Mechanism of Metformin: A Tale of Two Sites

Ruisheng Song

Diabetes Care 2016;39:187–189 | DOI: 10.2337/dci15-0013

Metformin (dimethylbiguanide) features as a current first-line pharmacological treatment for type 2 diabetes (T2D) in almost all guidelines and recommendations worldwide. It has been known that the antihyperglycemic effect of metformin is mainly due to the inhibition of hepatic glucose output, and therefore, the liver is presumably the primary site of metformin function. However, in this issue of *Diabetes Care*, Fineman and colleagues (1) demonstrate surprising results from their clinical trials that suggest the primary effect of metformin resides in the human gut.

Metformin is an orally administered drug used for lowering blood glucose concentrations in patients with T2D, particularly in those overweight and obese as well as those with normal renal function. Pharmacologically, metformin belongs to the biguanide class of antidiabetes drugs. The history of biguanides can be traced from the use of *Galega officinalis* (commonly known as galega) for treating diabetes in medieval Europe (2). Guanidine, the active component of galega, is the parent compound used to synthesize the biguanides. Among three main biguanides introduced for diabetes therapy in late 1950s, metformin (Fig. 1*A*) has a superior safety profile and is well tolerated. The other two biguanides, phenformin and buformin, were withdrawn in the early 1970s due to the risk of lactic acidosis and increased cardiac mortality. The incidence of lactic acidosis with metformin at therapeutic doses is rare (less than three cases per 100,000 patient-years) and is not greater than with nonmetformin therapies (3). Major clinical advantages of metformin include specific reduction of hepatic glucose output, with subsequent improvement of peripheral insulin sensitivity, and remarkable cardiovascular safety, but without increasing islet insulin secretion, inducing weight gain, or posing a risk of hypoglycemia. Moreover, metformin has also shown benefits in reducing cancer risk and improving cancer prognosis (4,5), as well as counteracting the cardiovascular complications associated with diabetes (6).

Although metformin has been widely prescribed to patients with T2D for over 50 years and has been found to be safe and efficacious both as monotherapy and in combination with other oral antidiabetes agents and insulin, the mechanism of metformin action is only partially explored and remains controversial. In mammals, oral bioavailability of metformin is ~50% and is absorbed through the upper small intestine (duodenum and jejunum) (7) and then is delivered to the liver, circulates unbound essentially, and finally is eliminated by the kidneys. Note that metformin is not metabolized and so is unchanged throughout the journey in the body. The concentration of metformin in the liver is three- to fivefold higher than that in the portal vein (40–70 μmol/L) after single therapeutic dose (20 mg/kg/day in humans or 250 mg/kg/day in mice) (3,8), and metformin in general circulation is 10–40 μmol/L (8). As the antihyperglycemic effect of metformin is mainly due to the inhibition of hepatic glucose output and the concentration of metformin in the hepatocytes is much higher than in the blood, the liver is therefore presumed to be the primary site of metformin function. Indeed, the liver has been the focus of the majority of metformin research by far, and hepatic mechanisms of metformin that have been suggested include the activation of AMPK through liver kinase B1 and decreased energy charge (9,10), the inhibition of glucagon-induced cAMP production by blocking adenylyl cyclase (11), the increase of the AMP/ATP ratio by restricting NADH-coenzyme Q oxidoreductase (complex I) in the mitochondrial electron transport chain (12) (albeit at high metformin concentrations, ~5 mmol/L), and, more recently, the reduction of lactate and glycerol metabolism to glucose through a redox change by inhibiting mitochondrial glycerophosphate dehydrogenase (13).

It is noteworthy that the remaining ~50% of metformin, which is unabsorbed, accumulates in the gut mucosa of the distal small intestine at concentrations 30- to

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, and Broad Institute of MIT and Harvard, Cambridge, MA

Corresponding author: Ruisheng Song, ruisheng_song@hms.harvard.edu.

See accompanying article, p. 198.

