Review Article

Metronomic chemotherapy

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ABSTRACT

Toxic effects and chemoresistance are major hurdles in chemotherapy and to avoid these problems caused by traditional chemotherapeutic regimens, a new modality of drug administration called "metronomic chemotherapy" has emerged. Such regimen involves the frequent administration of conventional chemotherapeutic agents at very low doses to target activated endothelial cells in tumors, the advantages of which include minimal adverse effects and a rare chance of developing acquired drug resistance. Previously it was thought that they act by targeting angiogenesis, but recently additional mechanisms have been discovered which has established metronomic chemotherapy as a type of multi-targeted therapy. The knowledge gained from the preclinical studies of metronomic chemotherapy, along with clinical experience, will help to design better therapeutic protocols against cancer. Detailed pharmacogenomic and pharmacoproteomic studies on tumor endothelial cells and large multi-centered clinical trials, integrating bio-marker analyzes, are needed to investigate and validate the best treatment combinations for each tumor type and patient population.

Key words: Anti-angiogenesis, conventional chemotherapy, metronomic chemotherapy, tumor dormancy

INTRODUCTION

Despite considerable progress in cancer research, current treatment protocols are unable to cure certain malignancies. Moreover, conventional chemotherapy and even new drug delivery systems lead to adverse drug reactions that restrict dosing and interferes the efficacy of anti-neoplastic drugs. So exploring new therapeutic targets as well as new therapeutic strategies are essential to improve cancer treatment. The endothelium of tumor vasculature has been recognized as a clinically validated therapeutic target and pioneering research work on this field was done by Folkman.[1] Over the past few decades, researchers have established that angiogenesis is the key factor in the local and metastatic growth of cancer. The discovery of the pivotal role of angiogenesis in tumor growth and metastasis has redefined chemotherapy and new protocols have emerged. In addition to rationally designed, molecularly targeted anti-angiogenic drugs such as anti-vascular endothelial growth factor (anti-VEGF) antibodies, many conventional and new pharmaceutical agents may have anti-angiogenic effects as an additional feature contributing to their treatment efficacy.[2,3] The recent preclinical studies have suggested that frequent administration of low doses ($1/10^{th}$-$1/3^{rd}$ of the maximum tolerated dose [MTD]) of certain anti-neoplastic drugs known as 'metronomic' chemotherapy, shows/enhances the anti-angiogenic property of the drugs. The effects of these metronomic protocols of anti-neoplastic agents may be further improved by concurrent administration of another agent that inhibits the processes of growth and tumor formation. The rationale behind the use of metronomic chemotherapy for longer duration is to minimize adverse drug reactions and to target both endothelial cells and tumor cells which are at proliferating stage. Hence, metronomic chemotherapy is

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defined as repeated administration of anti-neoplastic drugs at comparatively low doses frequently and without long drug-free period.[3] Previously, it was thought that they act by targeting angiogenesis, but recently additional mechanisms have been discovered which has established metronomic chemotherapy as a type of multi-targeted therapy.

THE CONCEPT

According to the conventional chemotherapy regimens, anticancer drugs are administered in cycles near or at the MDT and they alternate with long drug-free period to allow the patient to recover from adverse drug reactions. This strategy is successful in controlling the disease process in a significant number of patients (both adult and pediatric) but leads to some complications. In addition, despite initial improvement, recurrence is a common problem in metastatic and high-risk cancers. The rationale and effectiveness of conventional MTD-based chemotherapy regimens and dose modification strategies has been questioned for many years, especially in patients with poor-prognosis and scientifically convincing research data were needed to support the potential of an alternative therapeutic strategy.[4] Thirteen years ago Browder *et al*., published such much-awaited preclinical data from Judah Folkman's laboratory and it was confirmed in Robert Kerbel's laboratory.[5,6] For demonstrating the anti-angiogenic effect of low-dose chemotherapy, both the teams used transplantable tumors and xenograft models.[5,6] The first study revealed that metronomic regimen of cyclophosphamide (CPA) was more effective than conventional therapy and could overcome drug resistance.[5] Whereas, the second study explored the existence of synergism between continuous treatment with low-dose vinblastine and anti-VEGF receptor (VEGFR) therapy.[6] The scientific basis for metronomic chemotherapy is that conventional anti-neoplastic drugs target vascular endothelial cell proliferation but the anti-angiogenetic effect cannot be sustained because endothelial cells get a chance to recover during treatment breaks and this may be overcome by frequent treatment at low doses. Hanahan *et al*. coined the term 'metronomic' which is derived from the word "metronome", a musical instrument that produces regular, metrical ticks representing fixed, regular aural pulse.[7] Metronomic chemotherapy is the frequent administration of chemotherapy drugs at doses below the MTD and with no prolonged drug-free break. It thus achieves a sustained low blood level of the drug without significant toxic side-effects.[8]

The main characteristics of metronomic chemotherapy are:[9]

- Frequent (dose-dense) administration of chemotherapy without any interruptions
- Using a biological optimized dose instead MTD
- No application of hematopoietic growth factors
- Preference for oral drugs
- Low incidence of treatment related side-effects
- Potential for delayed development of resistance.

