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In vitro Effect of Sodium Warfarin on DNA and RNA Synthesis of Mouse L1210 Leukemic Cells and Walker Tumor Cells[1]

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Abstract. Sodium warfarin was studied for its *in vitro* effect on nuclei acid synthesis of L1210 leukemic cells and Walker 256 carcinosarcoma cells. Moderate inhibition was noted in the incorporation of thymidine and uridine into DNA and RNA in L1210 cells, but only at very high concentrations, and no inhibition was seen in Walker tumor cells. The survival of L1210-bearing mice was not influenced by various regimens of sodium warfarin treatment. These data indicate that sodium warfarin probably has no direct *in vivo* antitumor activity against L1210 leukemia of mice or Walker 256 carcinosarcoma of rats.

Key Words
Warfarin
Nuclei acid synthesis
L1210 leukemia
Walker 256 carcinosarcoma

The long-term administration of sodium warfarin, 3-(α-acetonylbenzyl)-4-hydroxycoumarin, has been reported to decrease the incidence of pulmonary metastases from the Lewis bladder carcinoma 150 in Swiss mice, the mammary adenocarcinoma in C3H/HeN mice, and Walker 256 carcinosarcoma of rats [1, 10, 14]. At the same time, the survival of these animals was also considerably increased. The incidence of cancer and its mortality has been studied in patients receiving anticoagulant therapy for thromboembolic disease, and the suggestion has been made that anticoagulant therapy may alter the natural course of cancer [8]. The effect of warfarin on tumor cells is thought to be due either to a change in motility mediated by the anticoagulation mechanism [5, 11] or to a direct cytotoxic activity against tumor cells [2]. Inhibition of local tumor growth by sodium warfarin or other anticoagulants was demonstrated in experimental tumors [2, 7, 14], yet there is still no convincing evidence of antitumor activity against human tumors. Because of these interesting reports, we have examined the

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possibility of a DNA and RNA inhibition in tumor cells by the drug, and studied the effect of sodium warfarin on nucleic acid synthesis in L1210 mouse ascitic leukemic cells and Walker 256 carcinosarcoma cells of rats.

Materials and Methods

In vitro *Effect on L 1210 Leukemic Cells*

L1210 mouse ascitic leukemic cells were harvested 5 days after the intraperitoneal inoculation of 2×10^5 cells into CDF_1 mice. A 1-percent cell suspension was made in Eagle's medium, containing 10% horse serum with sodium warfarin at concentrations ranging from 10^0 to 10^{-4} μM/ml. A control was also studied without sodium warfarin. The rate of DNA and RNA synthesis was also measured as previously described [3]. Briefly, ^{14}C-thymidine (TdR), ^{3}H-deoxyuridine (UdR) or ^{14}C-uridine (UR) was added to cell suspension and incubated at 37 °C with continuous agitation. 30 min later, the incubation was terminated by the addition of 10% cold trichloroacetic acid to precipitate nuclei acids, and the incorporation of radioisotopically labeled thymidine and deoxyuridine into DNA, and of uridine into RNA was determined by liquid scintillation counting. Changes in DNA and RNA synthesis resulting from exposure to various concentrations of sodium warfarin were plotted as percent of control values.

In vitro *Effect on Walker 256 Carcinosarcoma Cells*

Walker cells, 2×10^6, were injected intraperitoneally into 6-week-old Sprague-Dawley rats. 5 days later, these rats were sacrificed by cervical dislocation, and Walker cells were collected for the study of the effect of sodium warfarin on the nuclei acid synthesis, using the same procedure as for L1210 cells.

Effect of Sodium Warfarin on Survival of L 1210-bearing Mice

A total of 50 mice were transplanted intraperitoneally with 2×10^5 L1210 cells. These mice were divided into three groups: group 1, the sodium warfarin-treated group, with 30 mice; group 2, the saline-treated group, comprising 10 mice; group 3, the non-treated group, with 10 mice. Group 1 was treated intraperitoneally with sodium warfarin, 0.25 mg/kg/day (10 mice), 1.0 mg/kg/day (10 mice), and 2.5 mg/kg/day (10 mice) from the second day of the transplantation. Group 2 was injected with an equal volume of the diluent, and group 3 remained untreated for control purposes. The number of dead animals was recorded every day on each group, and the percent of dead animals calculated.

Results

Effect on L 1210 cells. The *in vitro* inhibition of sodium warfarin on the nucleic acid synthesis of L1210 cells was not apparent at concentrations of less than 10^{-2} μM/ml. At the concentration of 10^{-1} or 10^0 μM/ml, the drug resulted in 10 and 50% inhibition of TdR incorporation into DNA, and

15 and 62% inhibition of UR into RNA. In contrast, UdR incorporation was not influenced by the drug (fig. 1). These results indicate that sodium warfarin primarily inhibits the salvage pathway of DNA synthesis from thymidine without any detectable effect on the *de novo* pathway from deoxyuridine. RNA synthesis from UR was inhibited to the same degree as DNA synthesis from TdR.