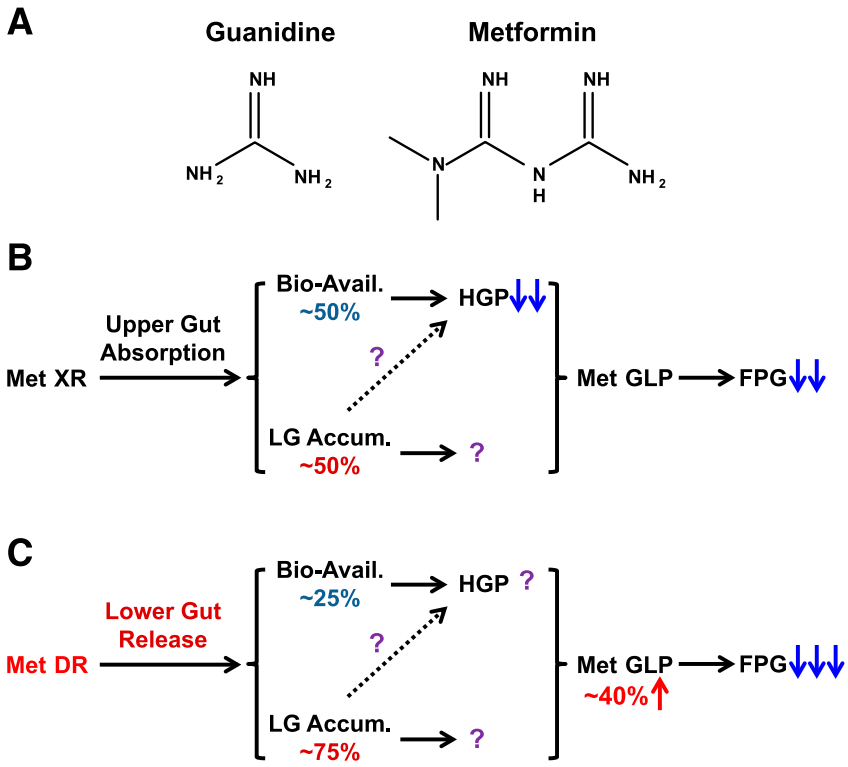

Figure 1—Mechanisms of metformin in humans. *A*: Chemical structures of guanidine and metformin (dimethylbiguanide). Schematic diagrams showing the pharmacokinetics of Met XR (*B*) and Met DR (*C*) in oral administration and the underlying mechanisms for their respective antihyperglycemic effects. Bio-Avail., bioavailability; HGP, hepatic glucose output; LG Accum., lower gut accumulation; Met GLP, metformin glucose-lowering potency; ?, unknown.

300-fold greater than in the plasma (14) and ultimately is eliminated with feces. However, in humans, gut effect of metformin remains largely obscure, although several proposals have been suggested from animal experiments including delayed intestinal glucose absorption (15), augmented lactate production by enterocytes (15), enhanced secretion of gastrointestinal hormones or peptides containing glucagon-like peptide 1 (16), bile acid metabolism (17), and potential roles of intestinal microbiota (18). Interestingly, Cabreiro et al. (19) recently demonstrated that metformin regulates systemic metabolism and retards aging in *Caenorhabditis elegans* by altering microbial folate and methionine metabolism, implying an important role of metformin in gut microbiota impacting systemic metabolism in higher organisms such as humans. Now, Fineman and colleagues (1) have offered clinical evidence suggesting the primary effect of metformin resides in the human gut. In their report, they described a novel formulation of metformin, namely, delayed-release metformin (Met DR). These metformin tablets comprise an immediate-release metformin hydrochloride core overlaid with a proprietary enteric coat, which is designed for delaying the release of metformin until pH reaches 6.5 in the distal small intestine or beyond, where absorption of metformin is very low. Thus, the bioavailability of the drug would be much decreased as compared with currently available metformin formulations Met IR (immediate-release) and Met XR (extended-release), and therefore a striking contrast of metformin concentrations in the gut and plasma could be made. Taking advantage of this, they hypothesized that gut exposure of metformin, but not circulation, accounts for most of its antihyperglycemic effect.

