Original Article

Overexpression of TIMP-2 mediated by recombinant adenovirus in rat abdominal aorta inhibits extracellular matrix degradation

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Summary **To investigate the effects of a tissue inhibitor of the matrix metalloproteinase-2 (TIMP-2) gene on the extracellular matrix of the abdominal aorta, models of rat abdominal aortic aneurysm were utilized and a solution of AdTIMP-2 was perfused into the abdominal aorta. The models of rat abdominal aortic aneurysm were constructed with intraluminal perfusion of the abdominal aorta with porcine pancreatic elastase, and an adenovirus solution carrying the TIMP-2 gene was transferred into the aorta by local perfusion. After 14 days, aortic wall elastin and collagen concentrations were measured with an image analysis system and fixed aortic tissues were examined by light microscopy for inflammation. No abdominal aortic aneurysms developed in TIMP-2 gene-transferred rat models, representing a marked decreased from control rats ($p < 0.01$). The elastic fibers and collagenous fibers were preserved with greater integrity in the AdTIMP-2 group than in the control group and inflammatory cells were seen in adventitial areas. The TIMP-2 gene mediated by adenovirus can renew the balance of degradation of the extracellular matrix caused by elastase and inhibit the formation of an abdominal aortic aneurysm. This finding provides a new strategy for treatment of abdominal aortic aneurysms.**

Keywords: Tissue inhibitor of matrix metalloproteinase, Abdominal aorta, Extracellular matrix, Gene therapy

1. Introduction

The formation of an abdominal aortic aneurysm (AAA) involves several pathological factors, the most important of which is the degradation of the extracellular matrix of the aorta wall resulting in the reformation of the vascular wall. A recombinant adenovirus vector-carrying tissue inhibitor of the matrix metalloproteinase-2 (TIMP-2) gene (*1*) was successfully constructed, and studies have proven that the TIMP-2 gene can inhibit vascular smooth muscle cell migration and proliferation *in vitro* (*2*). In this study, a rat model of AAA with local perfusion of a solution of the adenovirus vector was utilized to investigate the effects of the TIMP-2 gene on degradation of the aortic extracellular matrix with morphological and pathological techniques.

2. Materials and Methods

2.1. *Construction of rat AAA models and group assignments*

Twenty four grown male SD rats, weighing 350-400 g, were provided by the experimental animal center of the Second Military Medical University, Shanghai,

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China. The rat models were constructed by perfusing elastase into the abdominal aorta with reference to the literature (*3*). Porcine pancreatic elastase was purchased from EMD Chemicals Inc., San Diego, CA, USA. The rats were randomly divided into three groups: an AdTIMP-2 group, an AdCMV group, and a blank control group.

2.2. *Perfusion of the adenovirus solution*

Following perfusion of the adenovirus solution, an elastase solution was subsequently perfused into the rat abdominal aorta with a small-diameter syringe. The adenovirus solution was diluted to 10^9 pfu/mL and perfused at 1 mL into the rat aorta continuously for 30 min. After the blood flow through aorta was reestablished and the incision was closed, each rat was fed separately. The diameter of the rat abdominal aorta was measured before perfusion and immediately afterwards. Two weeks later, all of the rats were euthanized and surgery was performed to dissect the suprarenal segment of the abdominal aorta to evaluate the efficacy of systemic gene therapy with TIMP-2. The rats were killed after measuring the diameter of the aorta with vernier calipers. The dissected aorta segment was fixed in formalin and embedded in paraffin for morphological and pathological analyses.

2.3. *Pathological analyses*

Paraffin sections of vessel segments 4-5 μm thick were stained with hematoxylin-eosin and elastin-van Gieson. Morphometric analyses were performed on 3 to 4 cross sections for each vessel to microscopically determine the inflammation of the vessel wall. Computerized quantification of elastin degradation in the media was done using image analysis software.

2.4. *Statistical analysis*

The data were calculated as mean ± standard deviation. Comparisons between two groups were done using a Student's *t* test with $p < 0.05$ considered significant.