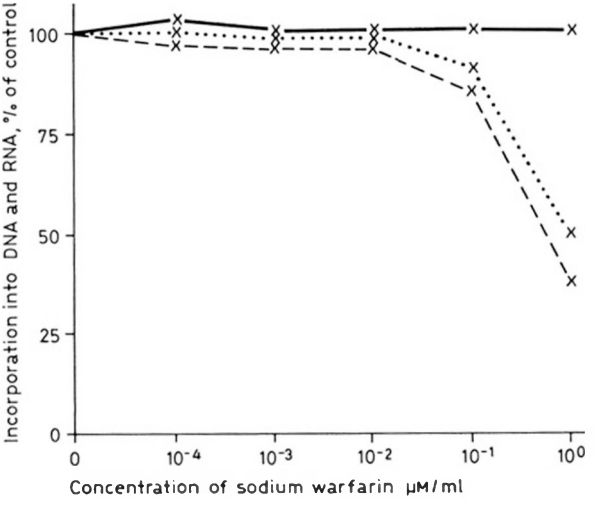

Fig. 1. In vitro (mouse L1210 cells) effect of sodium warfarin on DNA and RNA synthesis. Warfarin showed no effect on UdR (solid line) incorporation into DNA, but resulted in moderate inhibition of TdR (dotted line) incorporation into DNA and of UR (broken line) incorporation into RNA.

Effect on Walker 256 carcinosarcoma cells. The presence of various concentrations of sodium warfarin from 10^{-4} to 10^{0} µM/ml did not affect the rate of DNA and RNA synthesis by Walker 256 carcinosarcoma cells over the control values.

Survival of L1210-bearing mice. As shown in table I, on the eighth day of transplantation, 27% of the sodium warfarin-treated mice were dead, as were 10% of the saline-treated group and 10% of the non-treated group.

Table 1. Survival of L1210-bearing mice treated with sodium warfarin (SW)

Days after transplantation of L1210 cells	Dead animals, % SW-treated			Saline-treated	Nontreated
	A	B	C		
8	30	30	20	10	10
9	70	80	70	50	50
10	100	100	100	100	100

A = 0.25 mg SW/kg/day; B = 1.0 mg SW/kg/day; C = 2.5 mg/kg/day.

On the ninth day, 73% of the sodium warfarin-treated, 50% each of the saline-treated and the nontreated were dead. The different dose schedules of sodium warfarin produced no difference among the treatment groups. On the 10th day, all the animals were dead regardless of the modality of treatment. These results indicate that there was no increase in survival of L1210-bearing mice when they were treated with sodium warfarin.

Discussion

Recent studies of BOULOS *et al.* [2] showed that tumor growth rate was reduced and survival time considerably prolonged after long-term administration of sodium warfarin to rats with transplanted fibrosarcoma In contrast, HIGASHI and HEIDELBERGER [6] reported the lack of an inhibitory effect of sodium warfarin on cell growth of primary and metastatic L1210 leukemia and adenocarcinoma 755 in BDF_1 mice. Our present studies showed moderate inhibition of utilization of salvage pathway of thymidine for DNA synthesis and of RNA synthesis only in very high *in vitro* concentrations. Survival times of L1210-bearing mice after treatment with sodium warfarin were not influenced by various regimes of the drug. Apparently, *in vivo* DNA- and RNA-inhibiting capacity of the drug was not present at concentrations for anticoagulant therapy. Sodium warfarin did not show any effect on the DNA and RNA syntheyis of Walker 256 carcinosarcoma cells, in spite of a previous report of a significant decrease in pulmonary metastases and of an increase in survival times of rats with Walker 256 carcinosarcoma [1].

Experimentally, anticoagulation reduced the number and size of metastase [4, 12] and protamine increased the formation of experimental metastases [13]. O'MEARA and JACKSON [9] suggested that the growth of metastatic tumor cells was related to clumping or stickiness, which may be altered by fibrinogen or fibrin formation. If this is so, then substances which prevent fibrin formation might prevent metastases. This would offer an explanation for the reduction of spontaneous metastases of certain animal tumors by sodium warfarin and other anticoagulants.

The studies of HIGASHI and HEIDELBERGER [6] and ours failed to demonstrate direct inhibition of tumor cell growth by sodium warfarin. Since *in vitro* inhibition of DNA and RNA synthesis occurs only in high concentrations, which greatly exceed therapeutic concentrations in the human, it may have very little clinical promise as an antitumor drug.

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