

## Original Article

# Expression of tissue factor in rabbit pulmonary artery in an acute pulmonary embolism model

Jing-xia Zhang, Yong-li Chen, Yu-ling Zhou, Qian-yu Guo, Xian-pei Wang

Department of Cardiology, Tianjin Chest Hospital, Tianjin 300051, China

Corresponding Author: Jing-xia Zhang, Email: zhangjingxia001@126.com

**BACKGROUND:** Tissue factor (TF) is the initiation factor of the extrinsic coagulation pathway, and plays a critical role in the process of thrombosis. This study aimed to investigate the expression of TF and to explore their clinical effect on the pulmonary artery after acute pulmonary thromboembolism.

**METHODS:** Thirty-four Japanese white rabbits (Level II animals) supplied by Tianjin Medical University were randomly assigned into: group A, specimens of the pulmonary artery taken 3 hours after pulmonary embolism ( $n=8$ ); group B, specimens of the pulmonary artery taken 8 hours after pulmonary embolism ( $n=8$ ); group C, specimens of the pulmonary artery taken 24 hours after pulmonary embolism ( $n=8$ ); and control group, pseudo-operations performed without injection of autologous blood clots ( $n=10$ ). The animal model of pulmonary thrombo-embolism was established by injection of autologous blood clots into the jugular vein through a 5F catheter, and was confirmed by digital subtraction angiography. The mRNA expression of TF in different parts of the pulmonary artery was accessed by RT-PCR. The  $q$  test was used if there was a significant difference in a given continuous variable among the three groups assessed by ANOVA. The experiment equipment was supplied by the State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, the Chinese Academy of Medical Sciences and Peking Union Medical College.

**RESULTS:** The TF expression in the specimen adjacent to emboli was stable at 3, 8 or 24 hours after embolism. The mRNA expression of TF at 3 and 8 hours after embolism was lower in the specimens taken from the distal end of the morbid pulmonary artery than those adjacent to emboli. While at 24 hours after embolism, there were similar mRNA levels in specimens either adjacent or distal to emboli.

**CONCLUSION:** The high level of TF expression in pulmonary artery tissue adjacent to emboli could lead to locally increased coagulation activity, indicating the necessity of initiating anti-coagulation treatment as soon as possible after acute pulmonary embolism.

**KEY WORDS:** Pulmonary embolism; Tissue factor; Pulmonary; Gene expression; Thrombus; Anticoagulation; Thrombolysis; Rabbits

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## INTRODUCTION

Pulmonary embolism (PE) refers to the obstruction of the pulmonary artery or one of its branches by material (e.g., thrombus, tumor, air, or fat) that originated from elsewhere in the body. PE can be classified as acute or chronic. Patients with acute PE typically develop

symptoms and signs immediately after obstruction of pulmonary vessels. In contrast, patients with chronic PE tend to develop slowly progressive dyspnea over a period of years due to pulmonary hypertension. Acute PE is a common and often fatal disease, but its mortality can be reduced by prompt diagnosis and therapy because

the intrinsic fibrinolytic system in the pulmonary vascular bed can dissolve thrombus. However, in some patients, thrombus can't be completely dissolved even after treatment, and remains in the pulmonary vessel, leading to thromboembolus and chronic thromboembolic pulmonary hypertension. When acute PE occurs, the emboli strand in the pulmonary vasculature, which has been speculated to cause local hypercoagulation and in turn affects the outcome of emboli. Tissue factor (TF) is the initiation factor of the extrinsic coagulation pathway, and functions together with factor VII. Studies have shown that increased TF expression plays a critical role in the process of thrombosis. However, there are only few reports regarding pulmonary thromboembolism and TF expression. In the present study, based on the established animal models of acute PE, we aimed to detect the mRNA expression of TF, and to study the effects of local coagulation on the outcome of emboli.

## METHODS

### Experimental animals

Thirty-four male or female Japanese big ear white purebred rabbits (level II animals) were provided by Tianjin Medical University, weighing 2.0–2.5 kg each. They were randomly divided into: group A, specimens of the pulmonary artery taken 3 hours after pulmonary embolism ( $n=8$ ); group B, specimens of the pulmonary artery taken 8 hours after pulmonary embolism ( $n=8$ ); group C, specimens of the pulmonary artery taken 24 hours after pulmonary embolism ( $n=8$ ); and control group, pseudo-operations performed without injection of autologous blood clots ( $n=10$ ).

### Modeling pulmonary thromboembolism

Under sterile conditions, 2 mL blood was taken from the marginal ear vein of the rabbits. Twenty units of thrombin were added, and blood was kept at room temperature to form blood clots. Thromb at 2–4 mm in diameter and 7–10 mm in length were prepared from the clot. Using the sodium pentobarbital anesthesia (30 mg/kg, IV), the left jugular vein of the rabbit was isolated after local disinfection by iodine and alcohol, and a 5F catheter was used to inject the thrombus, which was followed by bolus injection of 5 mL saline to prevent local venous thrombosis. Each rabbit received the injection of two emboli, and pulmonary thromboembolism was confirmed by digital subtraction (Advantx, GE, USA). The control group did not receive thrombus injection, while other steps were the same as other groups.<sup>[1]</sup>

### Tissue sampling

Animals were sacrificed at 3, 8 or 24 hours after establishment of acute PE, and the lung tissues were removed and washed with saline. Dissection of the pulmonary artery revealed dilatation of the vessel at the embolization site and palpable intravascular emboli, freezer.

