

Hypothesis/Commentary

Is it Time to Test Metformin in Breast Cancer Clinical Trials?

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Abstract

Several studies have identified an increased risk of cancer in type 2 diabetic patients and this is in accordance with the hypothesis that increased insulin levels might promote cancer. Thus, there is a great interest in exploring the possibility that antidiabetic therapies lowering insulin levels could decrease cancer incidence or cancer-related mortality. Recent observational studies have shown that metformin, an oral safe and well-tolerated insulin-sensitizer antidiabetic drug, has been associated with reduced cancer risk. Recently, several preclinical studies have evaluated the effect of metformin *in vivo* on nude mice and showed a significant reduction of both breast epithelial cell proliferation and protein synthesis. Further investigations in the clinical setting are well-supported by the

promising results obtained thus far. At the European Institute of Oncology, the Division of Cancer Prevention and Genetics is planning to conduct a clinical trial to evaluate the activity of metformin on tumor cell proliferation in breast cancer patients undergoing surgery. It will be a presurgical randomized, double blind, placebo-controlled phase II biomarker trial: 100 histologically confirmed breast cancer patients will be randomly assigned to metformin (850 mg twice/daily) or placebo for 28 + 7 days till surgery to assess drug activity on tumor proliferation, as measured by Ki-67. The confirmation of the efficacy of metformin on cancer cell proliferation may lead the way to larger chemoprevention clinical trials. (Cancer Epidemiol Biomarkers Prev 2009;18(3):701–5)

Introduction

Despite major diagnostic and therapeutic innovations, treatment effect on breast cancer-related morbidity and mortality is still limited mainly because of the incomplete knowledge of the disease biology. Major research efforts continue to focus on identifying new agents able to treat or prevent breast carcinoma.

In the last two decades, there has been increasing evidence that physiologic concentrations of insulin may play a clinically important role in breast cancer development. A recent meta-analysis (1) has shown an increased risk of this disease in women with diabetes. In particular, the meta-analysis of 20 studies (5 case-control and 15 cohort studies) confirmed that diabetes was related with an increased risk of several kind of tumors and the combined results of 10 selected trials found that a high level of insulin was associated with a 13% and 25% increased risk of breast carcinoma in case-control and cohort studies, respectively. Moreover, it seems that insulin increased breast cancer recurrence and death (2, 3). Although the mechanism by which insulin influences breast carcinogenesis is still under investigation, it seems to activate several proliferative and antiapoptotic events through its own receptor (4). The

evidence that insulin influences breast carcinogenesis is extremely important because it raises the possibility of adopting insulin reduction for therapeutic and chemopreventive purposes in breast cancer.

Recent interest has been focused on metformin, a biguanide derivative, currently approved for the treatment of non insulin-dependent diabetes mellitus, and an insulin-sensitizing agent with potent antihyperglycemic properties. Its primary action is to inhibit hepatic glucose production, but it also increases the sensitivity of peripheral tissue to insulin (5). Thanks to these properties, metformin is an orally administered drug used to lower blood glucose concentration in patients with noninsulin-dependent diabetes mellitus and metabolic syndrome (6). Moreover, insulin resistance is one of the main features of the Polycystic Ovary Syndrome (PCOS), and the increase in insulin sensitivity metformin induces is used as PCOS therapy. PCOS is a disease characterized by the presence of chronic oligo-ovulation or anovulation, androgen excess, and polycystic ovaries, and is often associated with important metabolic derangements as diabetes mellitus and metabolic syndrome (7, 8).

Metformin and Cancer

Several studies have identified an increased risk of cancer in type 2 diabetes patients (9, 10) according to the hypothesis that increased insulin levels might promote cancer. Is indeed of great interest to explore the possibility that antidiabetic therapies (lowering

