STUDIES IN INTRAVASCULAR COAGULATION. II. A COMPARISON OF THE EFFECT OF DICUMAROL AND HEPARIN ON CLOT FORMATION IN ISOLATED VENOUS SEGMENTS

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Research Article

STUDIES IN INTRAVASCULAR COAGULATION. II. A COMPARISON OF THE EFFECT OF DICUMAROL AND HEPARIN ON CLOT FORMATION IN ISOLATED VENOUS SEGMENTS [1]

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In a previous report (1) a method of preparing isolated blood-filled venous segments in the dog was described. Intravascular coagulation was studied by determining clot appearance, prothrombin consumption, accelerator formation and platelet disappearance within these segments. Of particular interest was the observation that a fibrin clot developed prior to the disappearance of demonstrable amounts of prothrombin or the elaboration of measurable quantities of clot accelerators. Since dicumarol-induced hypoprothrombinemia is currently employed to inhibit or retard intravascular coagulation in man, it became important to ascertain whether induced hypoprothrombinemia actually was associated with retarded fibrin deposition *in vivo* under the same experimental conditions. For purposes of comparison the effect of heparin was also studied.

OBSERVATIONS

Dicumarol was administered orally to 18 dogs. After two-stage prothrombin concentrations (2) were decreased to values ranging from 29 to 2 per cent of the control level, the external jugular and femoral veins were exposed, isolated, and their contents examined as previously described (1). In brief, all demonstrable tributaries to these veins were carefully ligated and cut so that the vessels were entirely freed from surrounding structures. Continuity of each vein with the remainder of the circulation was then interrupted by the careful application of small clamps at each end of the freed segment. At selected intervals vein segments were removed from the animal and by release of one clamp the contents were emptied into clear shallow glass dishes containing 2 to 3 cc. of 2.5 per cent citrate. These dishes were carefully inspected for the presence or absence of a gross clot. Because in untreated animals clotting always occurred in

segments isolated for one and one-half hours (1), the failure of a clot to develop within 90 minutes after isolation of a segment was accepted as evidence of clot inhibition.

Since in clinical practice the degree of hypoprothrombinemia is usually estimated by the one-stage method, such determinations (3) also were carried out in eight animals. In each such instance prothrombic activity was reduced to less than 12 per cent. Although in most dogs the degree of induced hypoprothrombinemia was far in excess of that attained therapeutically in man, *in vivo* clotting in the isolated venous segment was not significantly retarded (*i.e.* more than 90 minutes) except in one animal

TABLE I

DICUMAROL DOSAGE	AT TIME VEIN ISOLATED PROTHROMBIN (%)		SPCA	GROSS CLOT WITHIN
mg/kg	2-stage	1-stage	%	90 MIN
17.6	2	1	0	0
13.4	5	5	-	↑
18.7	7	-	0	↑
4.4	8	13	20	↑
17.6	9	2	0	↑
18.0	10	4	0	↑
3.7	10	-	-	↑
9.0	11	-	0	↑
1.1	11	8	28	↑
18.7	15	-	0	↑
7.9	12	-	-	↑
18.3	12	-	0	↑
9.0	13	9	70	↑
13.4	15	-	0	↑
9.5	17	12	17	↑
3.1	26	-	-	↑
7.5	26	-	-	↑
4.0	29	-	-	↑

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(Table I). In this dog the coagulation defect was greater than in any of the other animals: the two-stage prothrombic activity was 2 per cent and the one-stage activity less than 1 per cent of the control values; the clotting time was prolonged to three hours.

The possibility was considered that serum prothrombin conversion accelerator activity (SPCA) had not been adequately depressed by the dicumarol and was responsible for the prompt clotting in the dicumarolized segments. However, estimations of serum SPCA activity by the method of Alexander (4) in twelve of the dicumarolized dogs revealed no SPCA activity in eight (Table I).

The effect of heparin in therapeutic doses was studied in 17 dogs (Table II). In ten of eleven animals, in which the vein was isolated 30 to 60 minutes after the administration of heparin, clotting did not appear within the vein segment for 90 or more minutes. In all eleven animals, Lee-White (5) clotting times at the moment of isolation ranged from four to more than 36 times the control values. When the period of time from the administration of heparin to the isolation of the segment was increased to two hours significant retardation of *in vivo* clotting could still be detected in three of six dogs. In none of these six animals did the Lee-White *in vitro* clotting times exceed twice the control value.

In the dicumarolized dogs there was such extensive oozing into the operative wounds, that it was impossible to maintain adequate hemostasis during the time period necessary for the isolation of the vein segments. Since bleeding to a similar extent did not occur in the heparinized dogs, it was felt that hemorrhage or its sequelae may have been a factor in the observed differences between the effects of the two anticoagulants. Consequently in eight additional animals, in which large doses of di-

TABLE II

HEPARIN DOSAGE MG/KG	INITIAL CLOTTING TIME (MIN)	AT TIME VEIN ISOLATED HRS. AFTER HEPARIN	CLOTTING TIME (MIN)	GROSS CLOT WITHIN 90 MIN
2.2	5	1/2	180 ≠	0
2.2	9	1/2	180 ≠	0
1.1	12	1	180 ≠	0
1.1	8	1/2	180 ≠	0
1.1	10	1	105	0
1.1	11	1	102	0
1.1	17	1	80	≠
1.1	10	1/2	60 ≠	0
1.1	9	1	57	0
1.1	6	1	24	0
0.55	10	1	39	0
1.1	17	2	31	≠
1.1	17	2	29	≠
1.1	17	2	23	0
1.1	15	2	20	≠
1.1	11	2	18	0
1.1	9	2	15	●

