

Metformin and Other Biguanides in Oncology: Advancing the Research Agenda

Michael Pollak

Abstract

Retrospective studies that may be impractical to confirm prospectively suggest that diabetics treated with metformin have a substantially reduced cancer burden compared with other diabetics. It is unclear if this reflects a chemopreventive effect, an effect on transformed cells, or both. It also remains to be established if these data have relevance to people without diabetes. Laboratory models, however, provide independent impressive evidence for the activity of metformin and other biguanides in both cancer treatment and chemoprevention. Investigations of mechanisms of action of biguanides have revealed considerable complexity and have identified important gaps in knowledge that should be addressed to ensure the optimal design of clinical trials of these agents. Such trials may define important new indications for biguanides in the prevention and/or treatment of many common cancers.

Introduction

The fascinating story leading to current interest in the use of metformin for cancer prevention and treatment began in medieval Europe. Herbalists of that time recognized that polyuria (excessive urination) could occasionally be alleviated by *Galega officinalis* (French lilac). Nothing was known concerning the active ingredient of the plant. Even the disease responsible for polyuria which responded to *G. officinalis*, diabetes mellitus, had not been defined. In the modern era, active fractions of the natural product were identified, leading to drug development efforts. After several false starts, the biguanides metformin, phenformin, and buformin were developed. Clinical experience showed that metformin has an excellent therapeutic index for diabetes, for which the drug was introduced in Europe in the 1970s and approved in the United States in 1995 (1).

Clinical trials showed that metformin has advantages over other agents used in the management of type II diabetes (2). This finding, in conjunction with the global diabetes epidemic, has made metformin one of the most frequently prescribed drugs on the planet, with an estimated 40 million prescriptions filled in the United States alone in 2008. Its safety has allowed further clinical trials, which documented effectiveness in other conditions including weight gain induced by antipsychotic medications (3), polycystic ovary disease (4), and diabetes prevention

for subjects at a high risk (5). Early laboratory studies of biguanides explored antimicrobial activity, and biguanide derivatives are now widely used in applications ranging from swimming pool decontamination to contact lens sterilization and are under study for AIDS treatment (6). This review outlines the basis for current interest in potential applications of biguanides in oncology.

Mechanisms of Actions of Biguanides for Current Indications

The precise mechanism of action of biguanides in the therapy of type II diabetes remains an active research topic. As these compounds lower both the hyperglycemia and the hyperinsulinemia associated with this disease, early investigations explored the concept that biguanides enhance insulin-receptor activation and downstream signaling, thus reducing insulin resistance, which is a key aspect of type II diabetes pathophysiology. More recent work showed that biguanides impair mitochondrial adenosine-5'-triphosphate (ATP) production (7, 8), which results in the activation of the liver kinase B1 (LKB1)-5' AMP-activated protein kinase (AMPK) signaling pathway. This pathway is central to the regulation of cellular energy homeostasis, and its activation under conditions of energy stress leads to physiologic downregulation of energy-consuming processes, such as protein synthesis and fatty acid synthesis, to restore ATP levels (9). This homeostatic system is conserved in evolution from yeast to humans, and LKB1 has previously been identified as a tumor suppressor gene (10).

In multicellular organisms, the LKB1-AMPK pathway has acquired further specialized roles in regulating energy metabolism at the level of the whole organism in addition to the cellular level. The system is involved in appetite control by the central nervous system (11), and in the

Author's Affiliation: Department of Oncology, McGill University and Jewish General Hospital, Montreal, Quebec, Canada

Corresponding Author: Michael Pollak, 3755 Cote-Ste.-Catherine, Montreal, Quebec, Canada H3T 1E2. Phone: 514-340-8222 ext. 4139; Fax: 514-340-8600; E-mail: michael.pollak@mcgill.ca.

special case of hepatocytes, activation of the LKB1-AMPK pathway downregulates gluconeogenesis, which represents the export of energy from hepatocytes to the organism in the form of glucose (12). This effect in turn reduces blood-glucose concentration, which results in a secondary decrease in insulin level. Although other mechanisms may also contribute, the inhibition of hepatic gluconeogenesis (by LKB1-AMPK activation and/or other processes; ref. 13) is now felt to be a key process underlying the utility of biguanides in the therapy of type II diabetes. Precise mechanisms of action in polycystic ovary disease and antipsychotic medication-induced obesity are unclear, but a reduction of insulin level is likely involved.

