



REVIEW ARTICLE

Metformin as a Novel Component of Metronomic Chemotherapeutic Use: A Hypothesis

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The hypoglycemic agent metformin has been found to possess chemopreventive and direct antitumor properties. Several clinical studies worldwide are using it as a monotherapy or as an add-on therapy with chemotherapeutic drugs to determine prospectively its efficacy and safety in treating human cancer. In terms of its mechanism of action, metformin moderately inhibits electron transport in mitochondria to cause increased AMP:ATP ratios, which antagonize gluconeogenesis in hepatocytes, and to promote catabolism in most tissues through activating AMP-activated kinase (AMPK). Inhibition of mammalian target of rapamycin signaling through activation of AMPK has been suggested to mediate the antitumor effects of metformin. However, AMPK-independent growth-inhibitory properties of metformin on tumor cells have also been described, suggesting that antagonizing electron transport *per se* may be cytostatic or cytotoxic to cancer cells. In addition, metformin was hypothesized to display antiviral and antimetastatic effects in 1950s, and recently it has been found to promote the generation of CD8 T memory lymphocytes, suggesting that its immune-activating effects may also contribute to its observed antitumor and chemopreventive properties. Chronic administration of metformin has an acceptable toxicity profile and is well tolerated by millions of patients with type 2 diabetes worldwide, suggesting that this agent could potentially be a therapeutic component with low intensity if given in continuous dosing/frequent usage schedules. These metronomic strategies show that metformin can inhibit tumor angiogenesis and activate antitumor immunity, indicating a potential therapeutic interaction with immune potentiation, antitumor effects, and an acceptable toxicity profile. Here, we review current knowledge on metformin's signaling, metabolic, and immune effects, as well as data from clinical drug trials, to discuss how the interplay may orchestrate the antitumor effects of this agent, particularly in combination with reduced-intensity or metronomic chemotherapeutic use.

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1. Introduction

Metformin, a biguanide, was approved by the United States Food and Drug Administration in 1995 as an oral hypoglycemic agent. Given alone or in combination with a sulfonylurea, metformin improves glycemic control and lipid concentrations in patients who respond poorly to dietary control or to a sulfonylurea alone.

In this review, we discuss evidence for metformin's potential use as an antitumor drug. We will review the major mechanisms related to its antitumor effects, clinical evidence of its antitumor and chemopreventive effects, metronomic chemotherapy—when less is more—and the interaction of metronomic chemotherapy and metformin.

2. Major mechanisms related to antitumor effects of metformin

The mechanisms underlying the action of metformin in exerting antitumor effects can be summarized as follows.

2.1. Metabolic and signaling effects

Anisimov et al, in their pioneer work to understand the antitumor mechanism of metformin, found that chronic administration of metformin to female transgenic HER2/neu mice significantly reduced the number and size of mammary adenocarcinomas, partly through downregulation of the insulin/insulin-like growth factor axis (IGF).¹ This mechanism has already been observed in patients with type 2 diabetes² and women affected by polycystic ovary syndrome.³

More recent experimental evidence indicates that this biguanide can activate AMP-dependent kinase (AMPK), either by

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suppressing the tumor suppressor kinase LKB1,⁴ or by promoting an increase in AMP:ATP ratios.⁵ Activated AMPK can in turn phosphorylate and activate TSC2, a negative regulator of mammalian target of rapamycin (mTOR).⁶ Inhibiting mTOR kinase activity can reduce signaling transduction through the kinase Akt, and decrease the efficiency of protein synthesis via decreased phosphorylation of the mTOR targets 4EBP-1 and S6K, which are essential components of the cap-dependent translation machinery.^{7,8} The inhibition of cap-dependent translation in response to metformin⁹ can result in decreased expression of the oncogene *Her2*¹⁰ and the cell cycle protein cyclin D1,¹¹ illustrating a potential avenue via which metformin can modulate signaling and cell cycle effects.

