Cancer Letters 400 (2017) 282–292

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

# Metronomic chemotherapy and immunotherapy in cancer treatment

Yu-Li Chen [a, b, c], Ming-Cheng Chang [c], Wen-Fang Cheng [c, d, e, *]

[a] Graduate Institute of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan
[b] Department of Obstetrics and Gynecology, National Taiwan University Hospital Hsin-Chu Branch, Hsin-Chu City, Taiwan
[c] Department of Obstetrics and Gynecology, College of Medicine, National Taiwan University, Taipei, Taiwan
[d] Graduate Institute of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan
[e] Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

*Article history:*
Received 22 October 2016
Received in revised form
26 January 2017
Accepted 26 January 2017

*Keywords:*
Metronomic chemotherapy
Immunotherapy
Cancer therapy

ABSTRACT

Systemic chemotherapy given at maximum tolerated doses (MTD) has been the mainstay of cancer treatment for more than half a century. In some chemosensitive diseases such as hematologic malignancies and solid tumors, MTD has led to complete remission and even cure. The combination of maintenance therapy and standard MTD also can generate good disease control; however, resistance to chemotherapy and disease metastasis still remain major obstacles to successful cancer treatment in the majority of advanced tumors. Metronomic chemotherapy, defined as frequent administration of chemotherapeutic agents at a non-toxic dose without extended rest periods, was originally designed to overcome drug resistance by shifting the therapeutic target from tumor cells to tumor endothelial cells. Metronomic chemotherapy also exerts anti-tumor effects on the immune system (immunomodulation) and tumor cells. The goal of immunotherapy is to enhance host anti-tumor immunities. Adding immunomodulators such as metronomic chemotherapy to immunotherapy can improve the clinical outcomes in a synergistic manner. Here, we review the anti-tumor mechanisms of metronomic chemotherapy and the preliminary research addressing the combination of immunotherapy and metronomic chemotherapy for cancer treatment in animal models and in clinical setting.



## Introduction

Systemic chemotherapy continues to be the mainstay of standard treatment for human cancer. All cytotoxic chemotherapeutic agents exert their effects by disrupting the cell cycle via one or more processes [1]. These agents are usually given at the maximum tolerated doses (MTD) with the goal of achieving total eradication of the cancer cells [2]. MTD schedules have led to complete remission or even cure in some chemosensitive malignancies, especially hematological malignancies such as pediatric acute lymphoblastic leukemia (ALL) and Hodgkin's lymphoma [3] and some solid tumors [1,4–7]. In addition, the combination of maintenance therapy and standard MTD may generate good disease control [8–10].

Despite the initial therapeutic responses after first-line MTD chemotherapy, a considerable number of patients experience disease relapse caused by resistance to chemotherapy. Therefore, an effective therapeutic strategy targeting specific mechanisms involved in tumorigenesis is needed [2]. A new era in cancer chemotherapy began in 2000 when Browder et al. demonstrated that an anti-angiogenic continuous low-dose schedule of cyclophosphamide is more effective than a conventional schedule in overcoming cultured breast cells with drug resistance [11]. In the same year, Klement et al. demonstrated that regular administration of low-dose vinblastine combined with anti-vascular endothelial growth factor (VEGF) receptor (VEGFR)2 antibody (DC101) produced sustained regression of tumors [12]. Following on this, Hanahan et al. coined the term “metronomic chemotherapy” introduced by the two aforementioned teams to describe this chronic, equally-spaced, low-dose administration of therapeutic agents without extended rest periods [11–13].

Metronomic scheduling was originally designed to overcome drug resistance by shifting the therapeutic target from tumor cells to the tumor vasculature, and more specifically, the tumor endothelium [14–16]. Additional mechanisms by which metronomic chemotherapy functions include activation of immunity (innate and adaptive), induction of tumor dormancy, and

* Corresponding author. Department of Obstetrics and Gynecology, College of Medicine, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei, Taiwan. Fax: +886 2 2391 5292.
*E-mail address:* wenfangcheng@yahoo.com (W.-F. Cheng).

http://dx.doi.org/10.1016/j.canlet.2017.01.040
0304-3835/

chemotherapy-driven dependency of cancer cells, which is known as the 4D effect [17–25]. Although both pre-clinical and clinical data support the use of a metronomic chemotherapy regimen as anti-cancer treatment, this strategy does not exclude the possibility of resistance without well-described mechanisms [26–28]. Some overexpressed proteins are associated with development of resistance to metronomic cyclophosphamide therapy [29], but a more comprehensive understanding of human tumor heterogeneity is warranted to explain and overcome treatment difficulties.

Several strategies, including metronomic chemotherapy, have the potential for immune modulation to enhance host immunity to overcome the immunosuppressive defense that tumors elicit. Current data show that adding chemotherapeutic agents to immunotherapy can trigger host production of durable and effective tumor antigen–specific T lymphocytes and synergistically optimize the anti-tumor effects [30,31]. Although few pre-clinical and clinical studies have been conducted to evaluate the efficacy and safety of combined metronomic chemotherapy and immunotherapy, these preliminary data hint at the feasibility of translating this combination into clinical application of future cancer treatment.