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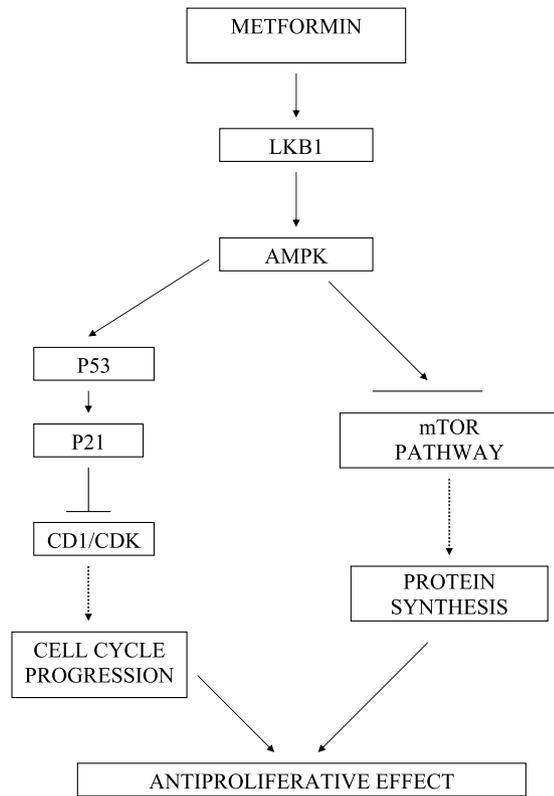


Figure 1. Complete mechanism of action of metformin on AMPK and proliferation.

insulin levels) could decrease cancer incidence or cancer-related mortality.

Recent observational studies have shown that metformin treatment has been associated with reduced cancer risk. In particular, Evans et al. (11) observed that diabetic patients treated with metformin had a lower incidence of any kind of cancer when compared with patients on other treatments. Interestingly, this effect seemed related also to breast carcinoma and seems to improve with higher doses. Another observational study (12) involving >10,000 diabetic patients treated with metformin or other antidiabetic agents showed a lower cancer-related mortality rate in the metformin group when compared with other drugs, such as sulfonylureas or insulin.

Although the mechanism of action of metformin is still under investigation to determine whether these observations on the antineoplastic action of metformin might be related to the direct action of this drug on cancer cells or to its indirect effect, such as insulin level reduction. At present, both these biological pathways seem related to its potential anticancer activity. In particular, it is biologically plausible that there may be a direct effect due to the influence of the physiologic levels of insulin on the proliferation of breast cancer cells, considering the uniform expression on breast carcinoma of its receptor. An indirect influence is also possible: metformin can suppress protein synthesis and cell proliferation with AMP-dependent protein kinase (AMPK) activation.

The Mechanism of Action of Metformin

As regards glucose homeostasis properties, the molecular mechanism of metformin has been studied in different tissues, such as muscle and fatty tissues and liver. Although, the effect on epithelial cells remains unclear. This aspect should be clarified for there is substantial evidence that the mechanism of action may involve AMPK activation (an AMP-activated protein kinase serving as an energy sensor) by means of an LKB1-dependent mechanism (13). LKB1 is a tumor suppressor gene with relevance to epithelial neoplasia; in particular, its activity loss is frequently associated with a syndrome, named Peutz-Jeghers, characterized by several gastrointestinal polyps and also by a significantly increased risk of various epithelial cancers, including breast cancer (14).

According to several published studies, AMPK activation strongly suppresses cell proliferation in both malignant and nonmalignant cells. These AMPK actions seem to be mediated by multiple mechanisms: cell cycle regulation and protein synthesis inhibition.

The first pathway is mediated by an up-regulation of the p53-p21 axis, the reduction of Cyclin D1 levels with the consequent inhibition of the correspondent cyclin-dependent kinases, eventually causing an antiproliferative effects by the G₁ cell cycle arrest. The second pathway involves the mammalian target of rapamycin. The complete mechanism of action on AMPK and proliferation of metformin is summarized in Fig. 1.

The hypothesis that metformin controls cellular proliferation as well as protein synthesis in epithelial cells has been shown in recent preclinical studies.

Potential Adverse Effects

In its clinical use, metformin has shown a good safety profile and has resulted well-tolerated in patients. The main limited side effect was gastrointestinal distress, such as nausea and diarrhea, and it was transient. Only few cases required discontinuation of the drug. Metformin may also promote hirsutism and can cause vitamin B12 malabsorption in long-term therapy (5). No teratogenic effects or adverse fetal outcomes were reported. The only potential major adverse event in metformin therapy is lactic acidosis. This condition is rare and is primarily confined in patients with concomitant renal and hepatic disorders (5).

Despite metformin has been used for many years in patients with type 2 diabetes and presented preclinical data on its potential as an anticancer agent are promising, there are no data regarding the potential short- and long-term effect of this drug in nondiabetic patients or affected by PCOS.

