in group E during surgical procedures. In group E, six rabbits died within 1 week after surgery (animals were euthanized when they showed certain clinical signs indicative of illness/injury/morbidity) and 4 rabbits survived; the survival rate was 40%.

3.1. Visual Observation of the AAA (Figure 1). Part of the blood vessels at the surgical site had no obvious expansion in groups A and B; group C showed slight expansion; compared with group A, groups D and E obviously expanded at the surgical site; the maximum rate of expansion was more than 100%.

3.2. Changes in the Diameter of the Abdominal Aorta upon Ultrasound Examination. The perfused segment of the abdominal aorta did not show local enlargement after surgery in group A, and no aneurysm was formed. In group B, the maximum aneurysm diameter in the perfused segment was slightly increased compared to that before surgery; the local dilation rate was 21.4–29.7%, which did not satisfy the criterion of aneurysm formation, and the aneurysm formation rate was 0%. In group C, the local dilation rate was 31.2–55.3%, and 3 rabbits showed a dilation rate above 50%, which satisfied the criterion of aneurysm formation in the abdominal aorta. Therefore, the aneurysm formation rate of group C was 33.3%. In group D, the perfusion segment dilation rate was 102.5–146.8%, and the aneurysm formation rate was 100%; thus, the aneurysm formation rate was 100%. In group E, the local dilation rate was 241.5–255.2%, and the aneurysm formation rate was 100%. The results are shown in Table 1 and Figure 2.

3.3. Histological Changes Are Shown in Figure 3. HE staining observed the vascular wall structure. As the figure shows, the vascular wall of group A is structurally complete, and the membrane smooth muscle is ranged neatly, without inflammatory cell infiltration. While the structure and the integrity of aneurysm wall from group E are poor and the smooth muscle in the membrane is disorderly arranged, with infiltrating inflammatory cells in vascular wall, CD68 positive cells can further reflect the infiltrating degree of the inflammatory cells in aneurysm wall. As the figure shows, the number of CD68 positive cells from group A to group E aneurysm wall increased significantly. The number of CD68 positive cells in each group is, respectively, as follows: group A 1.32 ± 0.47; group B 4.24 ± 0.39; group C 5.07 ± 0.21; group D 11.72 ± 0.41; group E 14.29 ± 0.23. Compared with group A, the number of CD68 positive cells in group D and group E increased significantly, P < 0.05; the difference was statistically significant (Figure 3(b)); EVG staining observed
Figure 2: The diameter of the abdominal aorta differentially changed in response to different concentrations of elastase, as shown by Doppler ultrasound examinations of the abdominal aorta. The aneurysm diameter increased with an increasing concentration of elastase.
Figure 3: Continued.
the destruction degree of elastic fiber tissue in each group. From the figure, we can see that the elastic fibrous tissue wavy in group A is clear, while that in group E is in disorder and fuzzy. The damage area percentage of elastic fiber to total observation area is, respectively, as follows: group A 1.10% ± 0.12%, group B 3.11% ± 0.21%, group C 10.61% ± 0.33%, group D 45.30% ± 0.27%, and group E 62.64% ± 0.45%; compared with group A, the destruction amount of elastic fiber in group D and group E obviously increased, \( P < 0.05 \); the difference was statistically significant (b). EVG staining showed the damage of the elastic fibers in group A is clear, while that in group E is in disorder and fuzzy. Compared with group A, the destruction amount of elastic fiber in group D and group E obviously increased, \( P < 0.05 \); the difference was statistically significant (c). Masson's stain shows that the collagen fiber content increased gradually from group A and group E. Compared with group A, the collagen fiber content in group D and group E increased significantly, \( P < 0.05 \); the difference was statistically significant (d).

**4. Discussion**

Abdominal aortic aneurysm (AAA), defined as a permanent localized aortic dilation with a diameter of 1.5 times the normal aorta diameter, is a silent degenerative disease that can be life-threatening [15]. The native AAA model induced by elastase perfusion is a standard aneurysm model for experimental research in rodents [1, 3, 16–21] and rabbits [22, 23]. Although the pathogenesis of AAA is still unknown, it is generally believed that the effects of elastase stimulated an elastolytic and inflammatory response in the aortic walls [24] and resulted in the continual progress of aneurysm development. In our study, we successfully developed an AAA model in rabbits by low-pressure perfusion of 100 IU/mL elastase.

We found that the aortic diameter increased with increasing elastase concentration. Lower concentration of elastase was unable to reliably induce AAA formation.

