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THROMBUS GROWTH UNDER THE INFLUENCE OF WARFARIN AND AFTER ABRUPT REVERSAL OF ITS EFFECTS*

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Recent evidence has suggested that mild depression of prothrombin activity may encourage thrombus formation,[1] and it has long been held that a similar effect due to "rebound" hypercoagulability occurs after sudden withdrawal of anticoagulant therapy.[2-5] This report assesses the effect of varying degrees of prothrombin depression and abrupt correction of hypoprothrombinemia with large doses of vitamin K_1 on thrombus growth *in vivo*.

MATERIALS AND METHODS

Studies were performed on adult New Zealand white rabbits ranging in weight from 3.5 to 6.0 kg and subsisting on Rockland Rabbit Ration. Warfarin sodium in the form of Coumadin and vitamin K_1 in the form of Aqua-Mephyton were utilized.

Thrombi were initiated in the ligated common carotid artery of the rabbit by applying a direct current of 2.5 mamp for 30 min through platinum electrodes (3 by 7 mm) placed central to the point of occlusion of the vessel. Thrombi were removed and weighed after growth had been allowed to continue for 6.5 hr in the obstructed vessel. Details of this method have been described elsewhere.[6]

Prothrombin activity was measured by a one-stage technique[7] utilizing Acuplastin as the source of thromboplastin. Control curves were constructed with pooled normal rabbit plasma, utilizing aluminum hydroxide adsorbed rabbit plasma as the diluent.

EXPERIMENTS

Effect of warfarin. After base line prothrombin determination and measurement of thrombus weight in one carotid artery, animals were injected with warfarin at 2- to 3-day intervals to maintain prothrombin depression. Depend-

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ing on the intensity of effect desired, and individual susceptibility, dosage ranged from 13 to 20 mg per kg per week. Thirty animals were treated for 10 to 14 days and seven were treated for 30 days. These differences in duration of therapy were chosen because Ashwin[8] had observed greater inhibition of thrombosis in rodents treated for 30 days than in those treated for a shorter time. At the end of treatment, thrombosis was produced in the second carotid artery, thrombus weight was obtained, and a final determination of prothrombin activity was carried out.

Reversal of warfarin effect with vitamin K_1. Warfarin was administered intramuscularly to 18 rabbits at 2- to 3-day intervals for 2 weeks (15 to 20 mg per kg per week) and then each animal was injected with 50 mg of vitamin K_1. Thrombus growth was measured in the first carotid artery 48 hr after K_1 in nine animals and 5 days after K_1 in the other nine rabbits. The control thrombus was produced in the second carotid artery 7 days after the initial study in both groups. Prothrombin time was determined before the first warfarin injection, before K_1 injection, and on the day of measurement of thrombus growth in the first carotid artery.

RESULTS

Effect of warfarin. Results were grouped according to prothrombin concentration at the time of thrombogenesis in the second carotid artery and compared to findings in a previously reported[6] group of control animals. Since

TABLE 1*

Effect of warfarin on thrombus growth

Group	Prothrombin Activity	*N*	Mean Thrombus Weight		Change in Thrombus Weight	Significance of Per Cent Change as Compared to No Warfarin Group (*p*)
			1st carotid	2nd carotid		
	%		*mg*		*mean % + S.E.M. and range*	
No warfarin†	100	19	42.8	51.8	+23.9 ± 9.6 (+)157 to (−)12	
Warfarin	>30	18	42.3	45.3	+10.1 ± 6.5 (+)60 to (−)36	>0.1
Warfarin	15–30	13	37.5	41.3	+10.9 ± 10.3 (+)64 to (−)65	>0.1
Warfarin	<15	6	37.7	28.8	−20.3 ± 15.8 (+)45 to (−)47	<0.01

* The influence of varying degrees of prothrombin depression on thrombus growth is documented here. Of note is the lack of evidence for hypercoagulability in the >30 per cent group.