DIFFERENCE FROM CONVENTIONAL CHEMOTHERAPY

- Conventional cytotoxic drugs are designed for use at MTD but in metronomic chemotherapy, doses lower than MTD are used
- Conventional therapy is usually administered at defined intervals (3 weekly, fortnightly, weekly) as determined by the recovery of bone marrow but in metronomic therapy dosing frequency is continuous, e.g., weekly, every other day, daily
- Metronomic therapy leads to sustained plasma concentration of the drug but rise and fall of the plasma concentration is the feature of conventional therapy
- Metronomic therapy is targeted to endothelial cells in the growing vasculature of the tumor whereas conventional therapy targets proliferating tumor cells
- The intention of convention therapy is to treat cancer directly by inhibiting or killing rapidly dividing tumor cells whereas metronomic chemotherapy is administered with the aim of achieving cancer control by targeting angiogenesis
- Metronomic chemotherapy achieves a sustained low blood level of the drug without significant toxic side-effects and hence there is reduced need for supportive therapy. However in case of conventional therapy toxicity is a concern as doses are used at MTD
- Conventional chemotherapy is, in general, more effective against the primary tumor than against metastasis. Most cytotoxic agents, even if given in combined schedules at MTD, achieve only palliation in patients with advanced cancer.

MECHANISM OF ACTION

Metronomic chemotherapy is a multi-targeted therapy. Metronomic chemotherapy exerts both direct and indirect effects on tumor cells and their microenvironment. It can inhibit tumor angiogenesis, stimulate anticancer immune response and also induces tumor dormancy.

Anti-angiogenic properties

Metronomic chemotherapy exerts its anti-cancer activity mainly by inhibiting tumor angiogenesis. Metronomic protocol of drug administration has been proved to increase the anti-angiogenic properties of some chemotherapeutic drugs *in-vitro* significantly, such as CPA and taxanes.[10]

The mechanisms of action of anti-angiogenic activity of metronomic chemotherapy have been demonstrated *in-vivo*. These include:[11]

- Proliferation and/or induction of apoptosis of activated endothelial cells is selectively inhibited
- Selective inhibition of migration of endothelial cell,
- Increase in the expression of thrombospondin-1, an endogenous inhibitor of angiogenesis
- Sustained decrease in levels and viability of bone marrow-derived endothelial progenitor cells (CEPs).

Endothelial toxicity is a common phenomenon in anti-cancer therapy. Anti-angiogenic dose of a drug that is higher than the dose required to kill malignant cells should not be considered anti-angiogenic. There are some well-defined criteria, which are necessary to be fulfilled for defining chemotherapy with anticancer agents as metronomic. The criteria for an anti-angiogenic approach of metronomic chemotherapy are:[9]

- Strong differential cytotoxicity between cancer cells and endothelial cells
- Dynamic contrast enhanced magnetic resonance imaging or contrast enhanced ultrasonic examinations detecting changes in the permeability and blood-flow in tumors suggesting altered function of endothelial cells
- Changes of mechanistic effects (e.g., biomarker changes: IL-1 and 6, VEGF, VEGFR1 and 2, bFGF, Ang 1 and 2, MMP-2 and 9, vessel density etc.)
- Inhibition of angiogenesis *in-vivo* and *in-vitro* (*in-vivo* models at best only with spontaneous, slow growing tumors).

Activation of immunity

Both innate and adaptive immune system have an important role in the development and control of cancer. The well-known adverse effects of chemotherapy on the immune system are neutropenia and lymphopenia but various studies have proved and reviewed that some cytotoxic drugs, such as anthracyclines, taxanes and CPA, possess immune-stimulatory properties.[12] In this context, the effect on regulatory T cells (T_{reg}) is quite relevant for metronomic treatments. T_{reg} are $CD4^+$ $CD25^+$ $Foxp3^+$ lymphocytes that can inhibit antigen-specific immune response both in a cytokine-dependent and cell contact-dependent manner.[13] T_{reg} can thus inhibit anti-tumor immune response by suppressing the activity of both tumor-specific ($CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T helper cells) and tumor-unspecific effector cells (natural killer [NK] and NK T cells).[13] In a variety of human cancers, T_{reg} cells have been found in increased proportions which can correlate with tumor progression and lack of treatment response.[14] So, impairment of T_{reg} activity by either specific blockade or depletion is a method to enhance immune response against tumor-associated antigens.[13] Many studies (preclinical and clinical) have documented the effect of low dose CPA on T_{reg} cells. It reduces the number of T_{reg} cells, suppresses the function of the T_{reg} cells and increases both lymphocyte proliferation and memory T cells.[15]

