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Warfarin Inhibits Tumor Metastasis Through an Immune-Modulatory, Coagulation-Independent Manner That Involves NK Cells: Possible Anti-Tumor Effects in Patients with OSCC?

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Abstract: Tumor patients show alterations of the clotting mechanism by means of a hypercoagulable state. Warfarin's anti-tumor action was reported in animal cancer models and in human studies with patients already receiving warfarin. An arrest of lung metastases was reported. The mechanism for this anti-tumor action seems to be coagulation-independent, probably involving immune-modulatory mechanisms related to NK function. It would be of great interest to evaluate the hypothesis that warfarin is able to modulate the generation of lymph-node metastases in OSCC by affecting NK receptors in an immune-modulatory manner.

Key words: Warfarin, OSCC, killer-cells, natural, immune-modulation

INTRODUCTION

The dysregulation of the clotting mechanism in cancer patients has been frequently reported in the literature. Animal and human studies have proven evidence of the presence of a hypercoagulable state and nearly all patients have dysregulations of the clotting mechanism (Sun *et al*., 1979; Rickles and Edwards, 1983). The 98% of the patients in a study by Sun showed one or more of the following dysregulations: elevated FDPs, prolonged thrombin time, thrombocytosis, hyperfibrinogenemia, prolongation of prothrombin time and elevation of the levels of specific coagulation factors (Rickles and Edwards, 1983).

Current concepts for the dysregulation of the clotting mechanism in cancer also involve the so-called Intravascular Coagulation and Fibrinolysis (ICF) that however, does not always lead to the generation of a clinically manifested Diffuse Intravascular Coagulation (DIC) (Rickles and Edwards, 1983). An important role for the generation of DIC, play the precipitating factors such as liver impairment and gram-negative sepsis.

In 1865, Armand Trousseau was the first who reported a high incidence of venous Thromboembolic Disease (TED) in patients with gastric carcinoma. Additionally in 1878, Billroth reported the presence of tumor cells in association with fibrin thrombi. The incidence of TED was described to be up to 11% (Soong and Miller, 1970) and it depends on the type of cancer as well as on the adjuvant therapy. Particularly, breast cancer patients treated with chemotherapy showed a frequency of TED of about 5%, a complication that mainly occurred during chemotherapy (Weiss *et al*., 1980).

A relationship between tumor cells, fibrin formation and platelet aggregation has been reported in the literature (Rickles and Edwards, 1983). Fibrin formation seems to play a crucial role in tumor progression. In particular, Fibrinopeptide A (FPA) levels have been related to tumor progression and worse clinical prognosis in cancer patients (Rickles and Edwards, 1983). Thus, FPA levels could be useful for the monitoring of cancer patients during treatment in order to assess response to treatment. The exact mechanism by which fibrin formation interferes with tumor metastases is yet unclear. Fibrin seems to play a different role in early stages of tumor formation and another in later. In particular, in an animal experiment with batroxobin, a snake venom that is a serine protease similar to thrombin, the administration of the enzyme 11 days after the implantation of 3LL tumor cells resulted into reduction in the number of pulmonary metastases with no effect on the size of the primary tumor. In contrast, pre-implantation administration resulted in an increase in the number of pulmonary metastases (Donati *et al*., 1978). In order to explain these results, it is important to understand the mechanism of action of this venom. Batroxobin or reptilase, acts similar to thrombin but does not activate Factor XIII which is essential for the generation of a stable thrombus. Thus, after fibrin formation, a fast defibrination follows that results in fibrinogen deficiency (Castro *et al*., 2004).

According to the above, in the initial states of tumor growth fibrin is important for the prevention of distant

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metastases in contrast to late stages where it plays a role in the generation of metastases. These results are important for future investigations that will address the effect of fibrinolytic drugs on tumor growth and metastases. There are two main hypotheses regarding the exact role of fibrin in tumor growth:

- H_1: one hypothesis is that peritumoral fibrin is essential for the accumulation of tumor cells, acting like glue and enhancing the extravasation process (Rickles and Edwards, 1983)
- H_2: the other hypothesis stresses that tumor cells accumulate near the vessel wall and secondary result into platelet aggregation and fibrin formation

Warfarin has been shown to exert anti-tumor activity. Ryan in their largestudy with mice with anaplastic sarcoma T241 of Lewis and mammary adenocarcinoma revealed a 3.2-10.0 fold increase in the frequency of lung metastases in mice of the control group in contrast to mice that received warfarin per gavage (Ryan *et al*., 1968). Similarly, Hilgard *et al*. (1977) showed that phenprocoumon when administered to mice per gavage from the first day of i.m. administration of 3LL (lewis lung carcinoma cells), inhibited primary tumor growth and reduced the number of spontaneous lung metastases.

