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Original Article

Effects of everolimus on platelet, D-dimer, and fibrinogen in cancer patients

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Abstract: Objective: Alterations in platelet count, D-dimer levels, and fibrinogen concentration were studied in the blood of patients with active tumors following the administration of everolimus, with the objective of elucidating their significance in relation to thrombosis. Methods: Fourteen patients treated with everolimus alone and fourteen healthy controls in our hospital between January 2020 and January 2025 were selected. The baseline plasma levels of D-dimer, fibrinogen, and platelet count on day 0 were compared to those after 21 days of treatment with everolimus tablets. Results: Platelet count, D-dimer and fibrinogen of patients with active tumors before treatment were higher than those of healthy controls ($P<0.05$). After 21 days of treatment, the plasma platelet count and D-dimer significantly decreased ($P<0.05$), whereas non-significant differences were observed in plasma levels of fibrinogen ($P>0.05$). Conclusion: The study confirmed that everolimus partially regulates cancer patients' coagulation profile, especially reducing elevated D-dimer, potentially lowering thrombotic risks. Limitations included small sample size, single-center design, short follow-up, and no tumor stage subgroup analysis. Future multi-center, long-term studies with stratified analyses and mechanistic/combination therapy exploration are needed to optimize coagulation and prognosis.

Keywords: Everolimus, cancer patients, hypercoagulability

Introduction

Everolimus, 40-O-(2-hydroxyethyl)-rapamycin, is a semisynthetic sirolimus derivative that inhibits mammalian rapamycin target (mTOR) [1]. Everolimus exerts its anti-tumor effect by a mechanism where, following cellular internalization, rapamycin first forms a complex with the immunoaffinity protein FKBP-12 in the cytoplasm and binds to mTORC1, thereby inhibiting its activity, resulting in cell cycle arrest during the G1 phase and promoting apoptosis in tumor cells. Studies have reported that mTOR can be activated through the AkT-independent Ras/MEK/ERK pathway to exert an anti-tumor effect [2]. Furthermore, abnormal activation of protein kinase B (AKT) was observed in patients with endocrine-resistant breast cancer, resulting in irregular activation of the PI3K/AKT/mTOR signaling pathway and exccessive tumor proliferation. Everolimus reverses endocrine resistance by blocking the PI3K/AKT/mTOR pathway to promote breast cancer treatment [3]. The PI3K/AKT signaling pathway plays a role in regulating the activation and aggregation of platelets (PLTs) and can initiate thrombotic events [4]. Consequently, this study compared the plasma levels of platelets (PLT), D-dimer, and fibrinogen in cancer patients before and after everolimus treatment. The results demonstrated that 21-day administration of everolimus tablets led to a significant reduction in both platelet count and D-dimer levels. Studies in solid tumor patients have indicated that D-dimer levels may rise transiently in the early stage of everolimus treatment, linked to tumor cell necrosis and coagulation activation. Conversely, long-term treatment often results in reduced D-dimer levels due to thrombocytopenia and suppressed coagula-

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tion, with heterogeneous outcomes across different patient populations. No direct, significant effect of everolimus on fibrinogen concentration has been identified; changes in fibrinogen levels are mostly secondary to altered coagulation status or concurrent medications. The key mechanism mediating these effects involves the suppression of megakaryocyte proliferation and differentiation, which disrupts the bone marrow hematopoietic microenvironment and reduces platelet synthesis [5].

Patients and methods

General data

A total of fourteen cancer patients who received treatment with everolimus alone and fourteen healthy controls at our institution between January 2020 and January 2025 were selected as research subjects. Among them, four had neuroendocrine tumors, three had breast cancer, and one had renal cell carcinoma. The selected patients comprise nine males and five females, averaging 52.14 ± 16.25 years (26-76 years). The healthy controls comprised eight males and seven females, averaging 50.74 ± 13.15 years (30-70 years). There was no significant difference in gender or age between the two groups (P>0.05). Informed written consent was obtained from all patients prior to the commencement of the study.