Because AMPK is the energy sensor of the cell, metformin also increases oxidative metabolism and reduces anabolism, resulting in decreased lipid synthesis, protein synthesis, and so on,¹² in part through direct phosphorylation effects on key metabolic targets such as acetyl CoA carboxylase (a committed step in fatty acid synthesis) and phosphofructokinase-2 (the master regulator of glycolysis).¹³ How metformin activates LKB1 remains unclear, but it has been shown that metformin can increase the AMP:ATP ratio, the canonical signal to activate AMPK, as a result of moderate inhibition of the electron transport chain at the entry point of NADH, the mitochondrial complex I.¹⁴

This latter evidence is also intriguing in light of Anisimov et al's original findings that metformin can also prolong the life span of *Her2* mice, because it highlights the possibility that the reduction in mitochondrial bioenergetics induced by metformin may be the cellular mimic of caloric restriction, a well-documented longevity and chemopreventive strategy.

2.2. Direct mitochondrial effects

Both the AMPK-independent antitumor effects of metformin action, such as the Rag GTPase-dependent inhibition of mTOR,¹⁵ and metformin-induced growth inhibition of AMPK-silenced ovarian cancer cells are important.¹⁶ Nearly 10 years ago, Owen et al described how metformin can inhibit the mitochondrial oxidation of complex I-dependent substrates in hepatocytes, an effect that can also be observed in isolated mitochondria.¹⁴ As discussed above, this inhibition of complex I may contribute to the activation of AMPK due to the decrease in capacity for oxidative phosphorylation and the subsequent increase in AMP:ATP ratio. This phenomenon may also account for the occasionally observed lactic acidosis in response to high doses of metformin,¹⁷ because pyruvate is converted to lactate rather than to acetyl CoA in the mitochondria.

The inhibition of hepatic gluconeogenesis in response to metformin is an AMPK-independent consequence of decreased intracellular ATP levels.⁵ This notion suggests that the pleiotropic effects of metformin could be the result of a targeted effect on electron transport in the mitochondria.⁵ In light of recent observations, this intriguing effect shows that inhibiting electron transport in cancer cells is a lethal insult,^{18–20} not because of an ensuing energetic catastrophe - cancer cells derive most of their ATP from glycolysis - but because the accumulation of NADH in the mitochondrial matrix can inhibit the Krebs cycle and the associated amphibolic reactions that support the generation of biomass.²¹

Electron transport, uncoupled from oxidative phosphorylation, antagonizes the onset of apoptosis in tumor cells.^{19,21,22} This supports the hypothesis that the antitumor and chemosensitizing effects of metformin could also be mediated through inhibiting electron transport in complex I.

2.3. Immune and hypothalamic effects

It is important to consider immune-modulating effects of metformin as a mechanism underlying antitumor activity. This concept was originally proposed in the 1950s by the Philippine physician Garcia,²³ who first recognized the important component of the antitumor effects of metformin. A recent thought-provoking report has shown that metformin can increase the number memory CD8 T cells in wildtype mice, and in consequence significantly improve the efficacy of an experimental anticancer vaccine.²⁴ This report suggested the mechanism that increased fatty acid oxidation in response to metformin can mediate the generation of CD8 T cells.

However, this notion does not agree with the observation that metformin inhibits electron transport in hepatocytes and hepatocyte mitochondria, and it is thus intriguing to hypothesize that metformin modulates tissue-specific responses in mitochondrial metabolism by inhibiting electron transport in hepatocytes instead of promoting fatty acid oxidation in lymphocytes. Irrespective of this, the mechanism for the generation of memory CD8 T cells could be a critical component of the antitumor action of metformin.

Lastly, Ropelle et al have shown that hypothalamic AMPK activation in response to metformin reverses cancer anorexia in tumor-bearing rats through inhibiting the production of proinflammatory molecules and controlling neuropeptide expression in the hypothalamus,²⁵ suggesting another potential benefit of the use of metformin as an adjuvant in cancer treatment, which warrants further clinical exploration.

2.4. Metformin might have multiple mechanisms in exerting its antitumor effect

Taken together, the observations described above indicate that the beneficial effects of metformin as an adjuvant in cancer treatment may be orchestrated via many (AMPK-dependent and -independent) mechanisms that might antagonize tumor initiation and/or progression, decrease cancer anorexia, and improve antitumor immunity.

