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Original Article

An animal model for venous thrombosis and spontaneous pulmonary embolism

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Study design: An animal model.
Objective: To test the natural sequence of venous thrombosis and pulmonary thromboembolism experimentally.
Setting: Veterans Administration Hospital, USA.
Method: In dogs, a venous thrombosis was induced in a isolated segment of the internal jugular vein by a 5 min exposure to sodium morrhuate and then re-establishing venous patency. A tracer, ^{125}I human fibrinogen, was administered through another vein 1 h prior to the end of each experiment when a blood sample, the venous thrombus, and the lungs were removed. Thrombi were described by age, weight, histology, and fibrin uptake (thrombus to blood radioactivity ratio, g/g). Pulmonary emboli (PE) were identified by autoradiography of lung slices or by microscopic examination of lung sections.
Results: Venous thrombosis developed in all experiments, duration 1–64, median 5 h ($n=12$). Histologically, younger thrombi were characterized by platelet aggregates surrounded by polymorphonuclear leukocytes (PMN), and uniform fibrin deposit; the older thrombi by platelet ghost cells, fewer PMN leukocytes, and broken fibrin strands and loops ($n=6$). Pulmonary thromboemboli were imaged as 'hot spots' in six of six experiments in which lung slices were autoradiographed and were identified microscopically in six of six experiments in which lung sections were taken. The number of PE diagnosed microscopically did not correlate with the age of the corresponding thrombus but was directly related to fibrin uptake ($n=5$, $r=0.99$, $P<0.01$).
Conclusion: An animal model for venous thrombosis that generates pulmonary thromboembolism has been described.
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Keywords: thromboembolism; pulmonary embolism; pulmonary infarction; venous thrombosis; experimental animal

Introduction

Animal models of venous thrombosis (VT) and pulmonary thromboembolism (PE) have been limited to one or the other of these events, failing to reproduce the sequence of both VT and PE.[1,2] Thus, the clinical impression that VT quickly embolizes,[3,4] and most dangerously embolizes at its onset[5,6] has not been testable by current animal preparations. The only reported comparison of anticoagulation for recurrent PE against untreated PE patients[7] cannot be replicated experimentally for lack of a suitable model. The following is a report of an animal model for VT that generates PE.

Methods

Mongrel dogs were anesthetized and sustained with 6.5% pentobarbital, given intravenously in total doses of 0.3–1.3 cm^3/kg body weight. The animals were intubated and maintained on a mechanical respirator for the duration of the procedure. The right jugular vein was exposed by dissection and a segment of approximately 3 cm in length was temporarily isolated from circulation with ligatures held in place with mosquito clamps. Sodium morrhuate was injected through a fine needle to distend the segment. After 5 min

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of exposure, the vein segment became discolored, the sodium morrhuate was aspirated, the ligatures were removed, and the flow of blood through the venous segment re-established. At intervals of 1–64 h after thrombus induction, the animals were killed with intravenous pentobarbital at doses of approximately 2 cm^3/kg. In the longer experiments, the animals were allowed to regain consciousness before being killed.

Radioiodinated human fibrinogen tracers were made and stored frozen prior to the animal experiments.[8] The animals were administered a dose of ^{125}I-fibrinogen (80–200 μCi) through the contralateral neck vein 1 or 4 h before the experiment was ended. Immediately prior to being killed, blood was collected into glass vacuum tubes with ethylene diamine tetraacetic acid anticoagulant. After being killed, the venous thrombus and the lungs were removed, and the thrombi were weighed. Radioactivity of the thrombus and 1 ml of blood sample was measured in a deep well scintillation counter. The counts were expressed per gram of tissue weight, and the ratio of thrombus to blood radioactivity was calculated as a measure of fibrin uptake by the thrombus.

Thrombi and lung sections were fixed in 10% formalin. These were embedded in paraffin, blocks were cut into 4 μm sections and slides made, stained in hematoxylin and eosin (H&E) or para-amino tungstic acid hematoxylin (PTAH). Microscopic descriptions of the thrombi were made with respect to platelets, polymorphonuclear (PMN) leukocytes, mononuclear cells, and erythrocytes (RBC) on H&E- and fibrin on PTAH-stained slides. Microscopic descriptions of the lung sections were made with respect to the presence or absence of pulmonary emboli and pulmonary infarction. An embolus was designated if a pulmonary artery contained more than one collection of white cells embedded in a red cell mass or if flow lines in the cell mass could be detected. The number of emboli on each section was counted by two examiners, one of whom (JH) did not know the age of the corresponding thrombus. Parenchymal infiltration with red cells or white cells or both were noted. Bronchial content was also described.