Inclusion criteria

Only patients with pathologically confirmed neuroendocrine tumors, breast cancer, and renal cell tumors were selected for the study. Additionally, the study included those who were currently taking everolimus tablets (10 mg once daily) as a single drug without any other drugs, with an interval between chemotherapy, targeted drugs, radiotherapy, and others of at least 21 days. The healthy control group comprised individuals with no history of relevant diseases and no medication use within the preceding 4 weeks.

Exclusion criteria

The exclusion criteria included the following: All patients who had abnormal blood routine or coagulation function; were unable to take continued medication due to side effects; had severe heart, liver, or kidney dysfunction; had received targeted therapy or chemotherapy within the past 21 days; or were using drugs in combination with other systemic diseases.

Methods

A total of 2-4 mL venous blood samples at fasting were withdrawn from the enrolled patients and healthy individuals for routine baseline blood and coagulation function tests before treatment. The Beckman COULTER LH780 hematology analyzer was utilized to measure the plasma platelet (PLT) count, with a normal reference range of 125-350 × 10^9/L. The plasma levels of fibrinogen (2.0 to 4.0 g/L) and D-dimer (0 to 0.5 mg/L) were found to be comparable. The patients were then placed over an everolimus (10 mg once daily) treatment regimen for 21 days, and the final levels of PLT, fibrinogen, and D-dimer were measured again on the 21st day of treatment.

Statistical analysis

All data were analyzed using SPSS 25.0 software. For measured data that did not adhere to a normal distribution, the rank sum test was employed, and the t-test was employed to describe data that conformed to the normal distribution. An independent samples t-test was employed to compare outcomes across different groups, whereas a paired samples t-test was utilized for longitudinal comparisons within the same group. All data were presented as the mean ± standard deviation ($\chi \pm s$). A *p*-value of less than 0.05 was considered significant.

Results

Significant differences were observed in platelet count (P=0.024), D-dimer (P=0.004), and fibrinogen (FIB) (P=0.015) between the healthy control group and the pretreatment patient group. The results showed that the PLT data were normally distributed, confirmed by the t-test. After 21 days of everolimus tablet treatment, the plasma PLT count significantly decreased (P<0.001). Significant differences were also identified relative to the healthy control group (P<0.001) (**Figure 1**). Discernible statistical variation (P=0.002<0.05) was noted in the plasma D-dimer concentrations. The data were normally distributed (t-test) after 21 days of everolimus treatment. Significant sta-

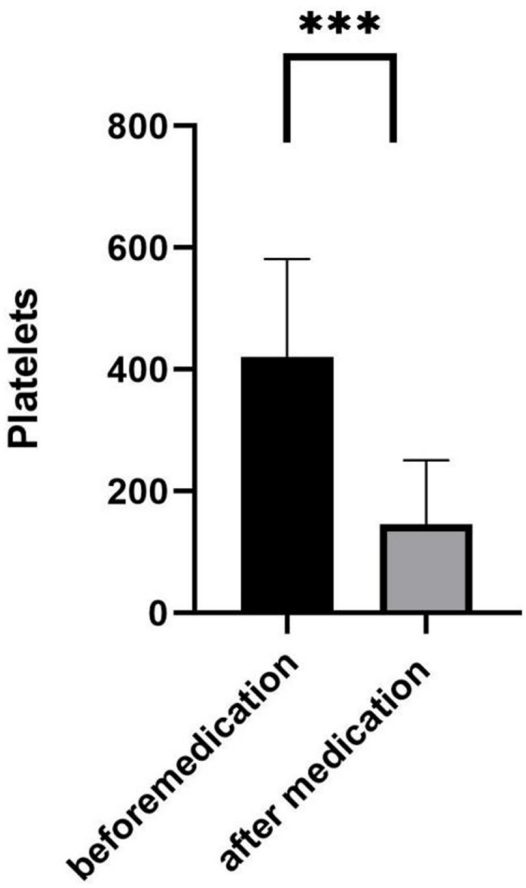

Figure 1. Comparison of platelet counts before and after everolimus treatment.