3. Clinical evidence for the antitumor and chemopreventive effects of metformin

Recently, the Zwolle Outpatient Diabetes Project Integrating Available Care (ZODIAC) study in The Netherlands reported that metformin exerts chemoprotective activity against all types of cancer in patients with type 2 diabetes.²⁶ Retrospective studies by a group from the University of Washington at Seattle, USA,²⁷ and another group from M. D. Anderson Cancer Center in Houston, USA,²⁸ confirmed that patients with type 2 diabetes treated with metformin had a reduced risk of prostate and pancreatic cancer, respectively.

Based on the available clinical and experimental data, Goodwin, in an editorial in the 2009 *Journal of Clinical Oncology*, proposed the use of metformin in the adjuvant treatment of breast cancer,²⁹ mainly citing the drug's ability to reduce hyperinsulinemia,³⁰ which she had reported to be a negative prognostic factor for recurrence.³¹ Of note, reduction in insulin level and the associated decrease in IGF-1 signaling transduction were also suggested to be a mechanism of action in the studies of Anisimov et al.¹

The first evidence of the efficacy and safety of metformin as an adjuvant in the treatment of breast cancer was reported by the breast medical oncology group at M. D. Anderson Cancer Center in a retrospective study of 68 diabetic patients taking metformin, 87 diabetic patients not taking metformin, and 2,374 nondiabetic patients.³² Their results showed that diabetic patients with breast cancer who received metformin and neoadjuvant therapy had

a higher pathological response rate than diabetic patients not receiving this agent. Importantly, the use of metformin was not associated with adverse effects in cancer patients receiving chemotherapy.³²

Subsequently, Stanosz reported that treatment with metformin in combination with hormonal agents in young women with well-defined stage 1 endometrial carcinoma led to complete remission of the disease after a 6-month treatment and 2-year follow-up.³³ These findings suggest that the potential therapeutic benefits of metformin are not limited to breast cancers. Most recently, a group at Yokohama City University reported that a 1-month treatment with metformin reduced the formation of aberrant crypt foci in nine patients without diabetes, suggesting a potential use of metformin in preventing colon tumorigenesis.³⁴

Taken together, the above-mentioned results and observations suggest that chronic use of metformin antagonizes the initiation and progression of cancer, as well as improving the outcome of traditional chemotherapeutic strategies.

4. Metronomic chemotherapy: When less is more

Traditional anticancer drugs are used at or near the maximum tolerated dose (MTD), with the goal of killing as many cancer cells as possible, but with unintended consequences that impair quality of life and cause serious, dose-accumulative toxic effects.³⁵ To balance the efficacy and safety of this “MTD approach” with particular emphasis on reducing myelosuppressive effects, high doses of chemotherapy are normally given once or on a few

consecutive days, followed by 3–4-week periods of rest to allow recovery of normal progenitor cells.³⁵ However, the fact that most cancer patients still suffer relapses suggests that high-dose chemotherapy is largely ineffective in killing 100% of tumor cells, and perhaps that the genomic instability caused by high-dose chemotherapy—as most chemotherapeutic agents directly damage DNA or the machinery necessary for its maintenance/replication—is the fire that the cellular heterogeneity of tumors fuels in choosing drug-resistant subclones that will no longer respond to the MTD approach. Moreover, the inhibitory effects of high-dose chemotherapy on the function of the immune system may provide a window of time for these drug-resistant cells to escape detection and/or to metastasize.³⁶

Interestingly, recent evidence shows that targeted anti-angiogenic agents provide a moderate therapeutic benefit in many cancer patients,³⁷ supporting a concept put forward nearly 40 years ago by Folkman, who proposed targeting the tumor vasculature instead of the tumor cell *per se* in order to inhibit the growth of primary tumors and the spread of malignant cells to distant sites.^{38,39} In this context, a preponderance of evidence suggests that low-dose, continuous infusion or frequent administration of certain chemotherapeutic agents—but not high-dose MTD approaches—can inhibit the proliferation and differentiation of tumor vasculature.^{40–43} In terms of its mechanism of action, the slowly proliferating phenotype of tumor vasculature, and the increased sensitivity of endothelial cells to cellular damage in response to cytotoxic agents likely makes the tumor endothelium more sensitive to continuous, low-dose exposure to chemotherapy