## Mechanisms of action of metronomic chemotherapy

### *Anti-angiogenic effects of metronomic chemotherapy*

Despite the initial satisfactory effects of tumor regression or remission, most patients, particularly those with high-risk tumors treated with MTD chemotherapy, will eventually have a recurrence and/or metastases [32]. In 1971, Folkman first advanced the hypothesis that tumor growth is angiogenesis dependent and established the concept of targeting tumor angiogenesis as an anti-cancer treatment [33].

Angiogenesis, the growth of new blood vessels from the existing vasculature, requires expansion of vascular endothelial cells. This process can occur locally by proliferation of differentiated endothelial cells or systemically by mobilization of bone marrow–derived endothelial progenitor cells, which enter the peripheral blood circulation, migrate to sites of angiogenesis, and are incorporated into growing vessels, thus playing an indispensable role in tumor progression and metastasis [14,15,34–36]. Therefore, it is reasonable to target tumor endothelial cells as an anti-neoplastic strategy.

A variety of stimulators and inhibitors precisely coordinate angiogenesis [21]. Stimulators of endothelial cell proliferation and migration are mainly receptor tyrosine kinase ligands [37], such as VEGF, fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor. Thrombospondin-1 (TSP-1), which modulates endothelial cell proliferation and motility, was the first angiogenic inhibitor to be described [38]. The equilibrium between activators and inhibitors regulates whether an endothelial cell will be in a quiescent or an angiogenic state, and it is believed that changes in the angiogenic balance mediate the angiogenic switch [21].

Conventional chemotherapeutic agents cause cell death by directly interfering with DNA or by targeting the key proteins required for cell division, but not specifically by targeting tumor cells [1]. Based on the relatively low rate of tumor endothelial cell division compared to tumor cells, standard chemotherapy causes only weak endothelial damage [32,39]. In addition, the proportion of endothelial cell proliferation in tumor-associated blood vessels is too low for chemotherapy to have a significant therapeutic impact [32]. Another important reason for the disappointing anti-angiogenic effects of chemotherapy is the conventional scheduling [11]. Browder et al. evaluated the anti-angiogenic and anti-tumor effects of cyclophosphamide in drug-resistant cancers and concluded that when cyclophosphamide is administered at the MTD, it causes apoptosis of endothelial cells in newly formed tumor microvessels. This anti-angiogenic effect could not translate into a therapeutic benefit because damaged endothelial cells can repair themselves during the rest period [11]. Thus, using a more compressed schedule (i.e., administration of cyclophosphamide at low and frequent [metronomic] doses) would be expected to suppress tumor angiogenesis simply by shifting the target from drug-resistant cancer cells to drug-sensitive endothelial cells [11,32,40,41]. A number of studies have also shown that activated endothelial cells in culture display selective sensitivity to extremely low concentrations (picomolar to nanomolar) of chemotherapeutic agents [42–47]. Protracted exposure of endothelial cells *in vitro* to low concentrations of several different anti-cancer agents causes marked expression of TSP-1, a potent endogenous glycoprotein inhibitor of angiogenesis, by binding to the CD36 receptors on endothelial cells [48–54].

Bone marrow–derived circulating endothelial progenitor cells (CEPs) are present in the peripheral blood, and they home to the neovascular bed of malignant tissues. These cells are thought to participate in the process of tumor vascularization and contribute to approximately 50% of tumor neovessels [15,55]. Bertolini et al. showed that immunodeficient mice injected with human lymphoma xenografts and treated with the MTD of cyclophosphamide exhibit vigorous CEP mobilization several days after the end of a cycle of drug administration, and tumors rapidly become drug resistant. In contrast, metronomic administration is associated with a consistent decrease in the number of CEPs to inhibit vasculogenesis-dependent mechanisms of tumor growth [14]. When tumor-bearing mice are treated with vascular-disrupting agents, this stress to the tumors would be expected to lead to an acute mobilization of CEPs homing to the viable tumor rim. Indeed, disruption of the CEP spike by anti-angiogenic drugs or genetic manipulation results in marked reductions in tumor rim size and blood flow [15].

In addition to the findings that the anti-angiogenic effects of metronomic chemotherapy can be induced indirectly by increasing TSP-1 expression and reducing the level and viability of CEPs, other mechanisms have been reported. Metronomic chemotherapy inhibits proliferation and/or induces apoptosis of activated endothelial cells and endothelial migration [42,44,56,57]. It also reduces the angiogenic potential of endothelial cells [46].

Another attractive target for anti-angiogenesis is hypoxia-inducible factor (HIF)-1α, which is a transcription factor essential for angiogenesis and tumor progression. Topoisomerase I inhibitors (topotecan and camptothecin) inhibit HIF-1α transcriptional activity [58], a finding that led to a pilot trial to determine whether or not oral metronomic topotecan can inhibit HIF-1α expression in tumor biopsies from patients with advanced solid tumors overexpressing HIF-1α [59]. These authors demonstrated a decreased expression of HIF-1α and its target genes (VEGF and GLUT-1) in response to treatment, although the sample size was relatively small ($n = 16$). Lee et al. further identified doxorubicin and daunorubicin as potent inhibitors of HIF-1α and reported reduced tumor vasculature in tumor-bearing mice treated with a 5-day, low-dose regimen using doxorubicin or daunorubicin [60].

Metronomic chemotherapy damages the source of angiogenesis stimulators, in contrast to conventional anti-angiogenic drugs, which target individual molecules or signaling pathways, thus with potentially more lasting effects [61]. In addition, metronomic chemotherapy can reduce the activity of essential angiogenesis factors.