In humans, the veterans administration cooperative study on the use of warfarin in the treatment of Small Cell Carcinoma of the Lung (SCCL) revealed that warfarin administration in addition to standard chemotherapy to cancer patients results in an increase in the median survival (50 weeks) compared to those patients that received chemotherapy alone (26 weeks) (Zacharski *et al*., 1981).

DISCUSSION

Brown hypothesized that deceleration of the clotting mechanism and a concomitant increase of the clearance of tumor cells from the lungs of mice treated with warfarin (as it was shown after i.v. injection of iododeoxyuridine-12Sl-labeled tumor cells), may be a feasible explanation for the anti-tumor action of warfarin. Cytotoxic effects and inhibition of tumor cell proliferation or migration were excluded due to the initial trapping of all injected cells into the lungs (Brown, 1973). The absence of coagulation-dependent mechanism for the anti-tumor action of phenprocoumon and vitamin K antagonists in general is being further supported by experiments conducted by Hilgard who have shown that initiation of vitamin K deficiency has the same tendency of reduction of pulmonary metastases as a high (0.3 mg day^{-1}) or low (0.03 mg day^{-1}) dose of phenprocoumon (Hilgard, 1977). Thus, vitamin K depletion could probably explain the anti-metastatic action of anticoagulants. An interesting and attractive mechanism is proposed by Hilgard that involves vitamin K mediated binding of Gamma-carboxyglutamic acid (Gla) to proteins, a prerequisite for coagulation factors to bind to Ca^{+} and phospholipids. The presence of Gla on tumor cells could offer an explanation for the inhibition of metastases formation after vitamin K depletion by anticoagulants.

The involvement of other mechanisms except from inhibition of clotting mechanism, becomes also obvious when looking to experiments with mice where human prothrombin complex concentrate, did not reverse the protection from metastases that was caused be warfarin administration (Hilgard and Maat, 1979). On the contrary, Brown has shown that administration of vitamin K succeeded in reversing the anti-metastatic action of warfarin (Brown, 1973).

An involvement of the NK cells in warfarin-induced inhibition of melanoma and mammary cancer metastases in mice was recently proven. The way by which NK cells could be involved in the anti-tumor effect of warfarin could be explained with the following mechanism: Paolino *et al*. (2014) have shown that genetic deletion of the E3 ubiquitin ligase Cbl-b or inactivation of the E3 ligase activity that is expressed on human and murine NK cells, results in inhibition of spontaneous lung metastases in melanoma animal models and in oncogenic-driven mammary cancer in mice. It seems to play an important role in NK cell anergy. TAM receptors are substrates for CBL-B ubiquitylation. Paolino *et al*. (2014) showed that the administration of a TAM inhibitor results in inhibition of lung metastases in mice in a NK-mediated manner. This was proven, after transfer of NK cells treated with this TAM inhibitor to B16F10-bearing mice (melanoma cell line). The results were compared with the results from mice that received untreated NK cells. An increased anti-tumor response was documented (Paolino *et al*., 2014). Warfarin is able to inhibit activation of TAM receptors by preventing gamma-carboxylation, rendering the activation of the receptors through Gas6 (a growth arrest-specific gene that is a vitamin K dependent protein) impossible (Paolino *et al*., 2014). The absence of NK cells abolishes the anti-metastatic effects of warfarin. Thus, a possible mechanism hidden behind warfarin-induced anti-tumor activity probably involves NK cells and TAM/Cbl-b (Paolino *et al*., 2014).

The timing and duration of administration seems to play a vital role because intermittent in contrast to continuous administration, inhibits primary tumor growth but did not influence metastasis formation (Hilgard *et al*., 1977).

CONCLUSION

Future investigations that will evaluate the involvement of the anti-metastatic effects of warfarin in OSCC patients that do not normally develop lung metastases will be very helpful in evaluating a possible anti-tumor effect of warfarin in OSCC. The hypothesis that warfarin may be able to interfere with other NK receptors, others than TAM, like CCR7 that are involved in the migration of NK cells to lymph nodes is very attractive and will help in understanding of the immune-modulatory actions of anticoagulants (Warnock *et al*., 2000). Other questions that remain to be elucidated include:

- Has the administration of anticoagulation drugs an influence on primary tumor growth and lymph-node metastases in OSCC?
- Is the timing of the administration, important for the anti-metastatic action in humans?
- Is the anti-tumor action of warfarin tumor-type dependent?

Future investigations that will investigate the effect of warfarin treatment in cancer progression in OSCC patients as well as of the prophylactic treatment with warfarin in OSCC carcinoma, promise to elucidate the presence of a possible immune-modulatory action of warfarin.

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