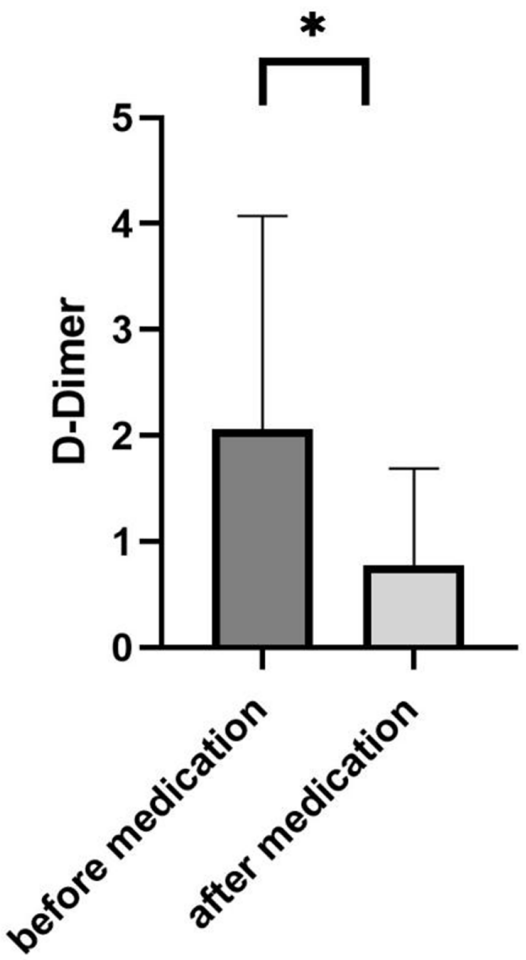

Figure 2. Comparison of D-dimer before and after everolimus treatment.

tistical differences were also identified relative to the healthy control group (P=0.003<0.05) (**Figure 2**). Furthermore, no discernible statistical variation (P=0.368>0.05) was noted in the plasma r fibrinogen concentrations before and after treatment on the 21st day and no significant difference was noted in comparison to the healthy control group (P=0.14>0.05), where the data were not normally distributed, as described by the rank sum test (**Figure 3**).

Discussion

The incidence of malignant tumors has increased in recent years, rising each year, and it has a complex progression mechanism. Malignant tumors are associated with abnormal coagulation function, whereas metastasis and invasion of surrounding tissues result in rapid progression of the tumor and are associated with abnormal coagulation mechanisms. Cancer patients experience different degrees of hypercoagulability in the blood, with thrombosis being the major and most common complication responsible for the higher death rate. It has been reported that almost 95% of cancer patients, especially those with advanced cancer, suffer one or more coagulation abnormalities [6]. Excessive platelet activation has been observed in certain malignancies, including colon cancer [7]. The interaction between cancer cells and platelets has been demonstrated to enhance their survival and metastatic potential [8]. This study demonstrated that cancer patients exhibited significant differences in platelet count, D-dimer, and fibrinogen compared to healthy individuals. The activation and apoptosis of platelets are intricately linked to the phosphatidylinositol 3-kinase/protein kinase B (PI3K/PKB or PI3K/Akt) signaling pathway. The PI3K family encompasses