TABLE III

DICUMAROL DOSAGE MG/KG	FINAL PROTHROMBIN 2-STAGE (%)	GROSS CLOT WITHIN 90 MIN	HEPARIN DOSAGE MG/KG	INITIAL CLOTTING TIME (MIN)	AT TIME VEIN ISOLATED HRS. AFTER HEPARIN	CLOTTING TIME (MIN)	GROSS CLOT WITHIN 90 MIN
13.4	5	≠	0.55	10	1	26	0
					2	23	0
					3	20	≠
18.7	7	≠	0.55	17	1	60	0
4.4	9	≠	1.1	18	1	180≠	0
					2	33	0
9.0	11	≠	1.1	10	1 1/2	19	0
1.1	11	≠	1.1	9	1	78	0
					2	16	0
18.0	12	≠	0.55	16	1	25	0
9.0	13	≠	1.1	18	1	77	0
					2	24	0
					3	20	≠
9.0	17	≠	1.1	11	1	162	0
					2	28	0
					3	15	≠

cumarol were given and in which the development of clot within 90 minutes was demonstrated, heparin was subsequently administered and additional segments isolated one, two and three hours after injection of the latter drug. In these animals clots did not develop until more than 90 minutes had elapsed in segments isolated one and two hours after heparinization (Table III). Coagulation was not significantly retarded in any vein segments isolated three hours after heparinization, despite the concomitant hypoprothrombinemia. There was, therefore, no evidence that dicumarol had even potentiated the clot retarding effect of heparin.

DISCUSSION

It has been previously reported from this laboratory that only small amounts of prothrombin need be present in the blood to produce a fibrin clot in the isolated vein segment of the dog (1). Data presented in this paper have shown that severe dicumarol-induced hypoprothrombinemia and SPCA deficiency do not retard markedly fibrin deposition in an isolated segment of vein unless sufficient dicumarol has been administered to prolong greatly the *in vitro* Lee-White clotting time of the blood. In contrast, heparin in therapeutic doses significantly retarded intravascular coagulation in similarly prepared venous segments.

It should be clearly understood that these observations do not preclude the possibility that dicumarol retards intravascular thrombosis nor do they demonstrate that heparin is an ideal anticoagulant. Failure to demonstrate clot inhibition with dicumarol may be inherent in the method employed. It can reasonably be argued that slight retardation of clotting in individual segments was overlooked by applying the rigid criteria of 90 minutes delay in gross clotting. Moreover, under the conditions of the experiment the blood in the segment was completely stagnant and isolated from the remainder of the circulation. Finally, the transient anticoagulant effect of heparin was readily apparent. The present study does show, however, that under comparable conditions the anticoagulant effect of heparin is superior to that of dicumarol. Similar findings have been reported by others (6, 7).

There is, of course, ample evidence that dicumarol does exert a retarding influence on blood coagulation *in vitro* (8–10) and on the development of experimental intravascular thrombosis in

animals (11–18). In addition, statistical studies in man indicate that the drug produces a significant reduction in thromboembolism (19–21). Bingham, Meyer and Pohle (22) and Rogers, Barrett and Lam (18) found, however, that moderate degrees of dicumarol-induced hypoprothrombinemia did not significantly reduce the incidence of thrombosis in dogs. When the one-stage prothrombic activity was less than three per cent by the method of Quick (23), Rogers, Barrett, and Lam (18) observed not only a marked inhibition of thrombosis but also an important increase in hemorrhagic complications. Richards and Cortell (12) also successfully inhibited intravascular thrombosis with dicumarol dosages that were associated with extensive hemorrhage.

It is important to recognize that there may be a profound dissociation between bleeding and clotting. Our dicumarolized dogs, for example, hemorrhaged profusely during the operative procedures required to expose the vein segments, yet clotting appeared unaffected within the isolated vein. In man, clinical states such as polycythemia vera (24) and thrombotic thrombocytopenic purpura (25) may be attended by both hemorrhage and thrombosis. It has been reported that a prothrombin concentration of between 5 and 10 per cent determined by the method of Rosenfield and Tuft (3) can usually be regarded as safe (26), and that at this level coagulation is retarded (27). What is not known is how effective such levels are against thrombotic disorders.

In the prophylaxis and treatment of thromboembolism there is a constant search for more effective therapy. That thrombosis may occasionally "break through" apparently adequate dicumarol therapy has been reported by Wright (28). Until it is clearly demonstrated that the pathogenesis of thrombosis is the same in congestive failure, in post-operative and post-partum states, in malignancy and in apparently healthy individuals, one cannot assume that hypoprothrombinemia will provide equally effective therapy in all these conditions. It is certainly not easy to translate the results of experimental studies in animals to clinical practice. Nevertheless, the significance of the study herein reported lies in the observation that substances such as dicumarol, which depress that part of the coagulation sequence concerned with

prothrombin conversion, may not provide as optimal an anticoagulant effect as heparin-like compounds which block fibrin deposition through other mechanisms (29–32).

SUMMARY

In experiments designed to study intravascular clotting in the isolated venous segment of the dog, the anticoagulant effect of heparin was found to be superior to that of dicumarol.

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