Metformin and Neoplastic Disease: Early Clues

Important early evidence suggesting that metformin is relevant to oncology came from population surveys examining the associations of diabetes and diabetes treatments with cancer. Several retrospective studies showed that diabetics had an increased cancer mortality compared with nondiabetics, and that diabetics on metformin had a substantially (~40%) reduced cancer burden compared with diabetics on other treatments (reviewed in refs. 14, 15). Although such associations are hypothesis-generating rather than definitive, the consistency of findings among different independent studies certainly contributed to the justification for further research.

Early laboratory studies also were supportive. Experiments showed that, whereas metformin activates the LKB1-AMPK pathway to inhibit gluconeogenesis in hepatocytes (12), it activates this pathway with different consequences in mouse embryo fibroblasts and human prostate, colon, and breast cancer cell lines. In these cells, the consequences of LKB1-AMPK activation include inhibition of mammalian target of rapamycin (mTOR) and protein synthesis (consistent with the need to reduce energy expenditure) and thereby a reduction in proliferation (16–20). Of note, despite the early view that metformin might act as an “insulin sensitizer” in classic insulin target tissues, the aforementioned experimental systems revealed the opposite effect in cancer cells: metformin inhibited insulin-stimulated mTOR activation and proliferation in an AMPK-dependent manner. The mechanisms of metformin are discussed further by Engelman and Cantley elsewhere in this issue of the journal (21).

Recent Findings Suggesting Oncologic Applications of Biguanides

Many laboratory models (for example, those reported in refs. 16, 17, 22–35) have been used to show the antineoplastic activity of metformin, either as a single agent or in combinations. Not all of these studies rigorously defined mechanisms, but many have provided molecular correlates of activity. There is evidence indicating that the growth of untransformed epithelial cells and a subset of

cancers is stimulated by insulin (reviewed in refs. 36, 37) and that the systemic insulin-lowering action of metformin might inhibit the proliferation of these cancers (22–24). The site of action of metformin that leads to insulin-lowering is the liver. Therefore, the insulin-lowering effect might operate *in vivo* even in the absence of detectable metformin in neoplastic tissue or, in the context of prevention, in at-risk tissue. This action of metformin shares mechanistic features with the manner by which caloric restriction inhibits the growth of certain cancers (38, 39). The antineoplastic action of biguanides attributable to insulin reduction is most likely to occur if the two following conditions are met: a lack of activating mutations in the signaling pathways downstream of the insulin receptor in neoplastic cells and hyperinsulinemia at baseline in the host. This mechanism might be relevant to prevention as well as treatment; indeed, the first condition is more likely to be satisfied in a prevention context.

Efforts to establish if the separate *direct* mechanism of action of metformin (involving LKB1-AMPK pathway activation within tumor cells described *in vitro*; refs. 16–20) also operates *in vivo*, have yielded surprising results. This mechanism may indeed play a role *in vivo*, but evidence also suggests that metformin could inhibit the growth of LKB1-deficient tumors by a separate mechanism involving the induction of cellular energy deficiency. In the context of LKB1 deficiency, the metformin-induced decrease in mitochondrial ATP production fails to trigger a fully compensatory decrease in energy expenditure in some models, resulting in an energy crisis and necrosis (40). Confirmation of this mechanism would imply an attractive therapeutic index of metformin for tumors with loss of *LKB1* tumor suppressor gene function and in Peutz-Jeghers syndrome; it also would suggest that agents which reduce ATP production have advantages over direct activators of AMPK (41). This implication is conceptually similar to a prior finding of enhanced activity of metformin in cancers with loss of function of p53 (25), as p53 is involved in mediating the antiproliferative, energy-conserving response to AMPK activation, and its loss accentuates metformin-induced energy stress.