In addition, fixed lower lobes or large section of lungs were sliced to expose the greatest surface, covered with thin plastic and placed on Type 57 Polaroid photographic film, and autoradiographed over periods of time optimized by trial and error. The number of 'hot spots' was counted for each preparation. Similarly, a few thrombi were autoradiographed. These experiments were approved by the research and animal studies committees of this institution.

Statistical comparisons were carried out for thrombus age to thrombus weight, thrombus age to fibrin uptake, and the average number of microscopic pulmonary emboli on lung sections to fibrin uptake. These comparisons were analyzed by the linear regression and correlation method. The software Primer of Biostatistics was used.[9]

Results

Experimental thrombi

In all, 12 experiments were carried out. The thrombosis preparations are summarized in Table 1. On microscopic inspection of longitudinal thrombus sections, the older thrombi were disintegrated in the center. Nevertheless, there was no clear correlation between the age and the weight of the thrombus ($n=12$, $r=-0.18$, $P=0.57$) or between age and fibrin uptake ($n=9$, $r=0.50$, $P=0.17$). The continuing but irregularity of fibrin uptake in an older thrombus is demonstrated by autoradiography in Figure 1.

Thrombus histology was described in six preparations, Table 2. The 1-h thrombus was characterized by large aggregates of platelets rimmed by PMN leukocytes, Figure 2. Fibrin was largely diffuse for the younger thrombi, very few fibers being discernable, even at the highest power of the light microscope. In contrast, the 64-h thrombus featured smaller aggregates of platelets, mostly transformed into ghost cells, fewer PMN leukocytes, and some mononuclear cells. Fibrin distribution had consolidated into fibrin bands and loops with numerous breaks in integrity (see Figure 3).

Pulmonary thromboemboli

Lower lobe slices were autoradiographed in eight experiments. Concentrations of radioactivity were found in all specimens, the average number of 'hot spots' per slice ranging from 2 to 12, median 5, for these experiments (see Figure 4). There was no correlation between the thrombus age and the number of emboli imaged ($r=0.07$, $P=0.86$).

Lung sections taken for microscopic examination ranged in number from 2 to 4, median 4, for six animals. Pulmonary emboli were found in each of these experiments by at least one of the two examiners. However, although the greatest number of emboli emanated from the youngest thrombus, the number normalized per section could not be correlated with the age of the thrombus. On the other hand, PE could be directly related to thrombus activity, expressed as fibrin uptake (see Table 3).

Microscopic examination of these sections also revealed small to massive pulmonary infarctions. The cell types infiltrating lung tissue were RBC, PMN, mononuclear cells, or mixtures of these. Some older preparations showed perivascular cuffing with mono-

Table 1 Characteristics of experimental thrombosis

	N	*Mean*	*SD*
Animal weight (kg)	11	15.1	5.1
Thrombus age (h)	11	14.1	20.2
Thrombus weight (g)	11	0.46	0.31
Thrombus/blood radioactivity	9	1.58	1.31

nuclear cells. The number of infarctions exceeded the number of PE in those experiments with the fewest PE. Bronchial accumulations were found in all preparations and reflected the surrounding infiltrations. Figure 5 shows a pulmonary embolus, associated pulmonary infiltrations, and bronchial secretions.

Discussion

VT has been created in dogs by inducing a localized phlebitis with sodium morrhuate. Histologically, these experimental thrombi look like thrombi taken from human subjects. The 1-h experiment reproduced the platelet accumulation that serves as a nidus for a thrombotic process and the repetitive accumulations

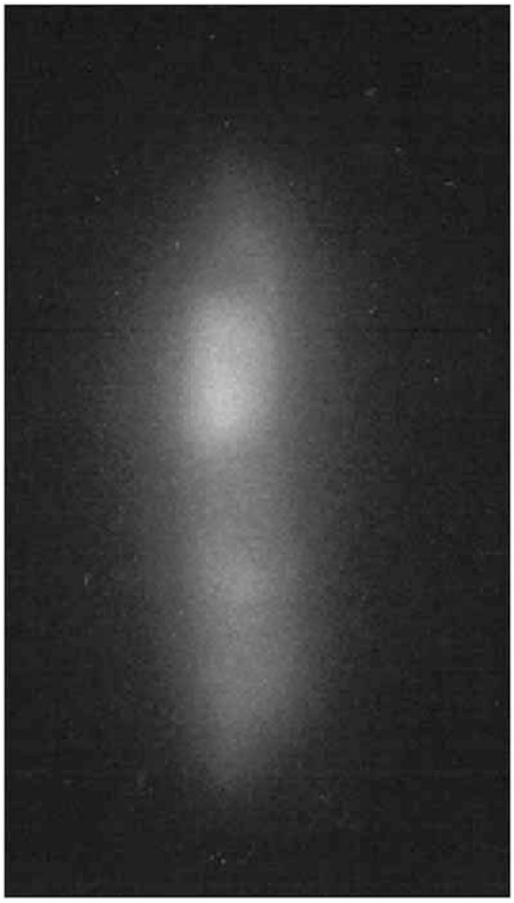

Figure 1 Gross autoradiography of a 24-h thrombus, showing linear sections of high and low radioactivities. The thrombus was measured 3 cm in length