Preclinical Studies

Sahra et al. (15) have recently investigated the effect of metformin on human prostate cancer cell proliferation both *in vitro* and *in vivo*. The action of different doses of the drug on three prostate cancer cell lines compared with one normal cell line. The authors have found out that metformin can lead to a dose-dependent strong

inhibition of cell proliferation in all prostate cell lines, blocking cell cycle by means of a strong decrease of cyclin D1 protein level. On the contrary, a moderate effect on normal cells has been noticed. These results suggest that metformin may specifically affect cancer cell proliferation compared with that of normal cells. Moreover, researchers have detected that metformin is able to reduce proliferation in colon and breast cancer cell lines. To confirm these data *in vivo*, metformin was either i.p. injected in nude mice or orally administered with water. This resulted in a tumor growth inhibition of 35% and 55%, respectively.

These data confirm the results published by Zakikhani et al. (16), according to which the AMPK pathway activation by means of metformin was observed in epithelial cells, together with a reduced proliferation and a general protein synthesis reduction. To evaluate the effect of different doses of the drug on proliferation, researchers have in particular analyzed the effect of this drug *in vitro* on a series of transformed and untransformed epithelial cell lines (breast, ovary, and prostate). Metformin was shown to act as a growth inhibitor rather than an insulin sensitizer, above all for MCF-7 human breast cancer cells. Moreover, Zakikhani found out that cell line exposure to the growth inhibitory concentrations of metformin can lead to a general decline in protein synthesis and ultimately to block both growth and proliferation. Interestingly, metformin is unlikely to directly affect those cancers inhibiting biallelic loss of LKB1 function. These data confirm that the effect of metformin on AMPK activation-mediated pathway require LKB1 suppressor gene activation.

After the results gathered on breast cancer cell lines, another study has tried to analyze the effect of metformin in different types of breast cancer cell lines, with particular attention to the hormonal characteristics of the disease. Phoenix et al. (17) confirmed that AMPK stimulation by metformin results in a significant repression of cell proliferation. She also found out that this effect was different in human breast cancer cell lines if related to either positive or negative estrogen receptors (ER). She detected complete cell growth repression in two ER-positive cell lines, although only a partial inhibition in ER-negative phenotypes. These data suggest that although both positive and negative ER breast cancer cells reduce cell growth under metformin treatment, the ER-negative cells are not as sensitive as the ER-positive ones. Moreover, researchers found a significant increase in vascular endothelial

growth factor ER-negative cell lines. This is of paramount importance because the increase of metformin-mediated vascular endothelial growth factor may override its antiproliferative effect in this particular phenotype and apparently results in proangiogenic stimuli. This mechanism could facilitate tumor progression as shown *in vivo* in the same study. However, these preclinical data need to be further investigated in the clinical setting.

Related Biomarkers

In general, an appropriate biomarker should be able to define risks and to identify the early stages of carcinogenesis. In addition, biomarkers are also able to make drug development more rational, thereby optimizing preclinical and clinical efforts and delivering timely benefits to patients. In previous preclinical studies, the effect of metformin was shown on some of these biomarkers. Sahra et al. (15) have shown the direct effect of this drug on cyclin D1. The effect of metformin on other biomarkers involved in cell cycle regulation and proliferation should be further investigated. Moreover, the possibility to better understand metformin-mediated effect on serum estrogen endogenous hormones and, in particular, on their cellular receptors has been discussed. Metformin has been proven to have an effect on ovarian androgens through the reduction of the circulating levels of insulin (insulin up-regulates ovarian steroidogenesis; ref. 18), may decrease ovarian androgen levels through a decrease in luteinizing hormone, may diminish androgen production due to an increase in tyrosine activity in metformin-exposed ovarian cells, therefore leading to a decrease in steroidogenic enzyme activity.

The insulin-like growth factor system (IGF) and its components (IGF-I and IGF-II) are peptides, whose mitogenic and antiapoptotic effects influence the proliferation of various cell types, including normal and transformed epithelial breast cells (19). These biomarkers have been thoroughly investigated in selected women with PCOS. In general, patients present lower levels of IGF binding protein-I, leading to increased ovarian bioavailability of IGF-I. On the basis of these observations, clinical trials have been conducted to assess whether metformin affects IGF pathways or its binding proteins (20-23). Unfortunately, this issue remains controversial. The most accredited hypothesis is that the drug reduces IGF-I concentration by indirectly increasing its binding protein (IGF binding protein-I), and not directly affecting IGF-I.