3. Results

3.1. *Common conditions after surgery*

A second surgery was performed on a total of 24 rats. The rats failed to move their lower limbs and activity decreased in the early stages after perfusion, usually recovering in 3 to 5 days.

3.2. *Measurement of the rat abdominal aorta diameter*

Table 1 shows the results of ADpre and ADpost separately for three groups of rats. As is apparent, there are no significant differences between the diameters of each group before and just after perfusion ($p > 0.05$). The diameter of the aorta on day 14 (AD14d) and the percent enlargement of the abdominal aorta (ADpet) were much lower for the AdTIMP-2 group than for the AdCMV and control groups. Similarly, the rate of AAA formation was also significantly lower for the AdTIMP-2 than for the other two groups ($p < 0.05$). AAA formation was completely prevented in the AdTIMP-2 treated group ($n = 8$), while all mice in the AdCMV and PBS control groups developed an AAA. These results demonstrate that overexpression of TIMP-2 in the vascular wall prevents AAA development in a rat model.

3.3. *Pathological analyses*

In the AdTIMP-2 group, the elastin fibers of media were preserved with integrity and degradation of collagen fibers was inhibited. Inflammatory cell infiltration was observed in the vessel wall (Figure 1A). The lumen of the abdominal aorta expanded and neointima formed in the AdCMV and control groups. In these two groups, the intima was split and elastin of the media was degraded. Collagen fibers were more badly degraded. Inflammatory

Table 1. Diameters of abdominal aortas in three groups of rats

Group	*n*	AD_{pre} (mm)	AD_{post} (mm)	AD_{14d} (mm)	AD_{pet} (%)	AAA (%)
AdTIMP-2 group	8	1.58 ± 0.04	2.03 ± 0.09	2.33 ± 0.06*	48 ± 4*	0**
AdCMV group	8	1.60 ± 0.04	2.09 ± 0.07	$3.52 \pm 0.11^{\Delta}$	120 ± 6	100
PBS group	8	1.57 ± 0.05	2.07 ± 0.08	$3.43 \pm 0.09^{\Delta}$	118 ± 5	100

Compared to the AdCMV and control test groups, * $p < 0.05$, ** $p < 0.01$; Compared to AD_{pre} and AD_{post}, $^{\Delta}p < 0.05$.

Table 2. Degrees of inflammation and degradation of rat abdominal aortic elastase-collagen in the three groups

Group	*n*	Inflammation of aorta			Degradation of elastin and collagen			
		(+)	(++)	(+++)	I	II	III	IV
AdTIMP-2 group	8	5	3	0	3	5	0	0*
AdCMV group	8	3	3	2	0	0	2	6
PBS group	8	3	2	3	0	1	1	6

Compared to the AdCMV and control test groups, * $p < 0.05$.

cells infiltrated through the vessel wall, especially in the outer layer (Figure 1B and C). Table 2 shows the degrees of inflammation and degradation of rat abdominal aortic elastin-collagen for the three groups of rats. The AdTIMP2 group displayed better preservation of elastin fibers and collagen, while the AdCMV and control groups displayed significant destruction of elastin fibers and the medial layer (Figure 2A-C).

