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Mini-review

Metronomic chemotherapy: A potent macerator of cancer by inducing angiogenesis suppression and antitumor immune activation

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ABSTRACT

Metronomic chemotherapy is a low dosing treatment strategy that attracts growing scientific and clinical interest. It refers to dense and uninterrupted administration of low doses of chemotherapeutic agents (without prolonged drug free intervals) over extended periods of time. Cancer chemotherapy is conventionally given in cycles of maximum tolerated doses (MTD) with the aim of inducing maximum cancer cell apoptosis. In contrast, the primary target of metronomic chemotherapy is the tumor's neovasculature. This is relevant to the emerging concept that tumors exist in a complex microenvironment of cancer cells, stromal cells and supporting vessels. In addition to its anti-angiogenetic properties, metronomic chemotherapy halts tumor growth by activating anti-tumor immunity, thus decreasing the acquired resistance to conventional chemotherapy. Herein, we present a review of the literature that provides a scientific basis for the merits of chemotherapy when administered on a metronomic schedule.

Introduction

Metronomic chemotherapy (MC) is the protracted and dense administration of low dose chemotherapy with no prolonged drug-free intervals [1]. MC was originally thought to mainly target tumor vasculature as opposed to conventional chemotherapy designed to kill tumor cells by administering the maximum tolerated dose (MTD) [2,3]. However tumors are not solely aggregates of cancer cells, but rather complex tissues comprised of cancer cells, stromal cells, immune cells and vessels. Tumors need nutrients and oxygen to grow, and an ability to discard metabolic wastes and carbon dioxide thus the formation of new vessels is indispensable [4]. This tumor-associated neovasculature is generated by the process of angiogenesis and addresses these needs.

Angiogenesis has been considered a hallmark of cancer [5] and an “organizing principle” in cancer drug discovery [6]. Inhibition of angiogenesis is one of the primary mechanisms of action of MC. It represents a treatment strategy that is able to circumvent acquired resistance to antineoplastic agents, as suggested by Kerbel twenty five years ago [7]. Thus, MC is an alternative approach of restoring tumor sensitivity to chemotherapy.

Immune escape is another hallmark of cancer [5]. Tumors evade immune destruction by activating mechanisms of immune tolerance and suppression [8]. Reversal of such mechanisms may promote tumor elimination. Treatments that eliminate immunosuppressive cell populations could possibly lead to the restoration of an antitumor immune response [9]. Low doses of chemotherapeutics, such as MC have been shown to selectively kill such immunosuppressive cell populations [10] and tilt the immune system from immunosuppression to immunostimulation [11] inducing tumor dormancy [12].

A growing body of evidence indicates a pleiotropic action for MC [10], making it an attractive alternative to conventional chemotherapy that requires scientific attention. This review discusses the antiangiogenic activity and the immune stimulatory properties of MC, in order to enlighten the different mechanisms of its action.

Materials and methods

Data from all original articles, reviews and clinical trials published were included using literature electronic databases searching (Pubmed, Medline and clinicaltrials.gov) A literature search was performed using the following combinations of terms: “MC”, “angiogenesis”, “antitumor immunity”, “endothelial cell”, “immunosuppression”. The search was restricted to English-language manuscripts. Search strategy was performed up to September 2016.

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Antiangiogenic role of metronomic chemotherapy

Metronomic chemotherapy was initially considered a treatment strategy that inhibits angiogenesis. The first evidence of the anti-angiogenic action of MC came in 2000. Browder et al. reported that murine tumors made of cyclophosphamide-resistant cancer cells were refractory to cyclophosphamide when administered in a conventional protocol [13]. On the contrary, tumors regressed when cyclophosphamide was administered at lower doses and more frequently, because of sustained endothelial cell apoptosis. The latter was absent with the conventional protocol, due to the rescue of endothelial cells during the drug-free periods. These data introduced the principle of constant, low dose, antiangiogenic scheduling. The same time, Klement et al. demonstrated that regular administration of non-cytotoxic doses of vinblastine inhibited tumor growth by impairing tumor vascularization [14]. Moreover in both studies, combination with an antiangiogenic agent acted synergistically [13,14]. Following these reports, this new schedule of chemotherapy was termed "metronomic" due to its characteristic regularity on drug administration [1]. Since then, various researchers have verified these original observations [15,16].

Microtubule-targeting agents, a class of drugs widely used in cancer chemotherapy, have been shown to possess antiangiogenic and vascular-disrupting properties at low concentrations through direct effect on endothelial cells [17–20]. We have previously demonstrated that low nanomolar metronomic concentrations of vinorelbine inhibited all critical steps of angiogenesis in vitro as assessed by various assays. In particular, metronomic vinorelbine inhibited endothelial cell proliferation, migration, tube formation and sprouting [21,22]. Notably, this action occurred with a clinically effective and non-toxic metronomic concentration that we previously determined in the clinical trial setting [23,24].

