

Atherosclerosis and acute arterial thrombosis in rabbits: a model using balloon desendothelization without dietary intervention

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Abstract

Acute thrombosis can be induced in rabbits by a triggering protocol using Russell's viper venom and histamine given after 8 months of a 1% cholesterol diet and balloon desendothelization. In the present study, we tested the hypothesis that aortic desendothelization performed 4 months before the triggering protocol without a high cholesterol diet is a highly effective and less expensive way of producing arterial atherosclerosis and thrombosis. Nineteen male New Zealand white rabbits on a normal diet were studied. The control group (N = 9) received no intervention during the 4-month observation period, while the other group (N = 10) was submitted to aortic balloon desendothelization using a 4F Fogarty catheter. At the end of this period, all animals were killed 48 h after receiving the first dose of the triggering treatment. Eight of 10 rabbits (80%) in the balloon-trauma group presented platelet-rich arterial thrombosis while none of the animals in the control group had thrombus formation (P<0.01). Thus, this model, using balloon desendothelization without dietary manipulation, induces arterial atherosclerosis and thrombosis and may provide possibilities to test new therapeutic approaches.

Key words

- Endothelium
- Histamine
- Russell's viper venom
- Coronary artery disease

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Plaque disruption and subsequent arterial thrombosis are currently considered to be the main mechanisms of acute ischemic coronary syndromes (1), and an animal model is needed for the study of these phenomena. In the sixties, Constantinides and Chakravarti (2) developed a model in which rabbits were fed a high cholesterol diet for 8 months to produce atherosclerosis, after which plaque disruption and thrombosis were triggered by intraperitoneal injection of Russell's viper venom, a procoagulant and endothelial toxin, followed by the intravenous injection of his-

tamine, which has a vasopressor effect in rabbits. Using this protocol, the rabbit aorta showed disrupted atherosclerotic plaques with overlying thrombi. Recently, Abela et al. (3) reproduced this model and were able to increase the *in vivo* proportion of thrombosis after 8 months by adding arterial balloon desendothelization to the high-cholesterol diet intervention. In the present study, we tested the hypothesis that aortic desendothelization performed only 4 months before the triggering protocol without a high cholesterol diet is an effective and less expensive way of pro-

ducing arterial atherosclerosis and thrombosis in the rabbit model.

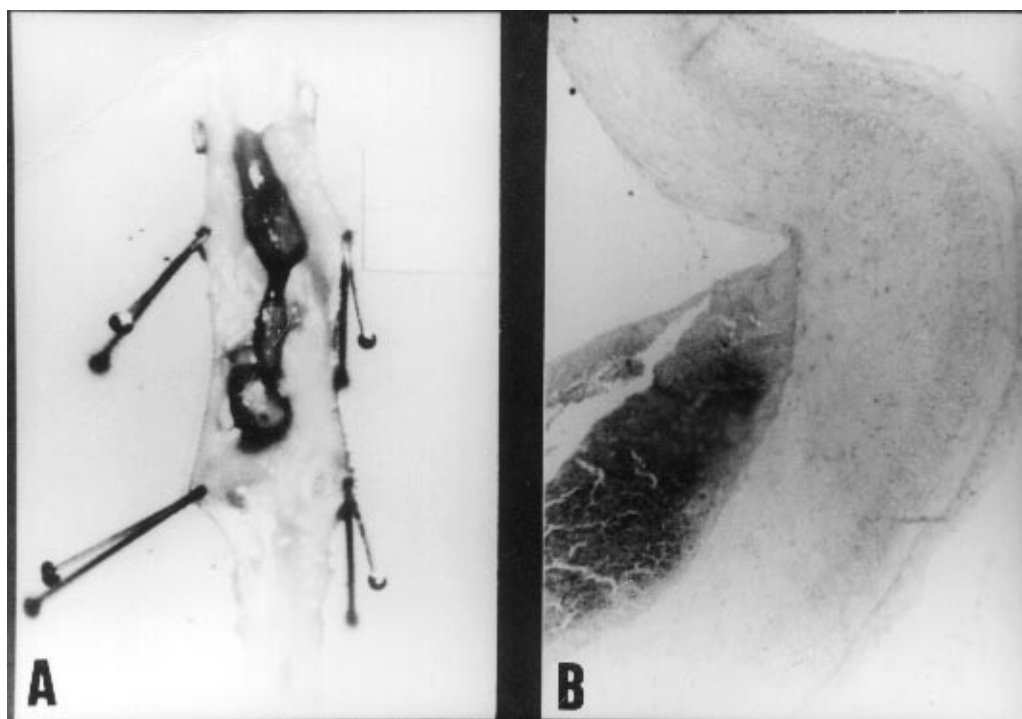
Nineteen male New Zealand white rabbits weighing 2 to 3 kg were assigned to 2 groups. Both groups received a regular diet for 4 months. Rabbits in the intervention group (N = 10) underwent balloon-induced arterial injury of the aorta using a 4F Fogarty catheter as previously described (3). The control group (N = 9) received no intervention during the observation period. After 4 months, all rabbits were killed 48 h after receiving the triggering protocol according to Constantinides and Chakravarti (2). Briefly, Russell's viper venom (0.15 mg/kg) was injected intraperitoneally, followed by intravenous administration of histamine (20 µg/kg) 48 and 24 h before the rabbits were killed with an overdose of intravenous pentobarbital. The aorta and ileofemoral arteries were excised and dissected, and the intimal surface was exposed by an anterior longitudinal incision of the vessel.