† First and second carotid experiments separated by 7 to 36 days.

TABLE 2*

Effect of abrupt correction of decreased prothombin activity on thrombus growth

Group	N	Mean Thrombus Weight		Change in Thrombus Weight	Mean Prothrombin Time		
		1st carotid	2nd carotid		Control	Warfarin	K_1
		mg		*mean % ± S.E.M.*	*sec*		
No warfarin†	20	42.6	51.7	+22.0 ± 5.7	11.5		
48 hr after K_1	9	46.0	56.6	+22.9 ± 7.1	11.8	19.5	12.1
5 days after K_1	9	33.8	41.3	+27.3 ± 17.3	11.9	22.8	12.0

* After K_1 administration, thrombi are not relatively heavier than in the control group. If they were, then there would have been little difference between first and second carotid thrombus weights in the K_1 group.

† First and second carotid experiments separated by 24 hr.

thrombi in any given prothrombin range were not smaller in those animals treated for 30 rather than 10 to 14 days, all 37 treated animals were considered together. As can be seen in table 1, thrombi in the second carotid artery of untreated animals were heavier than those in the first carotid (mean increase in thrombus weight +23.9 per cent with first and second carotid studies separated by 7 to 36 days). In treated animals with prothrombin concentrations in the 15 to 30 per cent or >30 per cent ranges, weights of second carotid thrombi were also greater than first (+10.9 and +10.1 per cent, respectively). While these changes were not as great as in untreated animals, the differences between controls and either treatment group were not statistically significant ($p > 0.1$). In animals with prothrombin concentration <15 per cent, however, second carotid thrombi were significantly decreased in weight (mean change, −20.3 per cent; $p < 0.01$).

Reversal of warfarin effect with vitamin K_1. Thrombus growth in the two groups of rabbits treated with vitamin K_1 was compared to that in control animals. If a thrombophilic state were induced by K_1, thrombi in the first carotid artery should have been relatively heavier than normal, thereby diminishing or obliterating the usual difference between second and first carotid weights. As can be seen in table 2, results in both groups of K_1-treated animals were the same as those observed in controls.* There was no suggestion of decrease in the mean per cent change in thrombus weight.

DISCUSSION

In our study of the effect of warfarin on thrombus growth the primary objective was to collect evidence bearing on the suggestion that "low dose" ther-

* Data presented here are based on findings in a group of normal rabbits with first and second carotid thrombi produced on consecutive days. It is felt that this is a valid control group since it has been demonstrated that second carotid thrombi average about 20 per cent heavier regardless of the interval separating them from first thrombi (see reference 6, and also table 1 in this paper).

apy enhances thrombus formation. Accordingly, more animals were maintained with minor prothrombin depression than in other groups. Yet, no evidence of thrombophilia appeared under these circumstances. In addition we observed no significant inhibition of thrombus growth in the group of rabbits with prothrombin concentration in the accepted therapeutic range. A substantial antithrombotic effect was apparent only in animals with prothrombin concentration less than 15 per cent of normal. These results are consistent with those obtained in previous similar investigations.[8-14] Coumarin therapy has been found to inhibit thrombosis only when prothrombin levels are severely depressed.

In none of the early studies with prothrombin-depressing agents was an effort made to collect data in animals with only mild disturbances of coagulation. The work of Murphy and colleagues[1] utilizing bifurcating plastic arteriovenous shunts in pigs provided quantitative information bearing directly on this area. In 22 animals treated with dicumarol for 8 to 14 days and with prothrombin time 1 to 1.5 times normal the mean weight of thrombotic deposits was found to be significantly greater than that found in controls. In discussing this finding the authors note that Ashwin[8] had observed a similar phenomenon in rats. On review of Ashwin's data, however, this finding is not impressive and has not been subjected to statistical evaluation. The reason for the discrepancy between our findings and those of Murphy *et al.* is most likely to be related to differences in methods for studying thrombosis. Specifically, differences in (1) surface on which thrombi grow and (2) duration of growth allowed deserve particular consideration. In shunt studies thrombi form on inert surfaces and therefore any influence which living vessel wall might exert on propagating thrombus is not measured. Rowsell's observation[15] that adenosine diphosphate-induced platelet aggregates in shunts do not break up whereas intravascular aggregates do suggests that some property of vessel wall tends to inhibit platelet accumulation. Therefore, mild increases in platelet adhesiveness, although detectable in shunts, might well be insignificant in arteries due to the "protective action" of vessel wall.

