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Letter to the Editor

Reduced Procoagulant Activity of Lewis Lung Carcinoma Cells from Mice Treated with Warfarin*

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COUMARIN anticoagulants have been found to reduce lung metastasis formation in a spontaneously metastasizing tumor, the Lewis lung carcinoma (3LL) and in some other experimental tumour models [1–4]. The mechanism of this effect, however, is still controversial, and the question is debated whether warfarin acts through induced systemic hypocoagulability or through a more direct interaction with cancer cells [1, 5].

3LL cells have a particular procoagulant activity, which has been recently characterized [6, 7]; this could contribute to the fibrin deposition observed at the primary tumour site [8], thus influencing the metastatic capacity of 3LL cells. Lately, Zacharski *et al.* [9] reported reduction of epithelial cell procoagulant activity in patients on chronic warfarin treatment, thus suggesting an additional, 'cellular' mechanism for warfarin anticoagulation.

In view of the supposed role of fibrin in dissemination processes, we investigated whether warfarin treatment of 3LL-bearing mice influenced the tumour cells' procoagulant (factor X-activating) activity. 3LL cells (1×10^5 per mouse) were injected i.m. into the hind paw pad of C57Bl/6J male mice at day 0. Warfarin (Coumadin®, Endo Laboratories, Garden City, NY, U.S.A.) was given in drinking water from day 7 according to a schedule capable of maintaining prothrombin complex activity (measured by the Thrombotest, Immuno S.p.A., Pisa, Italy) at less than 5% of control values [3]. Treatment was continued till the animals were killed on day 18, when the primary tumour weights were similar in control and warfarin-treated mice (5.4 ± 1.0 and 5.3 ± 0.9 g, respectively). Cell suspensions were obtained from excised tumours by trypsin treatment, washed extensively with and suspended in phosphate-buffered saline and immediately tested; procoagulant activity was assayed by a one-stage recalcification time of human factor VII-deficient plasma containing less than 1% factor VII. The clotting time of a mixture of cell suspension, plasma substrate and 0.025 M $CaCl_2$ was determined in plastic tubes at 37°C; this experimental system was especially devised for assaying direct factor X-activating activity [6].

Figure 1 shows that cells from warfarin-treated mice had lower factor X-activating activity (longer recalcification times) than those from untreated mice (means ± S.E.: 287

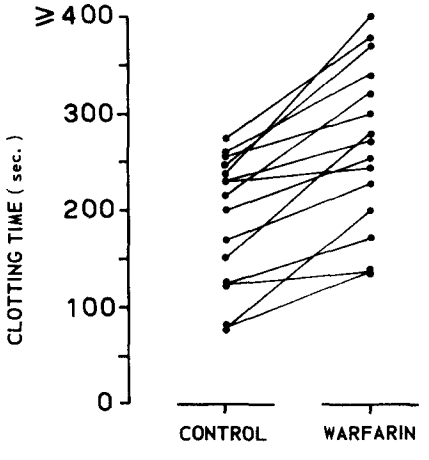

Fig. 1. Clotting times of test systems containing cells (15×10^6/ml) from control or warfarin-treated mice. Couples of one control and one treated mice were tested simultaneously.

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±34 vs 191±17 sec, $P<0.01$ by Student's *t*-test for paired comparisons). *In vitro* addition of warfarin (at various concentrations up to 1 mg/ml) to the 3LL cells from untreated animals did not influence the recalcification times.

Depression of the particular procoagulant activity of 3LL cells in warfarin treated mice, therefore, cannot be ascribed to a direct effect of warfarin on cell activity or on the assay system used. On the other hand, a direct *in vivo* cytotoxic effect of warfarin on 3LL cells is unlikely considering that the primary tumour weight was the same in treated and control mice at the time of cell harvest. Most probably, warfarin may induce some selective alteration of cell metabolism that impairs the cells' ability to synthesize the procoagulant activity. For instance, it is not yet known whether the synthesis of factor X activator in 3LL-bearing animals is a vitamin K-dependent process, as is the production of the so-called factors of the prothrombin complex.

Whatever the mechanism, the present observation of a 'cellular' anti-coagulant effect of warfarin on cancer cells could shed fresh light in our understanding of the antimetastatic activity of the drug and of the involvement of fibrin in dissemination processes.

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