According to Buzzai et al. (24), metformin can increase apoptosis and can therefore be selectively toxic in paired isogenic p53-deficient colon cancer cell lines. P53 could be another biomarker to investigate metformin's action in clinical studies.

Purpose

The promising results of observational and preclinical studies support further investigations on metformin in clinical settings and, in particular, in breast cancer

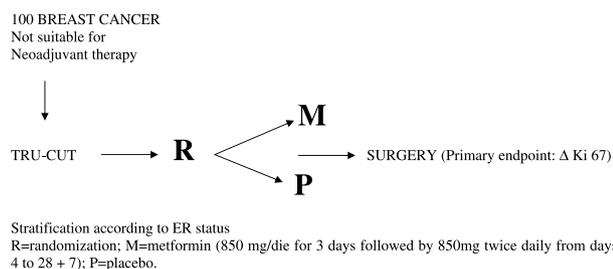


Figure 2. Design of presurgical metformin study.

treatment and prevention. Metformin could be investigated in two different settings:

- Presurgical model: to evaluate whether the use of different doses of metformin in early breast cancer preoperative settings can modulate tissue and serum biomarkers triggering tumor proliferation biomarkers. In this kind of studies, patients with a diagnostic core biopsy for intraductal neoplasia or invasive cancer are randomized to one of several doses in the time interval between biopsy and surgery (generally 4-6 wk). These phase II trials usually focus on establishing the lowest dose at which the drug modulates the risk and/or the mechanism of action of the biomarker. In spite of the significant problems with these presurgical models (i.e., difficulties of accrual, significant variations of the value of the biomarkers in different parts of the lesion), these approaches showed good feasibility and could serve as a springboard for future investigations on the presence of these biomarkers in healthy high risk women.
- Blind, randomized, placebo-controlled phase III study, in selected cohort of healthy high-risk women, harnessing the property of metformin to reduce or modulate hormonal status, in particular to assess its ability to decrease androgen levels, which are a recognized risk factor for breast cancer.

At present, in breast cancer settings, the substantial absence of clinical data on safety, on minimal dose efficacy, and on the effects on biomarkers, discourages an immediate phase III study. On the other hand, such lack of data highlights the pressing need for biomarker phase I to II studies. Our Division of Cancer Prevention and Genetics is going to start a presurgical study on women with histologically confirmed breast cancer. Our goal is to evaluate the real activity of metformin on tumor cell proliferation. We also aim to test the differences in the percentage change reduction in Ki-67 proliferation antigen evaluated before and after treatment and compare the data with placebo group. Secondary end points of the study will be to determine the interaction effect of metformin on Ki-67 by ER status as well as to observe the effect of this agent on some tissue biomarkers (Cyclin D1, p53, vascular endothelial growth factor).

Women with palpable breast cancer eligible for a tru-cut biopsy and not suitable for neoadjuvant therapy will be randomized and they will be given either metformin or placebo. The population target will be 100 women with histologically confirmed primary breast cancer, stage I/II/IIIa (T \geq 1 cm, N0-1, M0), not suitable for neoadjuvant therapy. After biopsy, patients will be randomly assigned to either metformin or placebo tablets 850 mg daily from day 1 to day 3 (to exclude treatment side effects), to be increased to 850 mg twice a day from day 4 to day 28 \pm 7. The complete design of our study is summarized in Fig. 2.

Given the short time of exposure to treatment, both ER-positive and ER-negative patients will be included to evaluate the potential different effect of the drug according to ER status as observed in the preclinical setting.

Conclusions

Metformin is an insulin-sensitizer agent, widely used to treat type 2 diabetes with a relatively favorable toxicity profile. In epithelial cells, metformin-induced AMP kinase activation has been shown to activate growth inhibitory and protein synthesis pathways. Moreover, it may have indirect antiproliferative properties through its insulin-lowering action, as suggested by population studies reporting an unexpectedly lower cancer incidence rate in diabetic patients treated with metformin compared with other therapies. As a consequence, insulin-lowering approaches by means of pharmacologic therapies may have potential in cancer prevention and prognosis.

Further studies are necessary to clarify the role of this agent in breast cancer prevention and therapy. Its favorable toxicity profile, its relative low cost, and evidence of its antiproliferative activity detected both *in vitro* and *in vivo* in preclinical studies should stimulate researchers to carry out further clinical trials on this agent.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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