Are Metformin Doses Used in Murine Cancer Models Clinically Relevant?

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An important mechanism of action of metformin as an antidiabetic drug involves inhibition of hepatic gluconeogenesis, but the molecular basis for this is controversial. A recent perspective (He and Wondisford, 2015) argued that direct action of the drug on AMPK is of key importance and occurs at clinically relevant concentrations of ~70 μM. However, that review cited conflicting studies, which concluded either that AMPK-independent mechanisms are important in metformin action, or that AMPK activation plays a role but is secondary to energetic stress resulting from inhibition of oxidative phosphorylation by the drug.

As work to define the molecular mechanisms of metformin in diabetes has proceeded, the hypothesis that biguanides may be "repurposed" for additional applications has received increased attention. This followed retrospective pharmacoepidemiologic studies that showed reduced cancer risk and/or improved cancer prognosis among diabetics treated with metformin relative to those treated with other drugs. Although some of these studies have been criticized on methodologic grounds, they nevertheless led to laboratory studies that revealed clear antineoplastic activity and provided a rationale for clinical trials (Buzzai et al., 2007). More than 100 trials designed to detect clinical utility of metformin in oncology are now ongoing. There is also experimental evidence that metformin extends lifespan in model organisms, sparking interest in the possibility that these studies have clinical relevance.

Not surprisingly in view of controversies regarding the molecular mechanisms of metformin action in diabetes, there is uncertainty regarding the mechanisms relevant to its putative antineoplastic and antiaging effects, and it cannot be assumed that these are identical to those involved in suppression of gluconeogenesis in diabetes (Foretz et al., 2014). One possibility is that the drug acts indirectly by altering the host endocrine milieu in a manner that inhibits the growth of a subset of cancers, for example, by lowering insulin levels. While this is possible, the magnitude of such changes is small and may or may not be sufficient to alter neoplastic behavior. Another possibility relates to direct actions of biguanides on cancer cells or cells at risk for transformation, for example, by inhibiting oxidative phosphorylation (Wheaton et al., 2014). However, it is unclear if clinically practical metformin doses are sufficient for such mechanisms to operate.

Most in vitro assays reveal that millimolar (mM) levels of metformin are required to have antiproliferative effects. At first glance, this implies that direct clinical antineoplastic activity is unlikely, as diabetic patients receiving typical doses of 1.5–2.5 g of metformin per day (~30 mg/kg) have plasma levels in the 10 μM range (Graham et al., 2011). However, sensitivity to metformin is known to vary greatly with nutrient availability, and in vitro culture conditions are unlikely to mimic the in vivo microenvironment in this respect, as glucose, serine, and glutamine, each of which reduces metformin sensitivity, are likely present in media at higher concentration than in poorly vascularized tumors (Gravel et al., 2014). Thus, we suggest that the requirement for millimolar metformin concentrations in vitro may not be useful for predicting the drug concentration required for activity in vivo or clinically. Nevertheless, it is important to determine the in vivo concentration of metformin in mouse models that demonstrates antineoplastic activity in order to determine if this level is achievable in patients.

We administered metformin to C57BL/6J mice at a dose of 250 mg/kg in drinking water for 2 weeks and used mass spectrometry to measure metformin concentrations at steady state. As shown in Figure S1 (available online), these mice had plasma concentrations in the 5 μM range and liver concentrations of ~40 μM. Accumulation in liver is plausible as hepatocytes highly express the organic cation transporters (OCTs) that import metformin. We also studied J:Nu mice bearing human HCT116 *p53*$^{-/-}$ colon cancer, an OCT-expressing tumor previously shown to be growth inhibited in vivo when mice were provided with drinking water as a 1.25 mg/mL solution of metformin, which provided a daily oral dose of ~250 mg/kg (Wheaton et al., 2014).

This represents a dose 10-fold higher than the human dose on a mg/kg basis. In this model, tumors became resistant to metformin-induced growth inhibition when transfected with metformin-resistant *Saccharomyces* cerevisiae NADH dehydrogenase, bypassing the need for respiratory complex I, suggesting that the drug acts by inhibiting oxidative phosphorylation in cancer cells (Wheaton et al., 2014). The plasma (n = 4) and tumor (n = 9) metformin concentrations were both in the range of 3.2–12.4 μM (Figures S1A and S1B), a level achieved in routine diabetes treatment (Graham et al., 2011). In an additional study, we utilized the metformin-sensitive human A549 lung adenocarcinoma. NMRI nu/nu mice were either intraperitoneally injected or given metformin (350 mg/kg) by mouth (P.O.) once daily for 2 or 3 weeks, respectively. After

the last dose, we measured the metformin concentration over a 24 hr period in the plasma, liver, and tumors of these mice (Figures S1C and S1D). Intraperitoneal (i.p.) dosing of metformin resulted in a peak liver and tumor concentration of approximately 100 μM while P.O. dosing resulted in a significantly higher liver peak metformin concentration (~1500 μM) than tumor metformin concentration (~200 μM). Time-averaged plasma concentrations were ~7.5 and ~47 μM following i.p. and P.O. dosing, respectively. The 350 mg/kg dose of metformin activates AMPK in vivo and diminishes tumorigenesis in mouse models of cancer (Lévy et al., 2015). Together, our findings suggest that the metformin plasma concentration in a murine model where the drug has antineoplastic activity attributable to inhibition of oxidative phosphorylation (Wheaton et al., 2014) is in the micromolar range, comparable to that of diabetic patients receiving a standard dose of metformin, despite the fact that the mg/kg dose in mice is considerably higher than the mg/kg dose in humans. This is in keeping with interspecies scaling of pharmacokinetics and suggests that ongoing clinical trials are reasonable from a pharmacokinetic perspective. It is also important to note that metformin is a cation and is predicted to accumulate 100- to 500-fold in the mitochondria due to the membrane potential. Thus, micromolar concentrations of metformin in the plasma may result in considerably higher concentrations in the mitochondria of cells that are capable of importing the drug.

However, the first formal blinded clinical trial of metformin with a survival endpoint showed no benefit, even though plasma drug levels in the micromolar range were observed (Kordes et al., 2015). This underlines the need to take into account both pharmacokinetic and biologic factors in evaluating antineoplastic activity of biguanides. Even in the presence of a potentially adequate plasma metformin concentration, antineoplastic activity likely varies with tumor-related factors, including expression of cell-surface transport molecules important in drug influx and efflux, mutational status of LKB1, and genes encoding mitochondrial complex I components (Algire et al., 2011; Birsoy et al., 2014). Therefore, further studies of pharmacokinetics and mechanism are needed to address the tantalizing possibility that metformin or novel biguanides may have a therapeutic role to play in treatment or prevention of diseases other than diabetes.

AUTHOR CONTRIBUTIONS

N.S.C., M.P., C.A., S.C., and A.H. designed the study. C.R.R. and S.E.W. performed the experiments in Figures S1A and S1B. D.A. did the metformin quantitation in Figures S1A and S1B. N.S.C. and M.P. wrote the paper. C.A. edited the paper. S.M. and R.N. performed the experiments in Figures S1C and S1D.

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