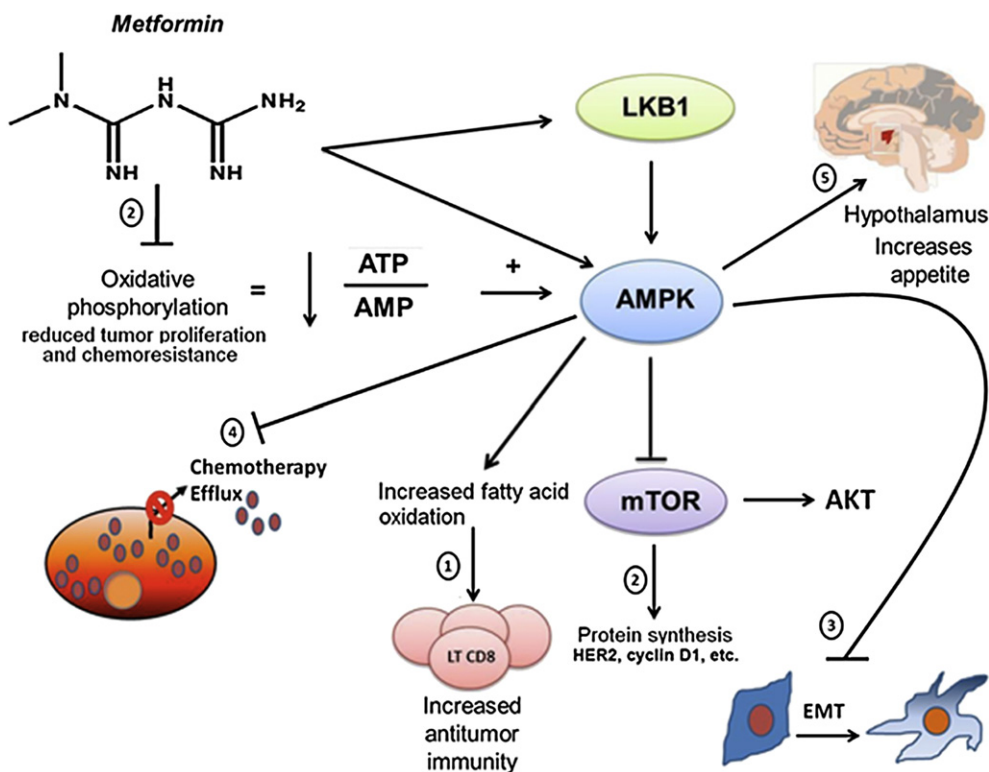


Figure 1 Diagrammatic representation of the mechanism of action of metformin, which may interact with metronomic chemotherapy at various levels. (1) Metformin can promote the generation of CD8 memory T lymphocytes, which would complement the reduction in T regulatory lymphocytes and enhance the maturation of dendritic cells induced by metronomic chemotherapy. (2) Inhibition of mTOR signaling coupled to the decrease in mitochondrial bioenergetics induced by metformin would generate more slowly proliferating, chemosensitive tumor cells that would be more appropriately targeted by reduced-intensity chemotherapeutic drugs. (3) Metformin inhibits the EMT transcriptional program, which effectively reduces the formation of tumor stroma and could potentiate the antiangiogenic effects of metronomic chemotherapy. (4) Metformin antagonizes the expression of P-glycoprotein (P-gp), which could maximize the cytotoxic effects of low doses of chemotherapy in cancer cells.⁴⁵ (5) Metformin activates hypothalamic AMPK and may antagonize cancer cachexia, a benefit most likely maximized in patients receiving metronomic regimens that are not anorexic or gastrotoxic.²⁵ AMP = adenosine monophosphate; AMPK = AMP-activated kinase; ATP = adenosine triphosphate; EMT = epithelial-mesenchymal transition; mTOR, mammalian target of rapamycin.

than to episodic near-MTD therapy. In 2000, Hanahan et al referred to this low-dose, continuous dosing strategy as “metronomic,” and suggested that its reduced systemic toxicity and its ability to target endothelial cells and slowly proliferating tumor cells could offer potential clinical benefits.⁴⁴