### RT-PCR method for detection of pulmonary tissue TF mRNA expression RNA extraction and purity test

Frozen tissue samples (from  $-80^{\circ}\text{C}$  freezer) were covered with TRIzol reagent (Invitrogen Company) and RNA was extracted in accordance with the manufacturer's instructions. M-MLV was provided by Invitrogen Company. The purity of the RNA was analyzed by OD260/OD280 ratio measured with ultraviolet spectrophotometry. RNAs with OD260/OD280 $>1.8$ , indicating the high purity of the RNA, were used in the following experiments. An aliquot of RNA underwent electrophoresis at 80–100 MV.

### Primer design and RT-PCR reaction

Frozen tissue samples (from  $-80^{\circ}\text{C}$  freezer) were covered with TRIzol reagent (Invitrogen Company) and RNA was extracted in accordance with the manufacturer's instructions. M-MLV was provided by Invitrogen Company. The purity of the RNA was analyzed by OD260/OD280 ratio measured with ultraviolet spectrophotometry. RNAs with OD260/OD280 $>1.8$ , indicating the high purity of the RNA, were used in the following experiments. An aliquot of RNA underwent electrophoresis at 80–100 MV.

According to the sequences of TF,  $\beta$ -actin cDNA, two pairs of primers were designed. Primers were synthesized by Shanghai Boya Biotechnology Company. TF upstream GGC AAG GAA CGG AAA CAC CA; downstream GCC GTA CCT GGA CAC AAA CC.  $\beta$ -actin upstream: AGG CCG TGC TGT CCC TGT AC; downstream: CTG GAA CAG CGC CTC GGG G. Cycling conditions:  $94^{\circ}\text{C}$  denaturation 5';  $94^{\circ}\text{C}$  45",  $60^{\circ}\text{C}$  30",  $72^{\circ}\text{C}$  1', 35 cycles;  $72^{\circ}\text{C}$  extends 7'. Products underwent 2% agarose gel electrophoresis containing 0.5  $\mu\text{g/mL}$  EB, and a gel imaging system was used to visualize and photograph the results. Using  $\beta$ -actin as the internal reference, TF expression was semi-quantitatively calculated by the computer system.

### Statistical analysis

Statistical software SPSS13.0 was used for statistical

analysis. Quantitative data were expressed as mean±SD. Multiple groups were compared by analysis of variance, and further by the paired *t* test. *P*<0.05 was considered as statistically significant.

## RESULTS

### RNA integrity

Electrophoresis showed 28 s and 18 s of RNA bands, and their rRNA fluorescence intensity ratio was 2:1, indicating that the RNA molecules maintained good integrity (Figure 1). In this experiment,  $\beta$ -actin was used as an internal reference, TF mRNA level was calculated as TF and  $\beta$ -actin ratio semi-quantitated by the Kodak digital image analysis software system. TF and  $\beta$ -actin RT-PCR products were separated by 2% agarose gel electrophoresis, their base pairs were 270 bp, 380 bp in length analyzed by the gel imaging analysis, consistent with the expected length, indicating the RT-PCR products were specifically amplified fragments.

### Tissue factor expression in pulmonary embolism

In this experiment, TF expression at the pulmonary embolization site didn't change significantly among the 3-, 8- and 24-hour groups. When compared to the embolization site and non-embolized artery (control group), TF expression at the distal end of the embolization was significantly lower (*P*<0.01) in 3 and 8 hour groups (group A: *q* value 6.429, 4.453; group B: *q* value 7.486, 8.120), and there was no statistical difference between the two groups (groups A and B, *q* values 2.323, 0.229, respectively). At 24 hours after the embolization, TF expression at the distal pulmonary

artery began to increase, and reached similar levels to the embolization site and the normal pulmonary artery (Table 1). The results of electrophoresis are shown in Figure 2.

## DISCUSSION

Distributed in the adventitia, organ capsule, skin and mucosa, etc, tissue factor is the initiating factor of the coagulation process. At the basal state, TF is expressed at a high level for normal hemostasis. Under normal circumstances, blood mononuclear cells and endothelial cells do not express TF.<sup>[2,3]</sup> However, under the atherosclerosis, septic shock and other pathological conditions, when vascular wall is damaged or endothelial cells are stimulated, endothelial cells can synthesize and express a large amount of TF that initiates the extrinsic coagulation pathway, causing intravascular thrombosis.<sup>[4]</sup> It is the main regulator of blood coagulation, hemostasis and thrombosis.