Histologically, the aneurysms are characterized by the near total destruction of the elastin matrix of the media. Busuttil et al. [25] had reported that elastase activity in the aneurysm wall was greater in intima media than in outer layers. White et al. [26, 27] had suggested that macrophages within the aortic media may be responsible for elastase secretion and subsequent aneurismal degeneration. They believed that medial injury with elastolysis was necessary for aneurysm formation, and the inflammatory response facilitated this process. The histological results of our study were coincidence with their. As shown by CD68 staining, the number of CD68 positive cells in the aortic wall increased from group A to group E. The more the positive cells, the more severe the inflammatory reaction whereas the less the positive cells, the less severe the inflammatory reaction. EVG staining showed the damage of the elastic fibers in the abdominal aortic wall. We can see that the elastic fibers of group A presented a wave-like texture without obvious damage. However, the texture of the elastic fibers in group E was obscured with residual elastic fibers. Compared to groups B, C, and D, group E showed the most severe damage to elastic fibers. Mason staining observed the changes of abdominal aortic aneurysm wall collagen fiber content in different groups. In our study, the collagen fiber content increased gradually from group A and group E. Compared with group A, the collagen fiber content in group D and group E increased significantly.

Our data suggested that the 100 U/mL group had the highest AAA formation rate and survival rate in the five groups with different perfusion concentrations. Azuma Junya and his colleagues reported that they had established a rat abdominal aortic aneurysm model by aorta perfusion, and
the perfusion concentration of porcine pancreatic elastic protease was 1.5 U/mL. Bi et al. [14] reported that they had observed the effects of different concentrations of elastin by using outer periphery embedding method to establish rabbit AAA model of abdominal aortic aneurysm. They divided rabbits into five groups with the concentration of porcine pancreatic proteases 0, 0.1, 0.5, 1, and 10 U/µL, respectively. Under the administration of perfusion pressure of 350 mmHg and perfusion concentration of 100 U/mL, Kobayashi [28] and his colleagues successfully established rabbit abdominal aortic aneurysm by using porcine pancreatic elastase. In our study, we used the same infusion concentration, but the perfusion pressure is much lower.

Low-pressure perfusion was performed in our study. At present, in order to cause injury of aortic wall, most of the researches give perfusion pressure 300–400 mmHg in the process of making animal abdominal aortic aneurysm. Yamaguchi et al. successively established rabbit AAA through giving peak pressure between 300 and 400 mmHg in the isolated segment [29]. In our preliminary experiments, we found that high-pressure perfusion (300 to 400 mmHg) might lead to entry of elastase into the blood circulation via the vessel wall microcirculation. This could increase the risk of acute pancreatitis and damage to other organs. Moreover, under high pressure elastase might leak outside of the lumen, leading to damage to the entire luminal layers and surrounding tissues. In our experiments, we only gave 100 mmHg intraluminal perfusion pressure. We found that low-pressure perfusion not only avoided hemorrhaging but also successfully induced an AAA.

Different experiments take different perfusion time in order to establish AAA models; the perfusion time varies from 20 min to 120 min [3, 13, 17, 28]. Anidjar et al. firstly established a rat AAA by perfusing elastase for 20 min. In Yamaguchi et al.'s study, 76 male Sprague Dawley rats were infused with a solution of elastase for 10, 20, 30, 60, or 120 min or with saline for 30 min. They concluded that it was possible to shorten the elastase infusion time from 120 min to 30 min in the elastase-induced rat aneurysm model and shortening of infusion time could reduce the experimental time and mortality rate [30]. Shortening the perfusion time was also considerable in our study; compared with them, we found that 100 U/mL concentration of elastase perfused for only 7 min was able to induce AAA formation, and that was favorable for increasing the survival rate of animals. Carsten et al. [31] indicated that a longer perfusion time resulted in longer obstruction of the abdominal aorta and prolonged ischemia of the organs below the level of the abdominal aorta and that would lower the survival rate of animals and even cause paralysis of the hind limb. Another research confirmed that 71% of the rabbits have been shown to develop paraplegia after temporary occlusion of the infrarenal aorta for 20 min [32]. One hour of ischemia had produced spinal cord infarction in all of the animals examined [1]. Thus, by reducing the perfusion time, we have greatly reduced the overall operation time and the death rate.

Ding et al. [33] reported that elastase-induced saccular aneurysm in carotid artery maintained stability and patency during 2- and 5-year follow-up. However, some research showed that AAA model induced by chemical substance injury, such as elastase, calcium chloride, or papain, might not result in progressive enlargement. Origuchi et al. [34] reported that aneurysms induced by adventitial elastolysis did not progress, gradually shrank over 42 days, and healed spontaneously by 90 days. Bi et al. [35] reported that aneurysms showed progression after day 5; however, long-term follow-up to study the further changes of the model was unwarranted, considering that there was no evidence of aneurysm stability beyond 21 days in the present experiments. In our preliminary experiment, we observed that rabbit AAA developed immediately after operation in the elastase-induced model. This fast-injury model enlarged within 10–20 days and aneurysm shrank thereafter. That is the reason why we choose to kill animals 14 days after the operation.

Anidjar et al. first introduced a method to create an AAA model in rats. It is quite obvious that rat AAA model induced by this method was too small to perform further research. In our research, we present an easy, efficient, and reproducible way to create rapid dilation of rabbit aortic arteries to form a model of AAA. This simpler aneurysm model will be more valuable for elucidating AAA mechanisms and therapeutic interventions. We believe that our rabbit aneurysms models are more suitable for the future research.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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