More recent clinical studies have shown that metronomic chemotherapy is a potential clinical alternative to either primary systemic therapy or maintenance therapy, and preclinical studies have suggested that, in addition to its well-established anti-angiogenic effects, metronomic chemotherapy can activate anti-tumor immunity. The mechanisms underlying the activation of antitumor immunity by metronomic chemotherapy have only recently been uncovered as most animal models are immunodeficient, but a number of published studies suggest that the effects are orchestrated by a reduction in T regulatory lymphocytes, and the maturation and activation of antigen-presenting dendritic cells.

5. How can metronomic chemotherapy interact with metformin?

As shown by Anisimov et al,¹ phenformin potentiates the effects of cyclophosphamide on various transplantable tumors, and recent evidence suggests that antagonizing mitochondrial bioenergetics potentiates the therapeutic effects of cytarabine in mice transplanted with human leukemia.¹⁹ Those findings support the notion that metformin could potentiate the effects of traditional chemotherapeutic agents. Indeed, Jiralerspong and colleagues showed retrospectively that metformin potentiates the pathological response to neoadjuvant chemotherapy in diabetic breast cancer patients without any evidence of increased toxicity.³²

Could metformin potentiate the effects of metronomic chemotherapy? The evidence discussed so far suggests that metformin can interact at various levels with metronomic chemotherapy. Figure 1 is a schematic illustration of the mechanisms of action that ought to be taken into consideration when incorporating metformin into a metronomic therapeutic strategy.

6. Conclusion

At the time of writing, a search of the Clinical Trials (www.ClinicalTrials.gov) website yielded 23 open, actively recruiting studies in North America evaluating the efficacy and/or safety of treating cancer patients with metformin. Eleven of these studies are aimed at breast cancer patients, three studies are enrolling colorectal cancer patients, and the remaining studies are enrolling patients with prostatic ($n=2$), pancreatic ($n=2$), esophageal ($n=1$), endometrial ($n=1$), head and neck ($n=1$), brain ($n=1$), advanced-stage ($n=1$), and other various types ($n=1$) of cancer.

One prostate cancer study and three breast studies are using metformin as a single agent before surgery to evaluate the molecular correlates of response (immunohistochemistry for cell cycle proteins, proliferation markers, etc.), while the other prostate cancer study is using metformin as a single agent in castration-resistant prostate cancer and evaluates prostate-specific antigen levels as a primary outcome. The remaining studies are evaluating the safety of combining metformin with tyrosine kinase (erlotinib or lapatinib), mTOR inhibitors (sirolimus, temsirolimus), and/or traditional high-dose chemotherapy (cisplatin, epirubicin, capecitabine, gemcitabine). To our knowledge, there are no registered clinical trials using metformin in combination with low-intensity metronomic regimens for the treatment of human malignancies.

Why combine metformin, which inhibits mTOR signaling on its own, with an mTOR inhibitor that causes immunosuppression? Why combine metformin with high-dose chemotherapy? Traditional anticancer treatments do not take into account the damage

that they do to the immune system, yet they continue to be a mainstay of cancer therapy. Moreover, the devastating effects of traditional treatment approaches, and the cost of dealing with the associated complications, reveal the urgency of developing chemotherapeutic strategies that lessen suffering, optimize costs, and allow the immune system to detect and destroy malignant cells.

Metformin is an effective and safe hypoglycemic drug with a potential new indication for managing and preventing cancer. The evidence presented here suggests that metformin displays single-agent therapeutic efficacy, at least in the setting of chemoprevention, and that it combines favorably with chemotherapy to provide cancer patients with a therapeutic benefit. The above therapeutic considerations, and in addition the low economic cost of metformin and metronomic chemotherapeutic regimens, warrant the initiation and support of additional clinical studies to evaluate the efficacy of metformin in patient populations that are not eligible for standard anticancer regimens. This may represent a novel paradigm for the treatment of human malignancies that reduces the costs of initial treatment and management of treatment-related complications, which place such a heavy burden on health systems around the globe.

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