In addition, this therapy also induces dendritic cell maturation and thus has the immune-stimulatory effect. Tanaka *et al*., found that metronomic chemotherapy regimens of some of the chemotherapeutic drugs such as vinblastine, paclitaxel and etoposide could promote dendritic cell maturation at non-toxic concentrations.[16]

Induction of tumor dormancy

Tumor dormancy occurs as a result of cell-cycle arrest or from a dynamic equilibrium state in which cell proliferation is balanced by induction of apoptosis. Tumor dormancy can be observed during the early phase of cancer progression and also after completion of anticancer treatments during the remission phase where tumors can resume their growth from remaining residual disease.[17] Suppression of angiogenesis, apoptosis of malignant cells and immune-surveillance are three main methods by which metronomic chemotherapy induces tumor dormancy.[8]

Induction of senescence

Lower grade of damage by chemotherapeutic agents may start senescence associated with antiproliferative responses. The cascades of caspase activity that induce cellular apoptosis are not activated. A dose of 250 nM doxorubicin causes apoptosis of prostatic cells, whereas its one-tenth dose (25 nM) triggers senescence. Deoxyribonucleic acid damage leading to single- and double-strand breaks have been induced by many senescence-inducing drugs. The induction of senescence in tumors can be achieved by repetitive, low-dose regimens of cytostatic drugs.[18,19]

Four-dimensional effect

Drug driven dependency/deprivation or a 4-D phenomenon has been hypothesized by André and Pasquier to explain the efficacy of the drug regimens using intermittent drug interruptions. According to this postulation, tumor cells become dependent on chemotherapeutic agents during long exposures and sudden withdrawal or replacement therapy may lead to cell death. This hypothesis may be used to explain the situations where multiple drugs are used with differing periods of administration.[20]

DRUGS USED IN METRONOMIC CHEMOTHERAPY

Metronomic chemotherapy regimen is usually a combination of various drugs of different classes having anti-angiogenic, immune-stimulatory and apoptotic properties. Frequent and repetitive administration of low dose of some antineoplastic drugs (CPA, methotrexate, etoposide, vinblastine, paclitaxel) is cytotoxic to both circulating endothelial cells and circulating CEPs but has no effect on non-endothelial cells and leucocytes.[5] Due to their relative genetic stability, endothelial

cells are inherently less susceptible to the development of drug resistance than are tumor cells.[6] It has been observed that in certain conditions when maximally tolerated doses of chemotherapy drugs are no longer effective, significant inhibition of tumor growth can be achieved by switching over to a metronomic regimen.

Metronomic chemotherapy in adults

Most of clinical trials on metronomic chemotherapy in adults have been conducted in patients with breast carcinoma.[21,22] Many investigators have used various metronomic chemotherapy regimen for patients with advanced and recurrent ovarian carcinoma, advanced multiple myeloma, hormone resistant prostate cancer, non-Hodgkin lymphoma and others.[23] Some well-studied metronomic regimens for adults have been listed in Table 1 and most of these studies have showed modest response rate to metronomic chemotherapy and overall clinical benefit.

Metronomic chemotherapy in pediatric patients

Metronomic chemotherapy has the potential to improve survival and quality of life of pediatric cancer patients by reducing the burden of adverse effects. number of clinical trials of metronomic chemotherapy in pediatric population is limited in number but some of the results are very promising [Table 2]. In a study published in 2002, Sterba *et al*., demonstrated the effect of metronomic temozolomide in combination with radiotherapy in children with medulloblastoma. Later they conducted a pilot study of a four-drug metronomic chemotherapy regimen in children with relapsed solid tumors.[32] Metronomic etoposide and metronomic temozolomide was evaluated through combined oral maintenance bio-differentiating and anti-angiogenic therapy protocol by Sterba *et al*., in 2006.[29] In another study, 20 children with recurrent or progressive cancer of various tumors were treated and evaluated with metronomic chemotherapy regimen, involving thalidomide and celecoxib with alternating metronomic etoposide and CPA.[30]

