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Anti-angiogenic effect of 5-Fluorouracil-based drugs against human colon cancer xenografts

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Abstract

In addition to the direct cytotoxic effects of chemotherapy agents on tumor cells, the anti-angiogenic activities attained by these agents by targeting proliferating endothelial cells in tumor blood vessels has attracted much research interest. In this study, we examined the antitumor activity of 5-Fluorouracil (5-FU)-based drugs (S-1 [1 M tegafur, 0.4 M 5-chloro-2,4-dihydroxypyridine and 1 M potassium oxonate] and capecitabine) on human colorectal cancer xenografts and evaluated their anti-angiogenic effects. Both drugs showed significant antitumor activities against COL-1 xenografts at a sub-maximum tolerated dose (sub-MTD), which was lower than the maximum tolerated dose (MTD). At the sub-MTD, a significant reduction in the microvessel number and the enhancement of tumor-associated microvessel endothelial cell apoptosis was seen in xenografts treated with S-1. In addition, we found that thrombospondin-1 (TSP-1) expression, known to be a mediator of the anti-angiogenic effects of metronomic chemotherapy, was significantly up-regulated in xenograft tumor tissues and plasma in animals treated with S-1 at a sub-MTD. Capecitabine also showed a trend toward the induction of TSP-1. These results suggest that 5-FU-based drugs inhibit tumor progression through different modes of action, including cytotoxic activity derived from 5-FU and the inhibition of angiogenesis through the induction of TSP-1.

Keywords: 5-Fluorouracil; TSP-1; Metronomic chemotheraphy

1. Introduction

Maximum tolerated dose (MTD) chemotherapy sometimes causes undesirable side effects normally associated with traditional cytotoxic chemotherapy regimens; during the break periods between successive cycles of MTD chemotherapy, the marked mobilization of hematopoietic progenitors from the marrow into the peripheral blood circulation sometimes occurs [1]. Kerbel et al. [2] and others [3] have shown that the use of chronic, low-dose chemotherapy administered at close regular intervals with no prolonged breaks (termed metronomic chemotherapy) inhibits angiogenesis by targeting the genetically

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stable endothelium of the neovasculature and circulating proangiogenic bone marrow-derived cells, including circulating endothelial progenitor cells (CEP) [4,5]. This treatment modality targets tumor cells indirectly by inhibiting angiogenesis through the continuous exposure of the more slowly proliferating endothelial cells to cytotoxic therapy, since cycling endothelial cells may actually be more sensitive to certain chemotherapeutic drugs than other types of normal cells [4,6,7]. Bertolini et al. found that MTD and low-dose metronomic cyclophosphamide (CTX) regimens have opposite effects on the mobilization and viability of CEPs [4]. Thus, metronomic chemotherapy may eradicate tumors via different mechanisms: (1) it may directly affect tumor cells; (2) it may cause direct endothelial cell death or growth inhibition; or (3) it may decrease the mobilization or viability of bone marrow-derived CEPs, which contribute to the tumor vasculature [5]. The effect of metronomic chemotherapy might also be associated with the endogeneous angiogenesis inhibitor thrombospondin-1 (TSP-1) in the tumor microenvironment. TSP-1 promotes endothelial cell apoptosis [8] and can suppress the mobilization of CEPs [9]. Thus, tumors that express a high basal level of TSP-1 may be more susceptible to tumor suppression by metronomic chemotherapy.

As shown by various studies, metronomic chemotherapy may offer several advantages over MTD therapy, including a treatment response irrespective of the resistance profile of the tumor cell population and a low toxicity [10–13]. Browder et al. suggested that the antitumor effects of chemotherapeutic drugs may be nullified by the long break periods between successive cycles of MTD therapy because the damage to the activated endothelial cells in the tumor vessels may be reversed by various mechanisms; he proposed a strategy for optimizing the anti-angiogenic effects of chemotherapy by chronically administering drugs on a more frequent schedule at a lower dose than the MTD with no long breaks [3]. Colleoni et al. first suggested that continuous low-dose CTX would be ideal for metronomic chemotherapy in breast cancer patients [14]. Furthermore, Munoz et al. also proposed that the combination of low-dose CTX and UFT, which can be administered orally more frequently – sometimes every day, might be useful for metronomic chemotherapy [15]. UFT, which is composed of tegafur (a 5-FU prodrug) and uracil at a molar ratio of 1:4, is presumed to help maintain the concentration of 5-FU for long periods and is classified as a dihydropyrimidine dehydrogenase (DPD)-inhibitory fluoropyrimidines (DIF). Yonekura et al. demonstrated that UFT has a stronger angiogenesis-inhibitory effect than 5-FU or 5′-DFUR [16]. Recently, randomized adjuvant clinical trials have been undertaken in patients with resected pathological stage I non-small cell lung cancer (NSCLC) [17]. These patients were given daily doses (250 mg/m^2) of oral UFT for 2 years; this treatment strategy resulted in both a survival benefit and a relatively low toxicity. A more recent study has shown that UFT administration improved the poor postoperative survival of patients with tumors exhibiting high microvessel densities [18]. These results suggested that UFT inhibits tumor-induced angiogenesis, possibly prolonging survival or contributing to the antimetastatic effects in postoperative UFT adjuvant studies.

S-1, which is a successor of UFT, is an oral anticancer agent based on the biochemical modulation of 5-FU; it consists of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP; a potent DPD inhibitor), and potassium oxonate (an orotate phosphoribosyltransferase inhibitor) at a molar ratio of 1:0.4:1. CDHP competitively inhibits DPD approximately 180 times more effectively than uracil [19–21], leading to the retention of 5-FU in the blood for prolonged periods. S-1 also showed a high clinical efficacy when used in patients with several tumors (one course consisted of the administration of 100 mg/day for 4 weeks and a 2-week withdrawal period) [22,23]. Recently, the results of an Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer (ACTS-GC) showed that for all randomized patients, the overall survival at 3 years was 80.5% for patients receiving S-1 and 70.1% for patients undergoing surgery alone, with a hazard ratio (HR) of 0.68 [24]. This trial proved that S-1 significantly reduced the relative risk of death in early-stage gastric cancer patients. The effectiveness of S-1 as an adjuvant chemotherapy and its pharmacokinetics struck us as being similar to the concept of metronomic chemotherapy, and we postulated that S-1 might have anti-angiogenic properties.

In this study, we verified the antitumor activity and the anti-angiogenic effect of S-1 and compared them with those of capecitabine, which is also classified as a fluoropyrimidine, on human colorectal cancer xenografts. In addition, we evaluated the association between the anti-angiogenic effect and TSP-1 and vascular endothelial growth factor (VEGF) expression.