The laboratory demonstration that dose and route influence benefit of metformin in the context of a lung cancer prevention model (reported in this issue of the journal; ref. 26) reminds us that pharmacokinetic considerations need to be addressed: one cannot assume that regimens optimized for diabetes control will be optimal for possible future oncology indications. Although this study (26) provides evidence that the observed indirect effect of metformin on levels of insulin and IGF-I, circulating hormones known to be involved in cancer risk (reviewed in ref. 36), is mechanistically important in prevention, a separate encouraging colon cancer prevention model favored a role for direct AMPK activation (27). The proposed direct and indirect mechanisms of action of metformin are not mutually exclusive. Factors including molecular pathology of tumors (or at-risk tissues), drug concentrations at the sites of action, and host characteristics such as the presence of

hyperinsulinemia and/or diabetes) might define settings where direct, indirect, both or neither mechanisms may operate, and which subjects will benefit. Results concerning the role of host metabolic factors provide an example of the uncertainties at this stage of investigation: although some experimental systems (such as that described in ref. 26) show the activity of metformin in the absence of documented host metabolic derangements, different models (for example, see refs. 22–24) suggest that maximal benefit is seen in hyperinsulinemic hosts.

Additional data of interest are provided by recent population studies. A retrospective study showed that the rate of pathologically complete response to neoadjuvant chemotherapy in patients with diabetes and breast cancer was significantly higher for patients taking metformin than for those taking other antidiabetic therapies (42), suggesting a role for metformin in sensitizing cancers to chemotherapy.

Support for investigations of metformin for breast cancer prevention is provided by a provocative study that found a 56% decrease in breast cancer among diabetics receiving metformin compared with diabetics on other therapies (43). This study is not definitive, however, as it involved a small sample size and was retrospective.

Gaps in Knowledge

The collective effect of the findings summarized here is to raise enthusiasm for further investigations of biguanides for cancer prevention and treatment, not only because the data suggest activity, but also because they identify important gaps in knowledge. Several key questions need to be addressed before clinical studies can be optimally designed.

What are the important mechanisms of action? What is the optimum compound and dosing regimen?

The question of mechanism is not merely academic because clarification of mode of action could facilitate clinical development. Candidate mechanisms are complex and involve both systemic (for example, insulin lowering) and direct effects. We know with certainty from diabetes experience that conventional metformin doses are sufficient to achieve concentrations in the liver that reduce glucose production, and, secondarily, reduce insulin levels for those patients who have hyperinsulinemia at baseline. One strategy to evaluate the importance of the systemic insulin-lowering actions of metformin will be to compare its efficacy with that of specific small molecule receptor tyrosine kinase inhibitors of the insulin/insulin-like growth factor 1 (IGF-I) receptor family (36). These agents block all the relevant receptors, whereas the systemic action of metformin merely reduces insulin levels, with little effect on IGF-I or IGF-II. Therefore, any *in vivo* evidence suggesting that the activity of metformin is superior to that of specific insulin-IGF receptor family inhibitors would imply mechanisms of action for biguanides beyond reduction of concentration of circulating insulin. A related point is

that, particularly for indications involving short-term treatment, any forthcoming evidence defining settings in which the insulin-lowering actions of metformin are mechanistically important will suggest applications for insulin/IGF-I receptor-blocking drugs, which may reduce signal transduction more effectively than would a decrease in concentration of one of the relevant ligands.

If the direct mechanism of action is important, there is a clear need for human pharmacokinetic and pharmacodynamic studies to identify the best compounds and dosing regimens for effects in extrahepatic tissues. The presence of the organic cation transporter 1 (OCT1) membrane transporter (44) may be important for cellular uptake of metformin, but less so for other biguanides, which may enter the cell in the absence of active transport. There are data implying that phenformin might be superior to metformin in activity for oncologic indications (28), and this may be due to differences in potency and pharmacokinetics. Although phenformin was withdrawn from the market because its risk of toxicity was felt to be unacceptable for long-term treatment of diabetes, it is safer than many antineoplastic agents in common use today. For cancer therapy, the toxicity risk of phenformin would likely be justified if it offered benefits over metformin in terms of efficacy, particularly if duration of therapy was limited, as might be the case in combinations with chemotherapy or radiotherapy. On the other hand, the risk of toxicity in the prevention setting must be minimal, and here, metformin has an obvious advantage, with large databases showing its long-term safety and tolerability; metformin is considered by many to be the safest available pharmacologic treatment for diabetes and to be safe enough for prevention indications (2, 5).

