PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 181, 275–276 (1986)

Prolongation by Warfarin of "Template" Bleeding Time in Rats: A Vitamin K-Dependent Effect (42253)

INE REYERS, GIOVANNI DE GAETANO, AND MARIA BENEDETTA DONATI

Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, Milan 20157, Italy

Abstract. Administration of warfarin to rats induced not only the well-known anticoagulant effect, but also an impairment of primary hemostasis as reflected by a significant prolongation of the "template" bleeding time. This effect was very closely associated with lowering of the prothrombin complex level and was reversed by administration of vitamin K. It is suggested that some of the clotting factors known to be vitamin K-dependent also play a role in primary hemostasis; alternatively, a putative vascular "bleeding factor" could be modulated by vitamin K availability.

We have previously shown that warfarin prevents thrombus formation in a vascular prosthesis model in rats (1). In the context of a larger study on the mechanism of warfarin's antithrombotic effect in rats, we observed that warfarin anticoagulation was accompanied by impairment of primary hemostasis as measured by the "template" bleeding time in the rat tail (2). This finding was somewhat unexpected, since the "template" bleeding time in rats is considered to reflect mainly the early steps of hemostatic plug formation involving blood platelets and the vascular wall function or tone (2). Indeed, in severe thrombocytopenia, some antiplatelet agents and vasodilators significantly prolonged the "template" bleeding time in rats (2). In contrast, heparin at full anticoagulant doses had no such effect (2).

We report here experiments designed to provide a better understanding of the mechanisms underlying the prolongation of "template" bleeding time in warfarin-treated rats.

Material and Methods. CD-COBS male rats (Charles River, Calco, Italy) weighing 250–280 g, were treated with racemic warfarin (Coumadin, Endo Laboratories, Garden City, N.Y.). The drug was dissolved in isotonic saline and intravenously injected at three different doses (0.2–0.4 or 1 mg/kg body wt) to achieve different degrees of anticoagulation. Bleeding time was measured 2 to 24 hr later by applying an automated device ("template") (3) on the rat tail (9 cm from the tip) as previously described (2, 4). The anticoagulant effect of warfarin was monitored immediately after bleeding time by the Thrombotest, adapted to native peripheral blood (Immuno S.p.A., Pisa, Italy). In a subsequent experiment Vitamin K1, dissolved in 6% Tween 80, was injected im 6 hr before the bleeding time test (1 mg/kg body wt vitamin K, 18 hr after 1 mg/kg body wt warfarin).

Results and Discussion. Warfarin treatment resulted within 24 hr in different levels of anticoagulation. The animals were subdivided into four groups according to their Thrombotest levels, as indicated in Fig. 1. A fifth group of rats (treated with isotonic saline) acted as a control.

The bleeding times were longer than those in the control group ($P < 0.01$ at Duncan new multiple range test) in the two groups with Thrombotest levels below 20%. Bleeding times longer than 500 sec were found in all rats but one with a Thrombotest level below 10% ($X = 28.51$, $P < 0.001$).

We then addressed the issue of whether or not the effect of warfarin on bleeding time was linked to its vitamin K antagonism. Administration of warfarin (1 mg/kg iv) 2 hr before testing did not prolong the bleeding time (controls 99 + 6 sec vs warfarin 98 + 14 sec) or affect Thrombotest levels. Moreover, administration of vitamin K to rats treated with warfarin 24 hr before completely corrected the Thrombotest and also the bleeding time (Table I).

In conclusion, administration of warfarin to rats markedly impaired primary hemostasis as measured by the "template" bleeding time test. Similar prolongation was observed with

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the same experimental system in severely thrombocytopenic rats (2, 4) or after treatment with pyrimido-pyrimidine compounds or prostacyclin (5). No effect on platelet number or on the amount of vascular prostacyclin antiaggregating activity was found in our study after warfarin treatment at the time of bleeding time measurement (data not shown). The effect shown here appears to be associated with warfarin-induced vitamin K antagonism; we do not know yet whether any of the clotting factors known to be vitamin K-dependent would also have some as yet undefined effect on primary hemostasis. In humans anticoagulated with warfarin, the possibility of factor VII playing a major role in primary hemostasis was ruled out by normal bleeding times when factor VII levels were well below the "therapeutic range" (6). This finding does not necessarily contradict the point raised in this paper, since the difference may be mainly due to the degree of the vitamin K antagonism. Alternatively, vitamin K availability could modulate some putative factor(s) crucial to the platelet–vessel wall interaction (such as a vascular "bleeding factor"). In this context it is of interest that vitamin K-dependent carboxylase activity has recently been detected in bovine vascular tissues, although its substrate has not yet been identified (7).

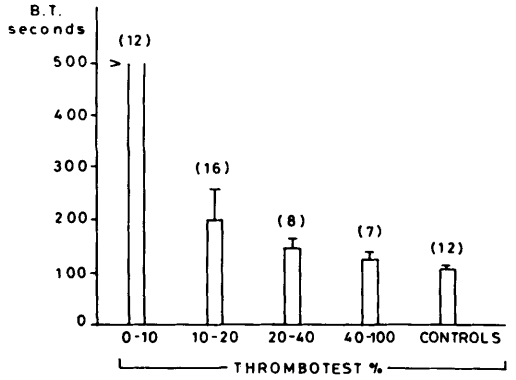

FIG. 1. "Template" bleeding times in groups of rats with different prothrombin complex levels (Thrombotest activity) after warfarin administration.

TABLE I. EFFECT OF TREATMENT WITH VITAMIN K ON WARFARIN-INDUCED LOWERING OF THROMBOTEST ACTIVITY AND PROLONGATION OF THE BLEEDING TIME[a]

	Thrombotest (%)	Bleeding time (sec)
Warfarin 1 mg/kg body wt (24 hr before)	3	500
Warfarin (24 hr before + vit K 6 hr before)	102 + 4	100 + 11

[a] Means ± SE of 12 values per group.

This work was partially supported by Italian National Research Council (Progetto Finalizzato "Medicina Preventiva," Sottoprogetto "Malattie Degenerative," Contract 84.02299.56). Judy Baggott, Ivana Garimoldi, Vincenzo and Felice de Ceglie, Vanna Pistotti, and the staff of G. A. Pfeiffer Memorial Library helped prepare the manuscript.

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Received July 8, 1985. P.S.E.B.M. 1986. Vol 181.
Accepted October 15, 1985.