Upon macroscopic evaluation of the aorta, the number of animals who presented arterial thrombosis was recorded. The mean area of

thrombi was measured by videoplanimetry using a videocamera interfaced with a Macintosh computer with a color image processing system (NIH Image). Reliability analysis among 3 independent investigators resulted in a Kendall coefficient of concordance of 0.92 for the thrombi measured by videoplanimetry. Segments of the aortic arch and thoracic and abdominal aorta which presented thrombosis were also analyzed by light microscopy. To quantify histological abnormalities, a pathologist who was unaware of the study groups graded the lesions from 0 to 4 according to the extent of intimal thickening, fibrosis, and medial rupture (4). Segments of the aortic arch, the thoracic and abdominal aortas, and the iliac artery were evaluated. A general histological score (range 0 to 32) was computed as the sum of all scores for each animal.

The sample size of 18 animals (9 for each group) was used to detect a difference of 50% in the incidence of thrombosis (P alpha = 0.05; power = 80%). Data are reported as mean ± SD. The number of animals presenting thrombosis in the two groups was com-

Figure 1 - A, Photograph of a representative aortic segment showing a white thrombus in the abdominal aorta. The aortic wall is thick and has lost its normal transparency. There are also 2 thrombi connected by a red fibrin and platelet-rich "bridge". B, Histology specimen (H&E) showing injured artery and thrombus.



pared using Fisher's exact test. The thrombus surface area was compared by the Wilcoxon test. The association between thrombus surface area and histological abnormality score was evaluated by Spearman rank correlation.

None of the animals died during the period of observation, and only one developed wound infection. In the balloon-trauma group, atherosclerotic lesions, fibrotic and calcified, with a large number of foamy smooth muscle cells were seen in aortic and iliac segments. In some areas, the endothelium appeared normal with fibrous thickening of the intimal layer. The histological quantification of the extent of injury resulted in a significantly ($P < 0.01$) higher mean score of 12 ± 10 for the balloon-trauma group compared to a mean score of 0.8 ± 9 for the control group. Eight of 10 rabbits (80%) in the balloon-trauma group demonstrated platelet-rich arterial thrombosis while none of the 9 animals in the control group had thrombus formation ($P < 0.01$) (Figure 1). Vascular thrombosis was seen in different stages of development. The surface area of white thrombi (mean of 3 measurements) ranged from 3 to 489 mm², averaging 194 ± 182 mm². When stained with hematoxylin-eosin, thrombi were found to be composed of white (platelets and fibrin) and red areas. A strong Spearman rank correlation coefficient of 0.82 ($P < 0.01$) was found between the histological score of arterial injury and thrombus area.

The exact mechanisms triggering arterial thrombosis are still under investigation. Most likely, endothelial injury plays a central role in this process. An animal model of *in vivo*

arterial thrombosis without acute manipulation of the vessel wall can be useful to study the process of thrombus formation and related acute ischemic events. The model presented here produced rates of thrombosis similar to those of the study of Abela et al. (3). The rabbit model of balloon desendothelization produced a large number of thrombi with minimal surgery-related complications. The proportion of animals presenting thrombosis was 80% in the balloon-trauma group, suggesting the possible participation of endothelial injury in the thrombosis cascade. Endothelial dysfunction related to the degree of arterial injury may still be present 4 weeks after catheter desendothelization, despite complete reendothelization of the injured aorta (5). In the present model, even 4 months after the mechanical lesion, the endothelium was still thrombogenic independent of the chemical (cholesterol) injury. Moreover, a strong correlation was found between the degree of histological injury and the thrombus area, suggesting that the model may be useful to test therapeutic interventions in a controlled environment.

In conclusion, this inexpensive *in vivo* model of arterial atherosclerosis and acute platelet-rich arterial thrombosis may offer a variety of possibilities to study the thrombotic process and ways of interfering with it. However, the fact that thrombus size was not uniform within the animals submitted to the experimental protocol may result in a model with little sensitivity to detect the effect of subtle therapeutic interventions.

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