Table 1: Metronomic chemotherapy regimens used in adults

Patient profile	Metronomic chemotherapy regimen
Metastatic breast cancer previously treated with conventional chemotherapy[21]	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly)
Untreated or previously treated breast cancer with conventional chemotherapy[24]	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly) (or) Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly) Thalidomide (oral, daily)
HER2+metastatic breast cancer, previously treated with trastuzumab and conventional chemotherapy[22]	Cyclophosphamide (oral, daily) Methotrexate (oral, twice weekly) Trastuzumab (every 3 weeks)
Recurrent ovarian cancer, previously treated with conventional chemotherapy[23]	Cyclophosphamide (oral, daily) Bevacizumab (every 2 weeks)
Hormone-refractory prostate cancer, previously treated by androgen deprivation[25]	Cyclophosphamide (oral, daily) Dexamethasone (oral, daily) (or) Dexamethasone (oral, daily) Celecoxib (oral, twice daily)
Aggressive relapsed or refractory non-Hodgkin's lymphoma[26]	Cyclophosphamide (oral, daily) Celecoxib (oral, twice daily)
Progressive multiple myeloma, previously treated with conventional chemotherapy[27]	Cyclophosphamide (oral, daily) Prednisone (oral, daily)
Metastatic or locally advanced neuroendocrine carcinoma[28]	5-fluorouracil (i.v., daily) Long-acting release octreotide (monthly)

OPTIMAL BIOLOGIC DOSE AND BIOMARKERS

Low-dose regimen of metronomic chemotherapy is a promising strategy in cancer treatment ensuring advantages such as low toxicity, increased efficacy, good activity against resistant tumors and ability to combine chemotherapeutic agents. However, an important disadvantage of this type of regimen is the empiricism in finding the optimal 'low-dose' or OBD and in monitoring therapeutic efficacy during the course of treatment. Because the predominant mechanism for metronomic chemotherapy is thought to be anti-angiogenesis, as a result of targeting both dividing endothelial cells in a tumor's growing vasculature and bone marrow derived circulating VEGFR-2$^+$ cells, including CEPs, Yuval Shaked *et al*., have investigated some cellular pharmacodynamic biomarkers for determining OBD of different metronomic regimens-(i) previous observations showing significant and sustained declines in circulating VEGFR-2$^+$ CEPs induced by prolonged daily low-dose metronomic chemotherapy; (ii) preclinical validation of measuring levels of such cells as a surrogate blood-based marker of angiogenesis and targeted anti-angiogenic drug activity, including optimal biologic dosing; and (iii) CEP

Table 2: Metronomic chemotherapy regimens used in pediatric age group

Patient profile	Metronomic chemotherapy regimen
Refractory, relapsing or "high risk of relapse" tumors of various types[29]	Etoposide (oral, daily, 3 weeks) alternating with Temozolomide (oral, daily, 6 weeks) Celecoxib (oral, daily) Retinoic acid (oral, daily, 2 weeks)
Refractory or relapsing tumors of various types[30]	Etoposide (oral, daily, 3 weeks) alternating with cyclophosphamide (oral, daily, 3 weeks) Thalidomide (oral, daily) Celecoxib (oral, daily)
Refractory relapsing tumors of various types[31]	Vinblastine (i.v., 3 times/week) Celecoxib (oral, daily) (or) Cyclophosphamide (oral, daily) Celecoxib (oral, daily)

potentially as a marker in the clinic of targeted anti-angiogenic drug activity. They also found a correlation between each OBD with the maximum reduction in viable peripheral blood circulating VEGFR-2$^+$ CEPs. These results suggest that CEPs may help as a surrogate biomarker to determine the OBD of metronomic chemotherapy regimens and can reduce the level of empiricism.[33]

TOXICITY OF METRONOMIC CHEMOTHERAPY

Most of the clinical trials demonstrated that metronomic chemotherapy was well-tolerated. In addition, metronomic chemotherapy may be a cost-effective treatment option as evaluated in various clinical trials.[30] High-grade toxic effects were either rare or not found. Most common toxic effects were mild nausea and/or vomiting, mild to moderate anemia, neutropenia, leucopoenia and lymphopenia as well as low-grade fatigue.[9] Overall, metronomic chemotherapy offers a significant clinical improvement including improvement in quality of life with minimal toxicity. However, data are still not adequate to draw definitive conclusions regarding the tolerance of these drug combinations.