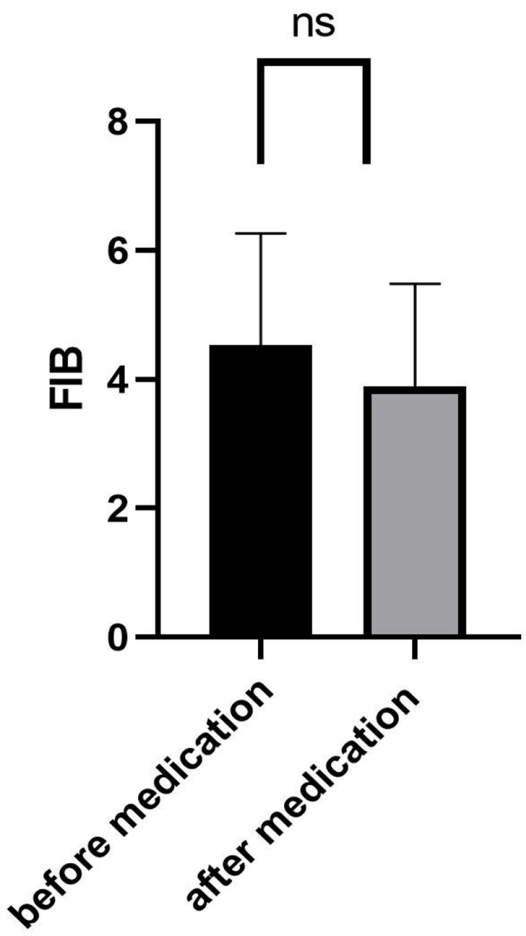

Figure 3. Comparison of FIB before and after everolimus treatment.

a diverse array of lipid kinases that adeptly receive and transduce signals from transforming growth factors, cytokines, and myriad other molecules into intricate intracellular signals, thereby orchestrating a multitude of signaling cascades to modulate an array of physiological functions and cellular processes, such as cell proliferation, growth, survival, and metabolic regulation [9]. The lipid metabolic enzyme phosphatidylinositol 3-kinase (PI3K), which is involved in platelet activation through the production of 3-phosphatidylinositol, has been considered a possible target for novel antithrombotic therapy [10]. The outer membrane of apoptotic platelets, when exposed to negatively charged phospholipids, serves as a crucial procoagulant factor that significantly expedites the thrombosis process [11]. Recent studies highlight the critical role of the PI3K/Akt signaling pathway in atherosclerosis development. By inducing Akt phosphorylation, PI3K effectively modulates downstream targets including B-cell lymphoma-2 gene-associated promoter (Bad), cysteinyl aspartate protease (caspase-9), glycogen synthase Ki-enzyme-3β (GSK-3β), and mammalian target of rapamycin (mTOR) [12]. Tumor cells and damaged endothelial cells activate the coagulation system by cell-cell interactions, directly releasing tissue factor (TF) and cancer procoagulant (CP) [13]. The 14 patients in this study exhibited a hypercoagulable state when compared to the healthy population.

Everolimus is a targeted mTOR blocker primarily used in treating various immune system diseases and organ transplantation [14]. It has also been shown to possess anti-tumor effects in different cancers and is currently the only oral mTOR inhibitor available on the market. It has garnered official endorsement for the therapeutic management of advanced renal cell carcinoma (RCC), advanced pancreatic neuroendocrine tumor (pNET), Tuberous Sclerosis-Linked Subependymal Giant Cell Astrocytoma (SEGA), renal angiomyolipoma (TSC-AML), postmenopausal women with advanced breast cancer characterized by estrogen receptor positivity and Her2 negativity, and other tumors. This study sought to explore the profound impact of everolimus administration on plasma levels of PLT, D-dimer, and FIB to explore its effect on coagulation function. Platelets are derived from mature megakaryocytes in the bone marrow and play a pivotal role in coagulation, hemostasis, and thrombosis, where the PLT count reflects the status of PLT production and decline [15]. The results revealed that everolimus decreased ($P<0.05$) the PLT count. The activation of the PI3K/Akt signaling pathway is closely related to PLT-related signal transduction pathways. It is located at the junction of mitochondrial-mediated PLT apoptosis pathways, which can directly or indirectly regulate PLT apoptosis by phosphorylation or interaction with related apoptotic proteins [16]. PLT autophagy is regulated by the PI3K/AKT/mTOR pathway, and mTOR plays a key negative regulatory role in this process [17].