Last, the possibility of novel mechanisms of action of biguanides, including immune system modulation (45), should be considered.

Are there rational combinations to investigate?

Early results and theoretical considerations suggest that biguanides might be more effective in combinations than as single agents for cancer treatment, combined not only with chemotherapy (29) and radiotherapy (30), but also with rapalogs (rapamycin-related compounds) or with other agents targeting energy metabolism (31). Many combinations might be effective in relatively short-term treatment durations, whereas single-agent treatment might be practical for longer durations, similar to current hormonal therapies for prostate or breast cancer. The improved rate of pathologic complete response to neoadjuvant chemotherapy in the presence of metformin (42) implies that at least some benefits of this biguanide do not require long-term exposure.

Metformin deserves investigation for prostate cancer therapy in the special context of preventing the hyperinsulinemia that arises as a consequence of castration (46), which might contribute to the progression to castration-resistant disease (47).

Combinations in the prevention setting are unexplored.

What are the relevant predictive host or tumor biomarkers?

The identification of predictive host or tumor biomarkers represents a critical issue in the contexts of both prevention and treatment, but few data are available. Possibilities include phosphatidylinositol 3-kinase, and LKB1-AMPK functional status in neoplastic tissue, and/or baseline fasting or postprandial serum insulin levels.

Because metformin has little effect on normal insulin levels, high baseline levels might predict an increased chance of any benefit mediated by a reduction in insulin concentration. Constitutive activation of phosphatidylinositol 3-kinase is an example of a tumor characteristic that would be expected to confer resistance to the insulin-lowering effects of metformin, but not necessarily to its direct actions involving mTOR inhibition. Indeed, metformin might have some advantages over rapamycin because its inhibition of mTOR does not seem to be associated with increased AKT activation in some *in vitro* models (32).

The functional status of the LKB1-AMPK pathway in neoplastic tissue may also predict activity, although it is difficult to predict the direction. Tumors or at-risk tissue in which this tumor suppressor pathway is operational might respond via the AMPK activation mechanism. On the other hand, if drug concentration is adequate, tumors or at-risk tissue with loss of function of this pathway might be sensitive to the mechanism involving metformin-induced energy stress.

Do studies of cancer incidence and mortality among diabetics in relation to metformin use have implications for nondiabetics? Do they imply a chemopreventive action?

The available population studies should be interpreted with caution as they are retrospective and, even more important, are confined to diabetics, in whom risk and prognostic factors that are relatively unimportant in the general population may have dominant roles. At present, we have no clinical evidence, even retrospective, for antineoplastic activity of biguanides in nondiabetics. Phase II clinical trials that address this issue will be of key importance in guiding subsequent clinical research.

Only a few *in vivo* models have treated host metabolic status as a variable to study its influence on the antineoplastic activity of biguanides. These models (22–24) suggest that metformin is most active in the context of a high-energy diet that leads to hyperinsulinemia. If confirmed, this finding would suggest that the insulin-lowering action of metformin is important and that hyperinsulinemic subjects would be of special interest for clinical trials.

Available data suggest that metformin-associated reductions in cancer mortality are similar in magnitude to reductions in cancer incidence, raising the possibility that the main effects may be preventive, notwithstanding the experimental evidence that biguanides have antiproliferative activity for established cancers.

What potential clinical oncologic applications of biguanides deserve study?

Available data suggest that biguanides have potential applications not only for adjuvant and palliative cancer treatment, but also in cancer prevention. Apart from this broad agenda, there is the challenge (and opportunity) related to the apparent general rather than organ-specific effects of biguanides. Preclinical data justify, as a minimum, further research in treating or preventing colon, prostate, breast, and lung cancer. Although many cancer research efforts are organized on a strictly organ-specific basis, coordination of biguanide research across disease sites might accelerate progress.