4. Discussion

Previous studies by the current authors have shown that

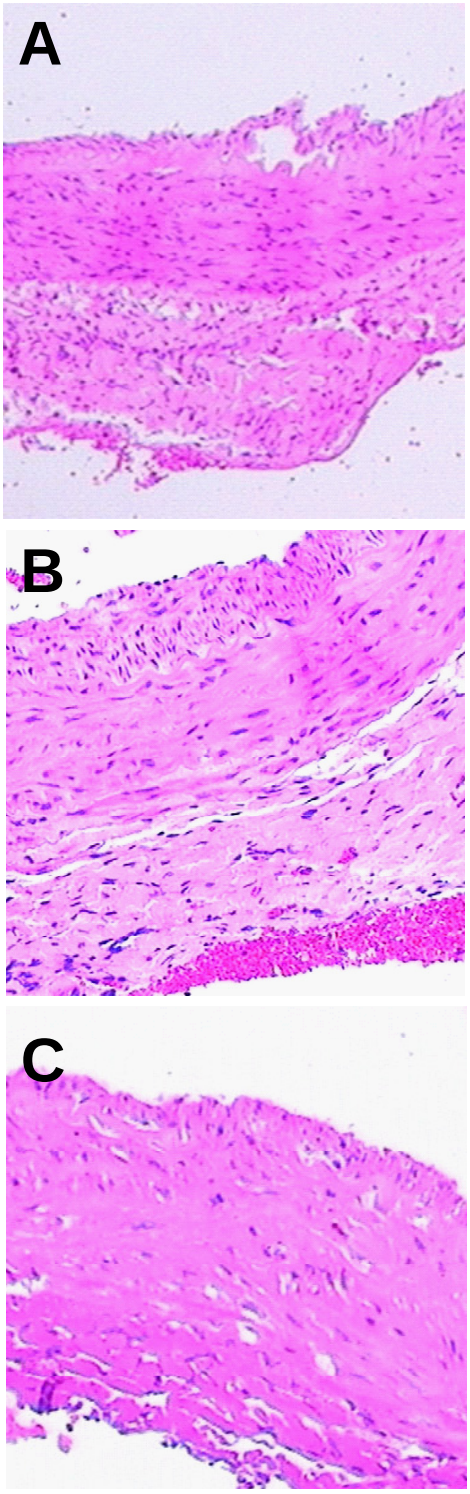

Figure 1. Histopathological changes in three groups of rats (magnification, × 200). (A) The AdTIMP2 group displayed better preservation of the medial layer; (B) The AdCMV group displayed hyperplasia of the intimal layer, destruction of the medial layer, and infiltration of inflammatory cells; (C) The PBS group displayed significant destruction of the medial layer and elastin fibers.

