Prevention of Mutagenesis: New Potential Mechanisms of Metformin Action in Neoplastic Cells

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Abstract

Several experimental and epidemiologic studies have shown that the antidiabetes drug metformin has antitumor properties. The report by Algire and colleagues in this issue of the journal (beginning on page 536) shows for the first time that metformin reduces mutagenesis induced by reactive oxygen species. This report offers new perspectives on metformin in cancer prevention and provides a new mechanism for the reduction of cancer risk in diabetic patients treated with this drug. Cancer Prev Res; 5(4); 503–6. ©2012 AACR.

Metformin (N,N’-dimethylbiguanide) belongs to the biguanide class of oral hypoglycemic agents. It is a widely used antidiabetes drug now prescribed to almost 120 million diabetic patients. In addition to its efficacy in lowering glucose levels, and consequently insulinemia, it has the clinical advantage of not inducing any risk of hypoglycemia at standard doses and of inducing few adverse secondary effects (1).

Accumulating evidence from epidemiologic and experimental studies shows that metformin exerts antitumor and antiproliferative effects (reviewed in ref. 2). Many epidemiologic studies have shown that metformin (compared with other antidiabetic drugs such as insulin or sulfonylureas) reduces the incidence of cancers in diabetic patients (2). It should be noted, however, that most of these studies have been retrospective and exclusively involved diabetic patients.

At the cellular level, metformin induces cell-cycle arrest, autophagy, and cell death, depending on the cancer cell origin (3–5). It also affects cancer cell metabolism and inhibits the activity of the mitochondrial complex I in hepatocytes and cancer cells (Fig. 1; refs. 6, 7). At the molecular level, metformin activates AMP-activated kinase (AMPK), a kinase regulated by liver kinase B1 (LKB1), a tumor suppressor gene. AMPK activation inhibits mTOR, which controls protein synthesis. Altogether, these observations suggest a direct action of metformin on cancer cell proliferation. Numerous preclinical studies have shown that metformin reduces tumor growth in mice models. Interestingly, an article by Vitale-Cross in this issue demonstrates that metformin prevents the development of oral squamous cell carcinoma (8). One of the disputed issues, however, is whether the in vivo effects of metformin are direct and/or indirect, that is, due to the associated decrease in insulin levels as a consequence of decreased hyperglycemia. Indeed, insulin as well as insulin-like growth factor-1 (IGF-1) are key factors in promoting cancer development (9, 10). Some preclinical studies in different mouse models found that decreased tumor growth was associated with a significant reduction of insulinemia (11, 12). One of the most interesting of these preclinical studies was recently published in this journal by Memmott and colleagues (13). They show that metformin prevents carcinogenesis induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butaneone (NNK), a well-known tobacco-specific human lung carcinogen that is notorious for causing DNA damage. The antitumorigenic effect of metformin in this study was attributed to a decrease in the circulating levels of growth factors such as insulin and IGF-I and to a reduction of IGF-1 signaling in lung tissue. Interestingly, a very encouraging new preclinical study showed that metformin significantly reduced the formation of hepatocarcinoma induced by diethylnitrosamine in mice, an effect attributed to a decrease of lipogenesis in the liver (14).

In this issue of the journal, Algire and colleagues (15) highlight a new mechanism that could explain the protective effect of metformin. Indeed, metformin could prevent cancer from DNA damage induced by NNK. The authors show that metformin attenuates the increase in reactive oxygen species (ROS) generated by the pesticide paraquat (which stimulates the formation of endogenous ROS by the mitochondrial complex I) but did not affect ROS generated by H2O2, which acts as an exogenous source of ROS. Importantly, metformin also reduces Ras-induced ROS production (Fig. 1). ROS induces a variety of abnormalities in DNA, including base oxidation, DNA strand breaks, and...
cross-links between DNA and proteins. Because these damaging effects occur at the onset of cellular transformation, protection against ROS-induced DNA damage represents a major issue during the initial steps of carcinogenesis. ROS also play a central role in the formation of advanced glycation end products (AGE) and the interaction of AGEs with their receptor, RAGE, enhances oxidative stress (16). This positive feedback loop further increases the risk of pathologies such as diabetes and cancer (Fig. 1).

Some reports have suggested that metformin possesses antioxidant properties (17, 18), and several in vivo and in vitro studies have shown that metformin prevents AGE formation (19-22). The structure of metformin could, in part, explain its glycation-inhibiting properties. Metformin has structural similarities with aminoguanidine, a drug which protects against the formation of AGE. Like aminoguanidine, metformin is able to trap carbonyl species and can directly react with methylglyoxal, a glucose derivative with high glycatnig potential (21). According to Bonnefont-Rousselot and colleagues, the aminoguanidine-like activity of metformin could indirectly participate to its antioxidant action (17).