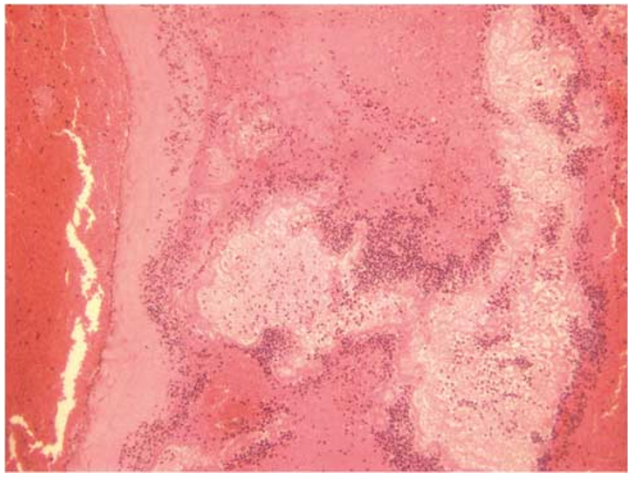

Figure 2 Histology of a 1-h thrombus, showing a platelet aggregation rimmed with PMN. H&E, original magnification $\times 100$

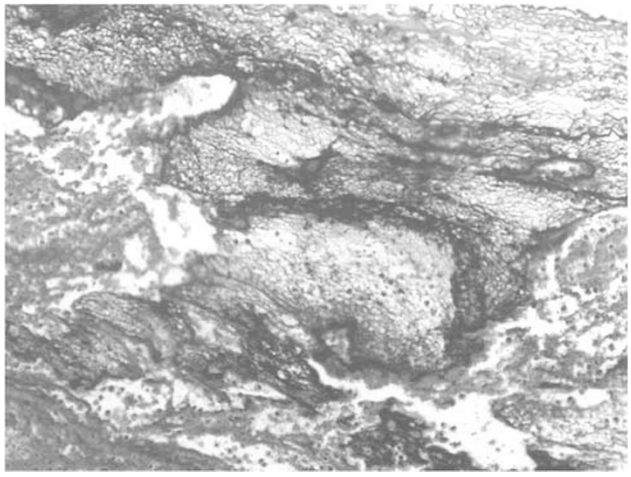

Figure 3 Histology of a 64-h thrombus showing fibrin stranding with loops around old platelet aggregates and breaks in the fibrin strands. PTAH, original magnification $\times 200$

Table 2 Histology of experimental thrombi

Age (h)	*Weight (g)*	*N*	*Cellular distribution (H&E stain)*	*Fibrin distribution (PTAH stain)*
1	0.73	1	Many platelet aggregates lined with PMN	Diffuse
4	1.03	2	Many platelet aggregates lined with PMN	Diffuse, loops
16	0.22, 0.65	2	Ghost aggregates of platelets with PMN	Loops
64	0.70	1	Smaller ghost aggregates, few PMN	Loops, strands and breaks

PTAH = phosphotungstic acid hematoxylin, PMN = polymorphonuclear leukocytes; loops = fibrin stain outlining platelet aggregates; strands = longitudinal strings of fibrin; breaks = discontinuity of strands and loops with ragged ends

Figure 4 Gross autoradiography of a lung lobe showing scattered foci of radioactivity 1 h after thrombus induction and simultaneous administration of radioactive fibrinogen. A blush of radioactivity extends from an embolus on the left side of this image, suggesting pulmonary infarction. The length of this lobe was 7 cm

Table 3 Comparison of thrombus age and thrombus activity with thromboembolism

Thrombus age (h)	*Thrombus to blood fibrin ratio (fibrin uptake)*	*Thromboembolism of the lungs (PE) (average number of emboli per lung section)*	
		Examiner 1	*Examiner 2*
1	1.8	6.0	3.5
4	0.18	0	0.3
4		0.5	0.5
16	1.27	4.8	0.8
16	0.21	0.5	0
64	0.12	0.5	0.3

Thrombus age *versus* thrombus to blood ratio; $n=5$, $r=-0.21$, $P=0.69$
Thrombus age *versus* PE: $n=6$, $r=-0.29$, $P=0.57$ (Examiner 1); $r=-0.37$, $P=0.46$ (Examiner 2)
Fibrin uptake *versus* PE: $n=5$, $r=0.99$, $P<0.01$ (Examiner 1); $r=0.88$, $P=0.05$ (Examiner 2)

that lead to the growth of the thrombus. It showed that PMN leukocytes were concentrated around the platelet accumulations (microscopic lines of Zahn). An accumulation of RBC made up the bulk of the thrombus. Fibrin ran throughout the thrombus with a morphology too small to be resolved by light microscopy. The older thrombi revealed cellular degeneration and a change in fibrin morphology to interrupted longitudinal strands and loops. This picture suggests that fibrin becomes consolidated and partially degraded with the maturation of the thrombus. In fact, the replacement of degraded fibrin as indicated by the uptake of fibrin was a better predictor of PE than was aging of the thrombus.