The lung is one of the organs that express the high levels of TF. Studies<sup>[5,6]</sup> have shown that TF mRNA expression can be rapidly induced in the lung under certain conditions. Armstead et al<sup>[7]</sup> established a mouse model of traumatic shock, and they found that 2 hours after trauma, lung tissue TF mRNA expression was significantly increased. TF has long been considered as a member of "immediate early genes" (early immediately gene), and its expression is rapidly induced in many diseases.<sup>[8,9]</sup> The present study showed that TF expression in the diseased state changes very quickly. After 3 hours of pulmonary embolism, TF mRNA expression was reduced at the distal pulmonary embolism artery, which extended to 8 hours. At 24 hours, TF expression at

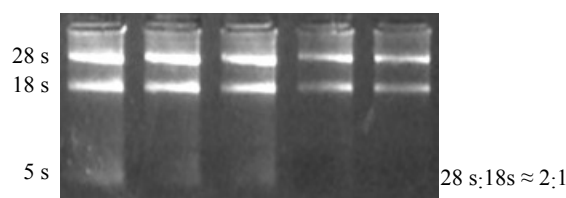


Figure 1. Electrophoresis of tissue RNA.

Table 1. Pulmonary TF mRNA expression levels

Groups	<i>n</i>	Pulmonary embolism	Distal pulmonary embolism	Normal pulmonary	<i>F</i>
A	8	1.10±0.21	0.40±0.29	—	10.72*
B	8	0.84±0.22	0.15±0.05	—	20.04*
C	8	0.72±0.55	1.15±0.27	—	2.14
D	10	—	—	0.86±0.34	

Compared with the control group, \**P*<0.01.

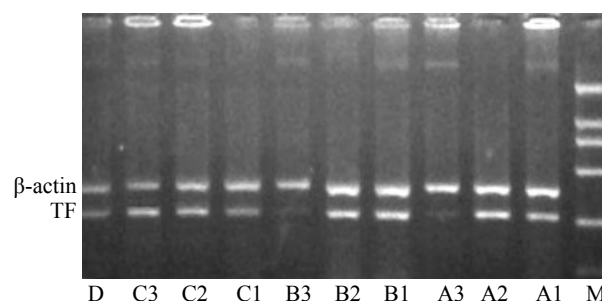


Figure 2. Pulmonary tissue factor (TF) RT-PCR products electrophoresis. A1: embolization 3-hour group—pulmonary embolism site; A2: experimental control group; A3: embolization 3-hour group—distal embolism pulmonary artery; B1: embolization 8-hour group—pulmonary embolism site; B2: experimental control group; B3: embolization 8-hour group—distal embolism pulmonary artery; C1: embolization 24-hour group—pulmonary embolism site; C2: experimental control group; C3: embolization 24-hour group—distal embolism pulmonary artery; D: experimental control group; M: DNA marker.

distal pulmonary embolism began to rise, reaching a level consistent with that of the pulmonary embolism site.

In this experiment, the expression of TF at the pulmonary embolism site was not significantly different from the normal pulmonary artery. But we found that at 3 and 8 hours after embolization, TF expression of the distal pulmonary embolism was significantly lower than that of the normal pulmonary artery and pulmonary embolism site. Measured by the RT-PCR method, TF expression was very low in some areas of the distal pulmonary embolism artery. One possible explanation is that body's self-protection mechanism was stimulated by the early stage of pulmonary thromboembolism to prevent thrombosis and further extension of the embolization to distal areas and to non-embolized areas. However, we also found that the expression of TF didn't drop at the embolization site, causing a local hypercoagulatory state that is not helpful for dissolving the thrombus.

Yildiz et al<sup>[10]</sup> reported that that 80% of deaths due to acute pulmonary embolism occur within 2 hours after the onset, so rapid treatment is crucial for patients. Thrombolysis and anticoagulation are the key treatments. Currently, thrombolytic therapy is the main treatment for fresh thrombus embolism within two weeks, with the greatest benefit being treatment within 48 hours after onset.<sup>[11,12]</sup> Recently, pharmacomechanical thrombolysis, as an emerging treatment option for symptomatic deep vein thrombosis, has been widely used.<sup>[13]</sup> Cuculi et al<sup>[14]</sup> reported the results of a novel, pharmacomechanical approach with prolonged infusion of urokinase in the occluded pulmonary arteries, and in 59% patients there was a reduction of thrombotic burden by >90%, and in 29% patients the reduction was 50% to 90%. Anticoagulant therapy administered immediately after pulmonary embolism has a clear therapeutic advantage. A study<sup>[15]</sup> confirmed that the mortality of patients was significantly reduced by early anticoagulant therapy during acute pulmonary embolism. Therefore, thrombolytic and anticoagulant therapies are effective treatments.

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**Ethical approval:** The study was approved by the Animal Care and Use Committee of Tianjin Chest Hospital, Tianjin, China.

**Conflicts of interest:** The authors declare that there is no conflict of interest.

**Contributors:** Zhang JX proposed the study, analyzed the data and wrote the first draft. All authors contributed to the design and interpretation of the study and to further drafts.

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