While using metronomic chemotherapy for a longer period, especially in children, some potential risks and concerns must be taken into consideration. Prolonged metronomic chemotherapy may lead cumulative toxicity of anticancer agents, which can lead to secondary diseases. For example, cumulative dose of etoposide or temozolomide can lead to secondary leukemia.[34,35] Although physiologic angiogenesis differs from tumor neoangiogenesis, special attention must be given to the growth and development of young children receiving metronomic chemotherapy. A recent study has found potential association of subdural hematoma in a child with medulloblastoma, with metronomic chemotherapy.[36] So careful monitoring of children receiving metronomic chemotherapy is must in any future studies.

RESISTANCE

One main obstacle in chemotherapy is the occurrence of resistance against the applied treatment regimen. Anti-angiogenic drug regimen targeting endothelial cells are unlikely to produce acquired drug resistance as endothelial cells were expected to be genetically stable. However, some experimental and clinical studies have established the development of escape mechanisms against anti-angiogenic treatment regimens also and support a hypothesis that two mechanisms of unconventional resistance can develop - evasive resistance, an adaptation to prevent specific anti-angiogenic effect; and intrinsic or pre-existing tolerance. Different methods in different tumor types may lead to manifestation of both evasive and intrinsic resistance. Cells developing resistance to metronomic chemotherapy undergo a complex molecular evolution. Changes in many different factors and pathways contribute to the formation of a resistant phenotype. One important category of resistance to anti-angiogenic therapy is "reduced vascular dependence." This resistance mechanism applies here, as the resistant tumor grows under conditions of hypoxia and restricted nutrients without the formation of additional tumor vessels. During anti-angiogenic treatment, blood vessels are destroyed and coagulation takes place. So anti-coagulation properties of the cells could be part of a complex resistance mechanism. So metronomic chemotherapy also can lead to resistance which demands assessment of their prevalence and importance.[37,38]

PHARMACOGENETICS

Data on pharmacogenetics of metronomic chemotherapy is still lacking. Gene expression profiling and comprehensive gene expression analysis of the resistant tumors can guide in choosing correct metronomic therapy. Gene expression of resistant tumors clearly differs from non-resistant tumors and to investigate the molecular basis of *in-vivo* resistance mechanism genome-wide microarray studies are required. Some studies revealed that expression of resistance-related genes *in vivo* differs from gene expression *in vitro*, indicating an involvement of micro-environmental factors leading to the observed *in vivo* resistance.[38]

Orlandi *et al*., evaluated the association between VEGF-A sequence variants and prostate-specific antigen progression, progression-free survival and overall survival, in advanced castration-resistant prostate cancer patients treated with metronomic CPA (CTX), celecoxib and dexamethasone.[39] Pharmacogenomics of bortezomib has been investigated by John D. Shaughnessy *et al*.[40] In another study, Mercurio *et al*., have quantified the expression of several genes (ICAM1, CRK, CD36 and IQGAP1) by reverse transcriptase quantitative polymerase chain reaction in pilocytic astrocytomas and glioblastomas. Those genes were all significantly over-expressed in hypothalamic-chiasmatic pilocytic astrocytomas relative to cerebellar pilocytic astrocytomas and CRK and ICAM1 were significantly over-expressed in pilocytic astrocytomas versus glioblastomas. These over-expressed genes are a new target of therapy and refractory multifocal pilocytic astrocytoma was treated with fluvastatin/celecoxib for 18 months successfully. This new strategy is promising for the treatment of refractory pilocytic astrocytoma.[41] Kubisch *et al*., worked on metronomic anti-angiogenic treatment of xenografted prostate cancer tumors in severe combined-immunodeficiency mice with CPA. To investigate the complex molecular changes occurring during resistance formation, they performed a comprehensive gene expression analysis of the resistant tumors *in vivo* and

observed a multitude of differentially expressed genes, e.g., PAS domain containing protein 1, annexin A3 (ANXA3), neurotensin, plasminogen activator tissue, when comparing resistant to *in-vivo* passaged tumor samples. Such studies can give insight into the mechanism of resistance and can explore new molecular targets of metronomic chemotherapy.[38]

FUTURE PERSPECTIVE

Evidence from pre-clinical and clinical studies has established metronomic chemotherapy as a new treatment strategy to control certain types of malignancies. However, optimizing a metronomic anticancer therapy is still a challenging task even after a decade of clinical investigation. So future cancer research should aim to identify the best agents to use according to tumor type, to calculate the doses of each agent to be used alone or in combination and to define the timing of drug administration. New strategies are being developed in the oncology clinic to combine metronomic chemotherapy with conventional chemotherapy, radiotherapy and/or targeted therapy and such strategies can explore infinite number of potential combinations. But in-depth pharmacogenetic and pharmacoproteomic studies on tumor endothelial cells are now needed to assess their sensitivity to metronomic chemotherapy and to determine the most effective drug combination to use in the clinic.

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