Fibrinogen is the major plasma entity responsible for hemostasis and thrombosis. It directly affects coagulation activity, and its plasma levels can severely affect blood coagulation and hemorheology [18]. Fibrinogen can activate inflammatory signaling pathways and sti-

mulate inflammatory cells, such as macrophages, to secrete different cytokines, including tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and nuclear factor-κB (NF-κB), thereby regulating the inflammatory response. Promoting the PI3K/Akt signaling pathway can enhance the activity of TNF-α-induced pro-inflammatory and adhesion factors, aggravate vascular wall damage, and easily lead to thrombosis. However, in our study, the effect of everolimus on fibrinogen level was non-significant ($P>0.05$). It is also plausible that the limited sample size introduced certain experimental errors.

D-dimer, a product of the dissolution of cross-linked fibrin clots by plasmin, is a biomarker for assessing fibrinolytic activity, and an increased or positive D-dimer is found in secondary hyperfibrinolytic function [19]. The blood vessels in the body exhibit activated thrombosis and fibrinolysis activities, resulting in an elevation of D-dimer levels. Research has shown that D-dimer levels are significantly elevated in patients with various solid tumors, including lung, liver, prostate, and colorectal cancers [20]. The elevation of D-dimer levels indicates an augmentation in secondary fibrinolysis, which serves as a crucial indicator of a hypercoagulable state and hyperfibrinolysis within the body. Studies have indicated that plasma D-dimer levels determine disease progression and are closely correlated with tumor staging and metastasis [21]. Some studies have indicated that D-dimer serves as an independent predictor for thrombosis in cancer patients, and it can effectively reflect the hypercoagulable state of these patients [22]. This study demonstrated that D-dimer levels were significantly higher in patients with active tumors compared to the healthy control group. While everolimus treatment resulted in a reduction in D-dimer levels, these values remained above the normal reference range. Thus, it is hypothesized that everolimus may exert certain antithrombotic effects in patients with active cancer.

Conclusion

This study systematically evaluated the effects of everolimus on three key coagulation-related indicators - platelets, D-dimer, and fibrinogen - in cancer patients, aiming to clarify the drug's regulatory role in the coagulation profile of this population. The results demonstrated that, compared to the healthy control group, cancer patients exhibited significant abnormalities in target indicators prior to everolimus treatment. After 21 days of treatment, there was a significant decrease in platelet counts and D-dimer levels, while fibrinogen levels remained unchanged with no significant statistical difference. This suggests the drug's effect on fibrinogen may be weaker than its effect on D-dimer or platelets, or that a longer treatment duration may be required to elicit a detectable response. Importantly, despite the favorable reductions in some indicators, D-dimer and platelets failed to return to the normal reference range, indicating that single-agent everolimus may not fully reverse the coagulation abnormalities caused by malignant tumors, possibly due to the persistent influence of tumor burden or the complex interplay between tumor cells and the coagulation system. Collectively, these findings confirm that everolimus exerts a partial regulatory effect on the coagulation profile of cancer patients, particularly in reducing elevated D-dimer levels, which may have clinical implications for lowering thrombotic risk and improving the systemic condition of patients. Nevertheless, this study had certain limitations, such as the small sample size, single-center design, short follow-up period, and lack of subgroup analysis by tumor stage. Future studies should employ larger multi-center cohorts, longer-term follow-up, and stratified analyses to validate the consistency of these findings across different cancer subtypes. Furthermore, exploring the mechanisms by which everolimus - via inhibiting mTOR signaling to modulate tumor cell-induced coagulation factor secretion and investigating combined everolimus plus anti-coagulant agents - may help optimize the normalization of coagulation function in cancer patients, improving their prognosis and quality of life.

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The written consent to publish was obtained from the patient. Verbal informed consent was obtained from all subjects before the study.

Disclosure of conflict of interest

None.

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