Also, thrombi in extracorporeal shunts, particularly in the early stages of their development, are composed mainly of platelets, and so it would not be surprising that the weight of deposits formed during short periods of flow (20 min) in such systems would be influenced primarily by the functional status of circulating thrombocytes. In fact this seems to be true, since Murphy has found the best *in vitro* correlation in his model to be with platelet adhesiveness. Our system on the other hand deals with thrombus growth over a 6½-hr period. The final weight of these thrombi is probably significantly influenced by nonplatelet factors. Also, for this reason, an increase in platelet adhesiveness might be apparent in shunts, but lost in our model.

Anticoagulant therapy is commonly "tapered off" because of the clinical impression that abrupt discontinuation of this treatment produces "rebound" thrombosis. Support for this impression can be mustered from a number of

clinical investigations,[2-5] but unfortunately these are not controlled adequately enough to allow definite conclusions. Approaching the clinical problem from another point of view, however, Sise *et al.*[16] found no evidence of rebound after elective discontinuation of therapy, but they did discover such a tendency in group of patients in whom treatment had been interrupted because of hemorrhage. Sevitt and Innes[17] reported an absence of increased venous thrombosis at necropsy in patients who died shortly after discontinuation of prophylactic anticoagulant therapy, and in some of these cases treatment had been terminated because of bleeding. Recently Van Cleve[18] found no difference in the frequency of thrombosis following abrupt as opposed to gradual cessation of long term anticoagulant therapy in patients with myocardial infarction.

Laboratory evidence of hypercoagulability after stoppage of anticoagulant therapy is scant. McGinty *et al.*[19] noted a mild overcorrection of prothrombin activity in the plasma of dogs recovering from the effects of dicumarol, and Lang *et al.*[20] observed a slight shortening of the one-stage prothrombin time in a small number of animals after discontinuation of a short acting anticoagulant. This finding has not been reproduced in several human studies. [21-23] However, Poller and Thomson[24] have reported a shortening of "cephalin time" after withdrawal of chronic therapy in man.

If slight changes in certain coagulation tests do occur after stopping anticoagulants, their significance can be judged best by *in vivo* studies. Unfortunately, only one such investigation had been carried out prior to the one reported here. Moschos *et al.*,[24] utilizing Wessler's technique of serum-induced thrombosis, found no evidence of a thrombophilic state in rabbits 48 hr after a standard hemorrhage, termination of warfarin injections, and administration of 10 mg of vitamin K_1 intravenously, but they did find a tendency toward increased thrombosis 5 days after anticoagulant hemorrhage. The authors chose the 5-day period because preliminary investigations had revealed that at this point A. H. G. levels, which had been elevated during therapy, returned to normal or subnormal, and because they postulate that decreased factor VIII activity indicates hypercoagulability (and vice versa).

Our study in rabbits reveals no thrombophilia either at 48 hr or 5 days after stopping warfarin and giving 50 mg of vitamin K_1 intravenously. It is unlikely that this result is due to our method being insensitive to increase in thrombotic tendency, since we have been able to demonstrate a thrombophilic state after bilateral carotid occlusion[6] utilizing the same technique. Thrombosis may occur after discontinuation of anticoagulant therapy due to persistence of local or systemic factors predisposing to its occurrence. The available evidence, however, indicates that it cannot be explained by the appearance of rebound hypercoagulability or thrombophilia.

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