In addition to its protective action against AGE, metformin has been shown to directly affect the activity of the membrane-bound enzyme complex nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, which generates superoxide anions (O$_2^-$; Fig. 1). In a recent study of Piwkowska and colleagues, metformin significantly reduced NADPH oxidase activity in renal podocytes (23) and, consequently, ROS production.

Algire and colleagues propose another hypothesis to explain the antioxidant properties of metformin. Because metformin has no direct scavenging effects toward hydroxyl radicals (24), they suggest that it inhibits ROS production through the inhibition of the mitochondrial complex I. This decrease of complex I activity would reduce the entry of electrons in the electron transport chain and therefore reduce ROS production by mitochondrial complexes I and III, the 2 main producers of ROS. This hypothesis proposes that metformin acts on mitochondria and more specifically on oxidative phosphorylation (a metabolic process that generates ROS) rather than acting through a classic antioxidant function. Taken together, the antioxidant properties of metformin could provide benefit against not only diabetes but cancer as well. Whether or not metformin should be prescribed as a preventive drug against adverse oxidative insults remains an open question.

The original and perhaps most interesting finding reported by Algire and colleagues (15) is that metformin reduces DNA damage associated with ROS production. Once again, a parallelism between diabetes and cancer leads to surprising discoveries. Indeed, a genome-wide association study conducted in a cohort of Scottish patients with type II diabetes identified a genetic variant of the Ataxia telangiectasia (ATM) gene, which modulates the anti-diabetic effect of metformin in humans (25). ATM is a serine/threonine kinase of the atypical phosphoinositide 3-kinase-related protein kinase family. ATM is activated by double strand DNA breaks and acts to induce cell-cycle arrest and to facilitate DNA repair (Fig. 1). From a cancer-oriented perspective, the association of metformin efficiency with ATM is a very interesting issue, prompting several laboratories to work on the potential role of metformin in activating the DNA damage response (DDR). Thus, Vazquez-Martin and colleagues found that metformin leads to the phosphorylation and activation of the ATM pathway (26). In addition, they showed that metformin treatment enhanced the phosphorylation of cAMP-response element binding protein, an ATM kinase-regulated event in response to oxidative DNA damage (27). Because DDR is a major component of the innate tumor suppressor mechanism, activation of the ATM pathway could contribute to metformin’s cancer preventive properties. Although a single nucleotide base mutation in the ATM region influences the success of metformin treatment in type II diabetes (25), a major remaining issue is to determine whether activation of the ATM/chk2 checkpoint is a critical event that prevents neoplastic transformation.
So far, no direct evidence has shown that metformin reduces DNA damage, and Onaran and colleagues showed that, to the contrary, metformin does not prevent DNA damage induced by cumene hydroperoxide in lymphocytes, despite its antioxidant activity (18). Algire and colleagues show that metformin treatment of AMPKα-/- and AMPKα-/+ fibroblasts following exposure to paraquat but not H2O2 significantly reduces the number of γH2AX-positive foci (a marker of DNA damage; ref. 16) independently of AMPK. In addition, they conducted a mutation assay showing that metformin impairs the mutagenic effects of paraquat.

Oncogenic transformation is associated with increased DNA damage, ROS production, and genomic instability (28). An important discovery made by Algire and colleagues is that metformin significantly reduces the number of foci with DNA damage in fibroblasts transformed by introduction of the oncogenic form of Ras.

The majority, if not all, of the ongoing clinical trials of metformin use it as an adjuvant to classic chemotherapeutic agents for treating existing cancers, and two metformin clinical trials have been published so far. Hadad and colleagues carried out a pilot study including a small number of patients with breast tumors (29). They showed that the percentage of cells staining for the proliferation marker Ki67 fell significantly with 2 weeks of metformin treatment, with parallel beneficial effects on genes of the cell-cycle pathways. Another short-term clinical trial, involving nondiabetic patients with a previous history of colorectal aberrant crypt foci (ACF), showed that metformin alone (at the low dose of 250 mg/d for 1 month) decreased the number and size of ACF, which are considered to be a precancerous lesion (30). New clinical results in the coming years should add to these early clinical trials in deciphering the true beneficial effect of metformin in the treatment of cancer.

Active research in cancer prevention includes metformin as one of the promising new approaches. Metformin has the advantage of being very safe and well tolerated. It is associated with only a very low incidence of lactic acidosis (<1 of 10,000) in patients with poor renal function (31), and some gastrointestinal problems are also described among minor side effects, such as diarrhea, bloating, and nausea, which can be partly prevented by progressively increasing the metformin dose to the desired level. Last, a deficiency in vitamin B12 is also a consequence of long-term metformin therapy (32). The use of metformin as a cancer preventive drug is an attractive clinical prospect, but long-term trials are needed to evaluate such potential use. One of the main obstacles to carrying out such trials would be to recruit a sufficient number of nondiabetic patients who would take an antidiabetes drug.

In conclusion, it is worth continuing to decipher the molecular and cellular mechanisms implicated in metformin action to justify a translational preventive study.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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