PE was found in all experiments, confirming its nature to recur. Although there were no control studies of the autoradiographed lung tissue, the shape of the 'hot spots' imaged suggested PE rather than pneumonia. (Fibrin tracer will detect inflammation as well as thrombosis,

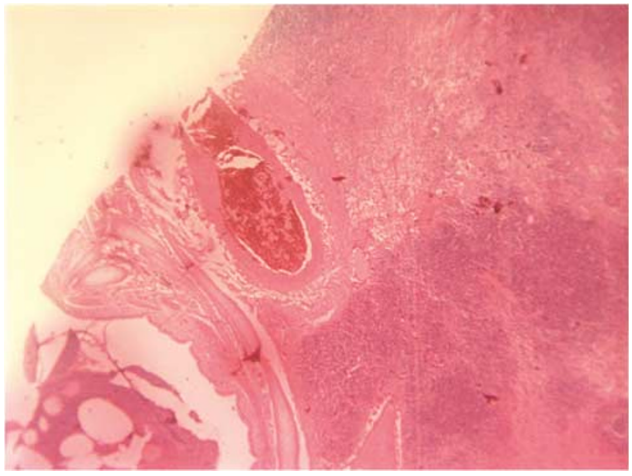

Figure 5 Microscopic view of a lung section with a pulmonary embolus (left center), hemorrhagic (right upper) and white cell (right lower) infiltrations, and pyogenic bronchial secretions (left lower). H&E, original magnification $\times 20$

since both processes accumulate fibrin.) Although interpretation varied between examiners of the microscopic sections – Examiner 1 generally seeing more PE than Examiner 2 – PE was found in all experiments and the trend of increasing PE with increasing fibrin uptake by the underlying thrombus was noted by both examiners. This trend, independent of thrombus age, was not known to either examiner, except in retrospect.

Pulmonary infarctions were prevalent in all experiments. All cellular infiltrates – whether predominately RBC, PMN leukocytes, or mononuclear cells – were interpreted as pulmonary infarctions. The coincidence of bronchopneumonia and pulmonary embolism could not be excluded. However, pulmonary infiltration with PMN leukocytes, monocytes, and macrophages has been described in experimental animals with PE.[10] Fibrin breakdown products, which are elevated in PE, are chemoattractants for PMN leukocytes.[11–13] Lung tissue ischemia elicits an inflammatory response that may not be hemorrhagic. The number of infarcted areas far exceeded the number of PE when PE were scarce, suggesting residua of earlier PE since resolved.

Physiologically, the induced VT simulated clinical thromboembolic disease because thromboembolism occurred spontaneously. Previous experimental models have artificially embolized thrombi.[1,2] The order of events in this model confirms the clinical impressions, suggesting that an ongoing thrombotic process, as indicated by greater fibrin uptake, generates more emboli than and a maturing one, one with less fibrin uptake. Fibrinolytic activity of a thrombotic process is probably continuous with thrombosis and related to entrapped plasminogen and PMN leukocytes.[14] Consequently, fibrinolytic activity causes thrombus fragmentation and generates thromboembolism. The continued embolism, the changing histology, and the 10-fold difference in fibrin uptake (high embolic *versus* low

embolic cases) observed over the 64 h span of thrombus ages indicate evolving thrombus metabolism. As the thrombus forms, its bonds are being lysed and thrombus fragments are embolizing, but at the same time the thrombus is 'repairing' with continuing fibrin uptake. The biological age of this process is not predicted by its chronological age. It is suggested, however, that PE closely reflects thrombotic activity or turnover. The effectiveness of anticoagulation, occasionally called into question,[15] would depend upon the extent of thrombus activity, which is variable.

The time frame of 64 h in this dog model spanned thrombus growth and embolism and thrombus resolution, for the most part. It is unlikely that this biological lifespan represents that of thrombotic events in human subjects, however. As an indication of a difference in thrombotic activity, it can be noted that the physiological survival of fibrinogen in dogs is much shorter than that in humans. The respective biological half-lives have been reported as 2.5 and 4.0 days.[16,17]

Conclusion

In an animal model, VT and PE are closely related in onset and activity. There is a rapid peaking and tapering of thrombosis and thromboembolism in concert over a 64-h observation period.

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