Metformin and Hepatic Carcinogenesis

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Abstract

Retrospective, hypothesis-generating population studies suggest that diabetics treated with metformin have a substantially reduced risk of several cancers, including hepatoma, relative to diabetics on other therapies. In this issue of the journal (beginning on page 544), Bhalla and colleagues contribute to the growing literature on metformin effects in experimental carcinogenesis models, showing reduced carcinogen-induced hepatoma in mice. The clinical need to develop novel prevention strategies for hepatoma is obvious, given an increasing prevalence and poor prognosis. The clues that metformin or related biguanides may have utility in this area justify accelerated laboratory research, as more data concerning mechanism, pharmacokinetics, and predictors of efficacy will help to optimize the design of clinical trials.

The literature on metformin and cancer has grown rapidly in the past few years. There is tantalizing evidence that this widely used, well-tolerated biguanide molecule may have applications in cancer prevention or treatment (1). In this issue of the journal, Bhalla and colleagues present interesting experimental evidence for an action of metformin in inhibiting the development of chemically induced hepatocellular carcinoma in mice (2), yet many important questions remain unanswered.

Several proposed mechanisms of action of metformin may underlie its activity as a cancer preventive agent. Some (but not all) of these mechanisms may overlap with the actions that are responsible for the utility of the compound in the treatment of type II diabetes. It is now thought that the pleiotropic effects of metformin originate with the primary actions of the drug on the mitochondria (3), where it inhibits oxidative phosphorylation (at respiratory complex I) in a manner that has not yet been described in detail. Sequelae to this inhibition occur at the local mitochondrial level, at the cellular level, and at the level of the whole organism. The best known local consequence is the decline of mitochondrial ATP production (1), but there is also recent evidence for altered redox status, a decrease in reactive oxygen species (ROS) production, and a decrease in mutations attributable to ROS (4). At the cellular level, the reduction in ATP production by oxidative phosphorylation leads to a degree of energy stress. This stress can have different effects depending on the cellular context.

Some cells have the ability to sense and respond to the stress by decreasing energy-consuming processes such as lipid synthesis, protein synthesis, and proliferation (1, 3). This decrease results in a new steady state characterized by reduced ATP production, reduced ATP consumption, and an energetically "sleepy" phenotype, which may tend to slow carcinogenesis and may also be cytostatic for a subset of established cancers. Another compensation involves increasing glycolysis to help correct the ATP deficit. This process may seem paradoxical at first, as it represents a situation of increased glucose uptake and a glycolytic phenotype associated with growth inhibition, thus contrasting with classic views originating with Warburg (5) that a shift to glycolysis is associated with more aggressive behavior and increased proliferation.

A special case of response to metformin-induced energy stress is seen in the hepatocyte. The process of gluconeogenesis, by which hepatocytes export glucose to the circulation, is an important part of whole-organism carbohydrate metabolism; from the point of view of the hepatocyte, gluconeogenesis represents export of energy, and so it is not surprising that metformin-induced energetic stress inhibits gluconeogenesis (3, 6, 7). This inhibition then improves hyperglycemia and hyperinsulinemia in patients with type II diabetes. These systemic effects might reduce the proliferation rate of any cancers or premalignant lesions that thrive in a high-glucose, high-insulin environment. The fact that metformin reduces glucose and insulin levels only when baseline levels are elevated contributes to its safety, but also implies that this mechanism can only contribute to antineoplastic activity in a metabolically defined subpopulation.

Many experimental studies demonstrate cancer treatment or prevention activity of metformin attributable to direct actions of the drug on target cells that are independent of

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systemic effects such as reduction of insulin and glucose levels. Reported cell autonomous actions include inhibition of lipid synthesis (8), as emphasized in the study by Bhalla and colleagues (2). Although this inhibition is a consequence of 5′ AMP-activated protein kinase (AMPK) activation due to metformin-induced energy stress in some models (9), this was not the case in the data reported by Bhalla and colleagues (2). Another direct action of metformin that arises as a consequence of AMPK activation is the inhibition of protein translation as a consequence of inhibition of mTOR (10). Inhibition of macromolecule (lipid and protein) synthesis is an expected consequence of a cellular response to an energy shortage. Work by Vitale-Cross et al. reported in this issue of the journal (11) is consistent with (but does not formally prove) that inhibition of mTOR underlies the cancer prevention activity of metformin in certain models. Other direct effects of metformin, including actions on miRNA relevant to stem cell biology (12), or new evidence for effects of metformin on Bambi expression (13) may also be relevant to its activity in cancer prevention models.