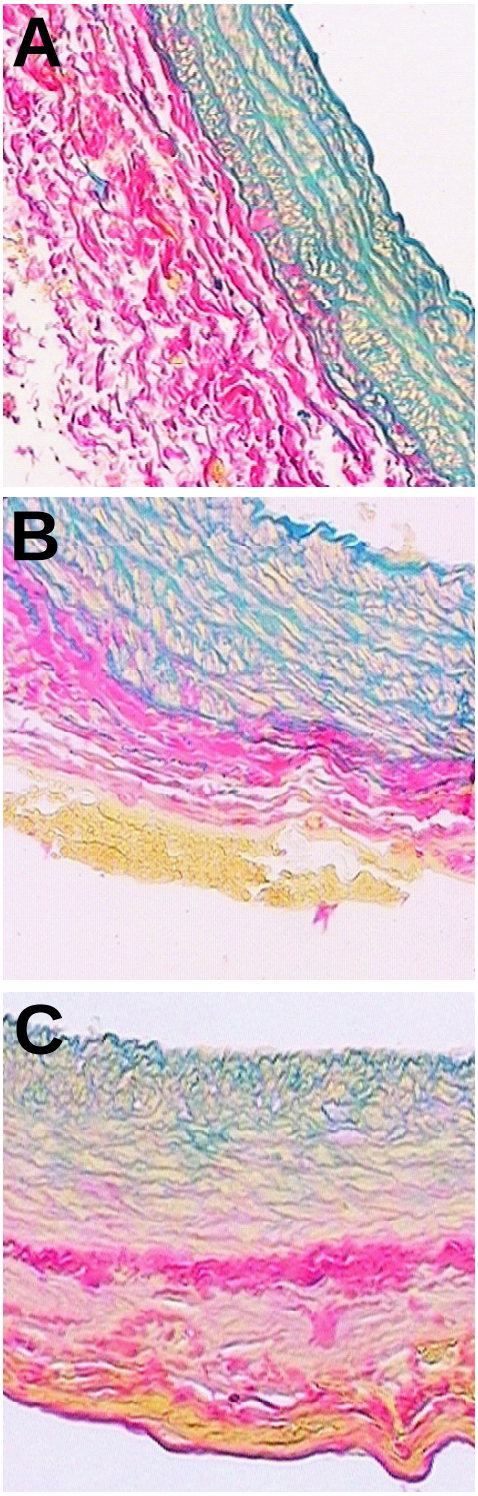

Figure 2. Verhoeff-van Gieson stain for elastin in three groups of rats (magnification, × 200). (A) The AdTIMP2 group displayed better preservation of elastin fibers and the medial layer; (B) The AdCMV group displayed significant destruction of elastin fibers and the medial layer; (C) The PBS group displayed disappearance of the medial layer and elastin fibers.

matrix metalloproteinases (MMPs) increased in the tissue of aneurysms and that their expression correlated with the inflammation of vessel walls, which can also be inhibited by tetracycline, thus inhibiting the formation of aneurysms (*4*). These results indicate that MMPs play an important role in the formation and progression of AAA in this rat model and provide valuable clues to the treatment of aneurysms. Presumably we can selectivity inhibit the MMPs activities in the vascular wall, the aneurysm can be cured fortunately.

Anidjar first reported creation of a rat AAA model by digesting the vessel wall with elastase (*3*). This model was similar to a human AAA in terms of histology and biology. Here, the TIMP-2 gene was delivered to the local area of vessel wall by gene transfer, consequently sustaining protein expression for a period of time. This can have a therapeutic effect and eliminate side effects in non-target organs.

Most adenovirus-mediated genes transferred to the vessel wall have delivery times of 20 min or longer or include 30 min incubation post-delivery from an infusion device (*5,6*). Regardless of the device used, most studies have delivered viral doses ranging from 1×10^9 to 1×10^{10} pfu/mL in delivery volumes of approximately 1 mL (*7*). Transduction efficacy can be improved by incubation of the vessel with a viral solution over an extended period of time. Although these conditions have been widely used, a lack of systematic study of delivery parameters suggests that delivery conditions were not necessarily optimal and that the clinical significance of such delivery is questionable. Here, a 10^9 pfu/mL viral solution was used and this concentration was found to have a therapeutic effect.

Impairment of ECM biosynthesis is thought to play a role in the pathogenesis of AAA because AAA is accompanied by a progressive decrease in the number of vascular smooth muscle cells that normally synthesize ECM (*8,9*). Disruption of elastin is sufficient for aneurysmal dilation of the aorta, and degradation of collagen is responsible for its rupture (*10,11*). Abundance studies have shown that MMPs were the main enzyme to digest elastin and collagen fibers of the vessel wall (*12,13*). Here, an adenovirus-mediated gene transfer of TIMP-2 to a local segment of rat aorta was utilized to block MMPs. The main components of ECM, elastin and collagen, were found to be preserved with greater integrity. Final results for TIMP-2 gene-transferred rats indicated a lower rate of AAA formation.

The current study also found that the inflammatory reaction was severe in the wall of artery although ECM degradation was inhibited. There was a great deal of inflammatory cell infiltration in the vessel wall, which suggests that other important factors may play roles in the formation of AAA. There may be two reasons for this: first, the adenovirus itself may be an immunogenic factor and stimulate an inflammatory reaction; second, vessels may be injured during model construction.

Other studies have found that mice deficient in MMP-9 and/or MMP-2 are protected from the development of AAA by infusion of elastase or $CaCl_2$ treatment of the aorta but are not protected from an inflammatory response (*12,13*). Doxycycline treatment inhibits the development of AAA experimentally, but it does not eliminate the inflammatory response (*14*), suggesting that the inflammatory response is maintained independently of MMP activity.

At present, the treatment of AAA relies on conventional surgery and there is no agent to inhibit the formation of AAA. This study has shown that adenovirus-mediated gene transfer of the TIMP-2 gene blocks degradation of ECM caused by proteinase, rebalanced the protein dissolution process and therefore preventing the formation of AAA, providing a new therapeutic strategy for the treatment of this condition.

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