PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 1–3 (1982)

Hypersensitivity to Warfarin in Rats with Walker 256 Carcinoma[1] (41297)

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Abstract. Walker 256 carcinoma cells were injected subcutaneously into rats that were then given warfarin intravenously. Depression of prothrombin and factors VII, IX, and X levels was much greater than in control rats given the same doses of warfarin. A somewhat similar hypersensitivity accompanied treatment of normal rats with subcutaneous injections of turpentine.

Considerable evidence indicates that the hemostatic process is involved in the growth of cancer, at least in experimental metastatic tumors (1). Thus, agents that inhibit clotting or platelet function or abet fibrinolysis limit the number of implants when an animal is given transplantable tumor cells intravenously. However, a recent attempt to alter the rate of growth of the primary implant of Walker 256 carcinosarcoma in rats by the use of heparin or warfarin was unsuccessful (2). This was in spite of the fact that the tumors accumulated significant amounts of ^{125}I from labeled fibrinogen and ^{51}Cr from labeled platelets given intravenously. During this study, it was observed that the tumor-bearing rat developed a remarkable sensitivity to the anticoagulant effects of warfarin.

Methods. With sterile precautions, Walker 256 carcinoma was excised from rats. The pink outer layer was removed and ground with mortar and pestle without adjuvants such as saline or antibiotics. One-tenth milliliter of the macerated material was injected subcutaneously into the lower left lateral abdominal wall of male Sprague–Dawley rats. The rats weighed 190 to 220 g. Tumors grew in more than 95% of the recipients. The tumors weighed about 1 g within 5 days, 4 g in 10 days, and 20 g in 20 days. During the first 3 days a pronounced hyperfibrinogenemia developed—more than 600 mg of fibrinogen per deciliter of plasma (normal 270 to 420 mg/dl). As the level of fibrinogen returned to normal, a modest thrombocytosis emerged; the platelet counts reached a peak of about 1,400,000/mm^3 (normal 750,000 to 1,150,000/mm^3) and then returned to normal within a week. Despite this reaction to the injection of the Walker cells, no change occurred in rectal temperature measured daily during the first week.

Turpentine in a dose of 0.1 ml was injected into the anterior thigh muscles of rats. A pronounced reaction associated with swelling reached a peak 5 or 6 days after the injection. Hyperfibrinogenemia and thrombocytosis were similar in timing and degree to those seen in tumor-injected rats. Like the rats with tumor, rats given turpentine experienced no fever.

Control rats were given subcutaneous injections of 0.15 *M* NaCl.

Warfarin sodium (Endo Laboratories, Inc., Garden City, N.Y.) was dissolved in 0.15 *M* NaCl such that the dose (0.025, 0.050, or 0.075 mg) was 0.5 to 1 ml. This was injected intravenously into a leg or tail vein either once or on four consecutive mornings. Coagulation factor assays were done on citrated plasma from blood collected 24 hr after the injection of the single dose or 24 hr after the last of the four doses.

Prothrombin was assayed by a two-stage test (3). Factor VII and X levels were measured by the prothrombin time test, and factor IX level was measured by the activated partial thromboplastin time using congenitally deficient human plasmas as substrates (4). Values are reported in per-

[1] This investigation was supported in part by a grant from the Eagles Cancer Fund.

0037-9727/82/010001-03$01.00/0

TABLE I. EFFECT OF SINGLE INTRAVENOUS INJECTION OF 0.05 mg OF WARFARIN SODIUM ON FOUR VITAMIN K-DEPENDENT FACTORS IN PLASMA[a]

Days after injection of tumor cells or turpentine	n	Coagulation factors (% of normal)			
		II	VII	IX	X
			Control rats		
—	12	92 ± 12	89 ± 15	62 ± 24	81 ± 13
			Rats with tumor		
2	7	61 ± 7[b]	45 ± 30[c]	28 ± 9[b]	59 ± 19[c]
4	8	38 ± 17[d]	18 ± 17[d]	18 ± 9[d]	17 ± 11[d]
7	7	40 ± 12[b]	43 ± 17[b]	30 ± 18[c]	51 ± 28[c]
			Rats given turpentine		
2	8	51 ± 20[b]	29 ± 28[c]	17 ± 9[d]	34 ± 11[b]
4	11	51 ± 29[c]	47 ± 29[c]	29 ± 15[b]	50 ± 26[c]
7	6	64 ± 23[c]	89 ± 13	83 ± 12	100 ± 11

[a] Warfarin was injected on the second, fourth, or seventh day after injection of tumor cells or turpentine, and coagulation assays were done 24 hr later. Control rats were assayed 24 hr after injection of warfarin. The results are mean ± SD. Depression greater than control value: [b]$P < 0.01$; [c]$P < 0.05$; [d]$P < 0.001$.

centage of the concentrations of coagulation factors in normal rat plasma assayed by the same methods and done concurrently with test plasmas.

Results. Normal adult rats readily tolerate a single dose of as much as 1 mg of sodium warfarin intravenously, although the levels of factors II, VII, IX, and X decrease below 10% of normal 24 hr later. Doses of 2 mg also have been tolerated (5). When tumor rats are given more than 0.1 mg of warfarin sodium, they tend to bleed within 24 hr, usually from the intestinal tract. As a result, 0.05 mg was selected for the single-dose treatment or 0.025 mg was selected for the daily multiple-dose treatment of rats with tumor. The effects of these dosages when warfarin was given once and the plasma was assayed 24 hr later are shown in Table I. With this single dose, control rats had almost no depression of the four vitamin K-dependent clotting factors, only factor IX decreasing below 80% of normal. By contrast, all four factors decreased sharply in the rats with tumor: factor II to 38%, VII to 18%, IX to 18%, and X to 17%, on average.