Uncertainty to what extent any of these or other direct actions occur clinically relates in large part to pharmacokinetics and drug uptake; metformin only accumulates in cells if the extracellular concentration is sufficient and if target cells have adequate specific cell surface transport molecules such as organic cation transporter 1 (OCT1; ref. 14). In many tissues, bioavailability of metformin following conventional oral dosing is not established. In the liver, however, it is clear that concentrations of the drug (via the portal circulation following oral ingestion) are high and OCT1 is highly expressed, making the liver a particularly interesting organ in which to examine metformin for cancer prevention.

Future research will have to address a few obvious issues. The dose of metformin (250 mg/kg/d) in the study of Bhalla and colleagues was an order of magnitude higher than the per kilogram dose used in humans for diabetes treatment; it is not clear if this dose was arbitrary or chosen because lower doses were ineffective. Induction of lipogenic gene expression in the model could rescue cells from growth-inhibiting effects of metformin. Although interesting, this finding does not establish that increasing lipid synthesis would be sufficient to block the ability of metformin to inhibit carcinogenesis. Indeed, the pleiotropic nature of metformin effects makes it challenging to isolate specific actions that are critical for antineoplastic effects. Furthermore, recent reports show that blockade of the Warburg effect may require concomitant inhibition of multiple components of cellular energy pathways and that AMPK activation can have an antiproliferative but prosurvival effect on cancer cells under energetically stressed conditions (15).

Caution is required in extrapolating activity in a chemical hepatocarcinogenesis model to other settings of hepatocellular carcinogenesis including those related to viral infection or type II diabetes, in which the steps leading to transformation may be different. It is intriguing, however, that separate research has shown that experimental hepatitis C infection is associated with downregulation of AMPK activity, and that restoring AMPK activation by metformin-induced energy stress reduces viral infection and some of its consequences (16). Another example of interesting research in this context is evidence that insulin (which reaches the liver in high concentrations in the hyperinsulinemic phase of type II diabetes) is sufficient to induce liver cancer (17, 18); thus, information concerning insulin levels and signaling downstream of the insulin receptor in the presence and absence of metformin in the model of Bhalla and colleagues (2) would be of interest. Another unanswered question is the relevance of the recently described metformin-induced inhibition of mitochondrial ROS production *in vitro* to step-wise carcinogenesis *in vivo* (4). And last, there is clear evidence for an important role of interleukin 6 in hepatocarcinogenesis (19), and it is unclear to what extent metformin influences inflammatory cytokines.

The clinical need for novel approaches to hepatocellular cancer risk reduction is substantial; as Bhalla and colleagues (2) point out, this disease is a growing public health challenge due to the increasing prevalence of predisposing factors such as hepatitis and type II diabetes. The established long-term safety (at conventional doses) and low cost of metformin make it an attractive candidate for use in cancer risk reduction. Indeed, its widespread availability as a generic drug has already permitted many preliminary clinical trials for various indications in cancer prevention and treatment (1). There are gaps in our knowledge, however, that need to be addressed before the design of clinical trials of this drug can be optimized. These gaps relate to details of mechanism of action, to cellular, and whole-organism pharmacokinetic factors that would allow a rational selection of dose (and even the best biguanide to use) for various proposed indications, and to predictive factors that might identify which individuals would be likely to benefit, either for treatment or prevention. Potential utility of metformin for prevention of hepatocellular carcinoma in high risk populations should be regarded as a high priority for investigation, because of favorable pharmacokinetics for this organ, encouraging predictions from laboratory models, and provocative results from cohort studies, such as a recent report from France that documented a substantial (>50%) decline in hepatitis C-associated hepatocellular carcinoma among metformin exposed subjects (20). Both cell-autonomous actions and systemic effects of metformin, particularly on insulin (21), deserve scrutiny in efforts to uncover the mechanism(s) underlying the cancer prevention activity of this agent observed in models such as the one reported by Bhalla et al. (2).

There have been major disappointments in large phase III clinical trials of micronutrients for the prevention of prostate, lung, or colon cancer—trials that were based on plausible hypotheses but supported by relatively little experimental data. With regard to liver cancer, there is a rather well defined and large population at a high risk of the disease that would be suitable for innovative prevention

trials of biguanides. It is therefore urgent to accelerate laboratory studies that will advance this exciting direction of research in a way that will guide clinical trial design.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Pollak, A.M. Gonzalez-Angulo.
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Administrative, technical, or material support: A.M. Gonzalez-Angulo.

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