Angiogenesis is a tumor supporting process, which is kept up aberrantly active through the influence of counterbalancing proteins that are produced both by the cancer cells and the microenvironment [25]. In response to hypoxia, endothelial and other cells of the microenvironment release growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGFb), platelet-derived growth factor (PDGF) and interleukin (IL)-8 which are responsible for the blood vessel formation [26]. These factors have different roles in this process, with VEGF initiating formation of sprouts [27] and FGFb providing signals for endothelial cell proliferation and tube formation [28], followed by vessel stabilization by PDGF. On the other hand, antiangiogenic growth factors such as thrombospondin (TSP)-1, maintain the angiogenetic process under control [29]. In the context of cancer however, several angiogenic stimulators, such as RAS, c-myc, and epidermal growth factor receptor (EGFR), overcome this TSP-1 inhibition of angiogenesis [30], leading to unrestrained release of angiogenesis-promoting signals.

Several studies have shown that MC inhibits the release of angiogenic factors; for example low doses of paclitaxel has been shown to decrease VEGF [31] while we have noted that metronomic vinorelbine suppresses IL-8 in vitro [21]. Additionally, low doses of metronomic doxorubicin were recently reported to block hypoxia inducible factor (HIF)-1α binding to DNA. HIF-1α is the first growth factor released from the tumor site and is responsible for the transcription of correlated genes such as VEGF, FGFb and IL-8 [32]. Inhibition of its activity increased destruction of the tumor vasculature and overcame resistance to antiangiogenic therapies [33]. Loges et al. reported that antiangiogenic treatment leads to a hypoxic microenvironment through restriction of the tumor blood supply [34]. Moreover, tumor hypoxia has been shown to be the initiating factor of secondary resistance to both antiangiogenic treatment [34] and chemotherapy [35]. We have recently reported that metronomic vinorelbine inhibited endothelial cell migration, sprouting and tube formation to the same extent in both normoxic and severe hypoxic conditions. Although severe hypoxia attenuated the antiproliferative action of metronomic vinorelbine, this effect was reversed by Akt inhibition [22].

Complementary to the in vitro studies of MC, clinical trials of low dose and dense administration of drugs have been conducted with encouraging results. In Table 1 we summarize the clinical effect of pure metronomic chemotherapy protocols that have been used over the last 6 years. Except for the trials that were designed to test the efficacy and the tolerability of MC, several studies have examined the levels of several pro- and anti-angiogenic molecules pre- and post- MC and their potential role as predictors of response. In a study by Zeng et al. metronomic therapy with cyclophosphamide, prednisone and etoposide led to a decrease in serum circulating endothelial cells (CECs) and VEGF levels [36]. Interestingly enough, in those patients that response was noted, VEGF and CECs levels remained relatively low even at 12 months post treatment [36]. In another study, the combination of daily dalteparin, oral cyclophosphamide, twice-weekly methotrexate, and daily prednisone (dalCMP), VEGF and soluble VEGF receptors (sVEGFRs) -1 and -2 were used as predictive biomarkers. VEGF levels were found to be decreased though not significantly, whereas sVEGFR-1 and -2 levels increased significantly after 2 weeks of therapy. No correlation was noted between response and VEGF, sVEGFR-1, or sVEGFR-2 levels, thus their role as predictive biomarkers could not be established [37]. In contrast, in a recent phase II study of metronomic cyclophosphamide and celecoxib in patients with advanced breast cancer patients (ABCP), serum baseline VEGF and VEGF/sVEGFR2 ratio were associated with time to progression (TTP) suggesting that they could be useful markers of early predictors of response [38]. Earlier, the same group had reported that VEGF/sVEGFR-2 and VEGF/TSP-1 ratios were associated with TTP and therefore could serve as potential biomarkers of response in ABCP treated metronomically with the same regimen [39]. Briasoulis et al., also reported on potential biomarkers in the context of MC. Low pre-treatment blood concentrations of FGF2 and IL8 predicted favorable response to metronomic vinorelbine, while high levels of TEK gene transcript predicted treatment resistance [23].

It is worth highlighting the remark made by Kareva et al. regarding the different mechanisms of the anti-angiogenic effects of conventional anti-angiogenic drugs that target individual molecules and the anti-angiogenic actions of MC that targets endothelial cells and inhibit the production of growth factors at the source [40]. More specifically, drugs such as bevacizumab bind to extracellular VEGF and switch off the molecular pathway; thus sprout formation is inhibited [41]. On the other hand, MC damages cells like fibroblasts and endothelial cells [3,14]. Therefore, the different mechanisms MC uses to inhibit angiogenesis and the primary target may be the reason why MC has more lasting effects in time [40].

Metronomic chemotherapy decreases the acquired therapeutic resistance

Until recently, treatment of cancer was based on the concept of the MTD, consisting of the highest survivable (minimum lethal) dose, steered to kill as many tumor cells as possible. This strategy has resulted in increased response rates and occasionally increased survival rates in recent years. However, the majority of cancer patients, after a period of disease regression or stabilization, experience tumor progression [42]. The efficacy of conventional schedules is circumvented by several factors, one of them being the heterogeneity and the genomic instability of tumor cells [43]. Tumors are characterized by high levels of both genotypic and