Rats given turpentine also received a single dose of 0.05 mg of warfarin sodium to determine if they were more warfarin-sensitive than normal. Lowest mean values achieved were 51% for factor II, 29% for factor VII, 17% for factor IX, and 34% for factor X. Peak sensitivity was at 2 days in the rats given turpentine and was even greater at 4 days in rats with tumor. By the seventh day after receiving turpentine, the rats had reverted to normal warfarin sensitivity, but the rats with tumor still exhibited hypersensitivity.

The study was repeated with 0.025 mg of warfarin given daily for 4 days, the assays being conducted 24 hr after the last dose. The warfarin was begun 2 days before injection of tumor cells or turpentine, on the same day, on the third day after the injection, or on the seventh day. To permit comparisons, normal rats were given daily doses of 0.025, 0.050, or 0.075 mg of warfarin (Table II). If the responses of the control rats to these three doses are plotted and the depressions of clotting factors in the rats with tumor are then interpolated, the sensitivity of the tumor rats to warfarin averaged 1.8 times the control for prothrombin, 2.1 times for factor VII, 2.4 times for factor IX, and 2.0 times for factor X. The rats given turpentine also showed hypersensitivity, but it had disappeared a week after the injection of the turpentine. Further, the depression of clotting factors tended to be less than in the rats with tumor. Curiously, factor X showed virtually no effect from the turpentine treatment.

The prothrombin time, reflecting three of the four vitamin K-dependent factors (II, VII, and X), lengthened as these factors de-

TABLE II. EFFECT OF FOUR DAILY INTRAVENOUS INJECTIONS OF 0.025 mg OF WARFARIN SODIUM ON FOUR VITAMIN K-DEPENDENT FACTORS 24 HR AFTER LAST DOSE

Age of tumor or turpentine abscess when first dose of warfarin given[a] (days)	n	Coagulation factors (% of normal)			
		II	VII	IX	X
			Control rats		
Normals					
0.025 mg	6	86 ± 2	75 ± 12	64 ± 21	72 ± 13
0.050 mg	7	19 ± 2	41 ± 21	16 ± 7	29 ± 5
0.075 mg	4	11 ± 2	4 ± 2	19 ± 3	7 ± 2
			Rats with tumor		
−2	6	18 ± 15[b]	29 ± 21[b]	7 ± 5[c]	33 ± 22[b]
0	8	21 ± 18[b]	34 ± 17[d]	14 ± 6[c]	29 ± 21[b]
+3	7	55 ± 14	19 ± 12[c]	9 ± 6[c]	29 ± 15[b]
+7	8	42 ± 28	59 ± 6	16 ± 10[b]	55 ± 21
			Rats given turpentine		
−2	6	37 ± 11[b]	43 ± 8[d]	11 ± 3[c]	64 ± 11
0	5	18 ± 11[b]	43 ± 21[d]	10 ± 7[c]	48 ± 37
+3	3	85 ± 15	31 ± 10[b]	26 ± 15[d]	73 ± 30
+7	3	85 ± 7	104 ± 47	54 ± 24	98 ± 14

[a] The first dose was given 2 days before injection of tumor or turpentine (−2), on the day of injection (0), on the third day after injection (+3), or on the seventh day (+7). For comparison, controls were given doses two and three times larger as well. Results are mean ± SD. Depression greater than control value: [b]$P < 0.01$; [c]$P < 0.001$; [d]$P < 0.05$.

creased during warfarin treatment. Normal rats had a prolongation of their prothrombin times from a normal of 12.0 to 13.2 seconds when 0.025 mg of warfarin was given daily for 4 days, to 18 sec with 0.050 mg/day, and to 25.5 sec with 0.075 mg/day. The prothrombin times of the tumor rats or rats given turpentine were approximately 24 sec after 4 days of 0.025 mg of warfarin per day.

Discussion. The hypersensitivity to warfarin of rats injected with Walker 256 carcinoma cells was unexpected. Since the precise mechanism by which warfarin interferes with gamma carboxylation of glutamic residues of proteins by vitamin K is unknown, and since the basis for the opposite condition—warfarin resistance—in man or rat is equally obscure, a clearer understanding of the warfarin–vitamin K interaction is needed. Alteration of the body's metabolic state by modifying thyroid function clearly affects catabolism of coagulation factors II, VII, and X in the rat (6). Perhaps the cancer cells and the nonspecific irritant turpentine act through some general metabolic process, although this was not manifested by an elevation in body temperature.

The effect of cancer on the liver may not be limited to the coagulation proteins. Hepatic tissue from patients with extrahepatic malignancies has an increased capacity for synthesizing albumin (7). Further, the response of the body to cancer may extend to organs other than the liver. Thus, these rats also developed thrombocytosis. However, one might speculate that the megakaryocytes are merely responding to the excessive production of a hepatic stimulant.

Mr. Joseph E. Standing and Mr. Curtis Grabau provided valuable technical assistance.

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Received July 27, 1981. P.S.E.B.M. 1982, Vol. 169.