For all indications, one of the key challenges in clinical trial design is deciding who is likely enough to benefit to be included in a trial. One possibility, using the available epidemiologic clues, would be to confine early studies to diabetics. In practice, however, this would be difficult for many reasons, including the possibility that some physicians or patients would not be at equipoise for a randomization that might exclude metformin. A subgroup in which randomization might be acceptable, and in which biguanides might have a higher chance of efficacy than in an unselected population, would be individuals with insulin resistance and hyperinsulinemia but no formal diabetes diagnosis. Of course, it is easiest to carry out trials in unselected populations, but such studies must be large if they are to have sufficient power to detect benefits that are confined to subgroups of special interest, such as hyperinsulinemic individuals or, in an extreme example, people with Peutz-Jeghers syndrome.

The Way Forward

A substantial body of evidence justifies further research regarding oncologic applications of biguanides, but considerable gaps remain in the knowledge base required to optimize designs of clinical trials of these agents. We need to ascertain the best biguanide or related compound, determine the appropriate dose, and determine biologically based eligibility criteria that define subpopulations likely to benefit before conducting a costly phase III trial involving thousands of patients and prolonged follow-up. Therefore, early steps should include small-scale trials involving pharmacokinetic and pharmacodynamic end points and acceleration of ongoing laboratory and epidemiologic studies. These steps may yield data that will not only contribute to the rationale for major phase III trials, but may also help optimize their design.

The role of the private sector in the exploration of new indications for metformin might be problematic because the drug is off-patent, but mechanisms have been proposed to justify investment in new indications for approved agents (48). On the other hand, there is a clear incentive from an intellectual property perspective to screen libraries of biguanides and related compounds to

identify patentable molecules that may have advantages over metformin for cancer treatment.

With respect to cancer prevention, it is noteworthy that a recently reported pilot clinical trial (49) provides preliminary evidence that even low-dose metformin (250 mg once daily) can suppress aberrant crypt formation in the colon. If confirmed, this will represent an important milestone in translational research concerning applications of metformin in oncology. However, this study also points to the need for more data with respect to pharmacokinetics. The half-life of metformin is approximately 6 hours (50), and a typical dose in diabetes is 500 mg three times daily, so the tissue drug concentrations (and therefore the mechanisms) responsible for the observed effect on aberrant crypts are unclear. The gastrointestinal tract may be a special case, as there is indirect evidence from FDG PET scanning (showing metformin-stimulated glucose uptake by normal colon, as would be expected following AMPK activation) that metformin might act locally from the lumen following oral administration (51). Would the slow-release metformin preparations developed for dosing convenience (for example, see ref. 50) enhance benefits by achieving more continuous exposure? Would higher doses achieve more impressive effects? As is the case with other potential applications of biguanides in oncology, design of definitive clinical trials capable of measuring maximal achievable benefits will be facilitated when optimal dosing regimes are clarified using forthcoming pharmacokinetic, pharmacodynamic- and even pharmacogenomic (52, 53) data in the context of mechanism.

Metformin may have broad activity against carcinogenesis in general, unlike intensively studied compounds

that have utility only for a specific site (e.g., tamoxifen or raloxifene for the breast and finasteride or dutasteride for the prostate). If such broad spectrum activity is confirmed, metformin could become a far more attractive chemoprevention agent than are disease site-specific compounds. Metformin also may have benefits in the context of aging-related diseases (33) in addition to known benefits in diabetes prevention and treatment. Therefore, if the next research steps provide sufficient justification for large-scale cancer prevention trials, end points should include overall health outcomes. Such trials would be most justifiable, however, if future research provides criteria to identify a specific high-risk population in which metformin is expected to provide benefit.

We now have both motivating data and the tools of epidemiology, laboratory studies, and translational research to pursue specific settings in which biguanides might be useful for cancer control.

Disclosure of Potential Conflicts of Interest

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