To test this hypothesis, Fineman and colleagues (1) conducted two studies. Study 1 was a randomized, four-period, crossover pharmacokinetic study in 20 subjects (BMI 25–35 kg/m^2), and every subject in a randomized sequence received 1-day dosing for each of four treatments: 500 mg Met DR BID, 1,000 mg Met DR BID, 1,000 mg Met IR BID, and 2,000 mg Met XR QD. Treatments were separated by a 3- to 7-day washout interval. Plasma metformin concentrations were measured over a 36.5-h period (including five standardized meals). Pharmacokinetic parameters were determined using noncompartmental analysis. Study 1 was constructed to have 90% power to detect a difference of area under the curve of at least 25% between 1,000 mg Met DR BID and 2,000 mg Met XR QD. Study 2 was a phase 2, 12-week, randomized, placebo-controlled, dose-response study conducted in 240 T2D subjects, and subjects were randomized to six treatment groups consisting of placebo or 600, 800, or 1,000 mg Met DR QD in the morning or 1,000 or 2,000 mg Met XR QD in the evening (positive references). The primary end point was the change in fasting plasma glucose (FPG) at 4 weeks of treatment, and the secondary end points included the changes in FPG at 4, 8, and 12 weeks of treatment. Accordingly, fasting metformin (week 1, 2, 3, 4, 8, and 12) and plasma HbA_{1c} and lactate (week 12) were also measured. A sample size of 40 subjects per group provided ~80% power to detect a difference in week 4 FPG values between at least one Met DR group and placebo. The results were as they expected (Fig. 1*B* and *C*). In study 1, the bioavailability of Met DR BID was ~50% (1,000 mg) that of Met IR and Met XR after 1-day dose. In study 2, first, both Met DR and Met XR displayed a clear dose response, and second, all Met DR treatments (600, 800, or 1,000 mg QD) produced not only a statistically significant, clinically relevant, and sustained reduction in FPG over 12 weeks compared with placebo but also a stronger FPG lowering than Met XR (1,000 mg QD), while the metformin concentrations in plasma were much lower than that of Met XR. As a result, a ~40% increase of glucose-lowering potency was found in Met DR compared with Met XR (Fig. 1*B*). Third, plasma lactate levels were significantly decreased in Met DR arms, although they were in normal ranges in all groups. Fourth, placebo-subtracted changes in HbA_{1c} were consistent with FPG changes. Additionally, similar to current available metformin, Met DR was generally well tolerated and adverse events were consistent with published prescribing information.

These observations by Fineman and colleagues (1) are important, because they, for the first time, have demonstrated in humans that metformin effect selectively biased toward gut is actually even stronger than systemic effect where hepatic effect is thought to be dominant and therefore conceptually suggested that gut is the primary site of metformin action. The demonstration is clear and straightforward, and the results may have a great impact not only on our understanding of metformin mechanism in humans but also on future metformin therapy in clinic, for example, using gut-released metformin (Met DR) instead of the current formulation (Met XR). In addition, achieving low plasma exposure of metformin by using Met DR might be particularly useful in patients with conditions that increase the life-threatening risk of metformin-associated lactic acidosis, including renal impairment, cardiac dysfunction, hepatic insufficiency, or intercurrent illness such as dehydration. Despite the strengths discussed here, there are still limitations of their article, as acknowledged by the authors. First, the dose-ranging efficacy trial Fineman and colleagues conducted was short term (12 weeks), although it seems long enough for the article. A longer investigation is still required to test safety, tolerability, and adverse effects of Met DR more sophisticatedly for future clinical application. Second, the mechanism underlying the striking effect of Met DR is unknown. How does gut effect impact the whole body? And is the liver involved in the gut mechanism of metformin? The results obtained by Fineman and colleagues cannot rule out the systemic effect because the bioavailability of Met DR is not zero, despite it being low. In fact, it is conceivable that a certain value of systemic exposure may be essential for metformin action. However, metformin still works even after gut effect is removed by intravenous administration (13), indicating gut exposure could be bypassed for glucose-lowering effect of metformin. Nevertheless, Fineman and colleagues (1) developed a novel gut-release metformin Met DR and demonstrated for the first time that the primary effect of metformin resides in the human gut, at least when orally administrated. Ultimately, these interesting results offered not only a conceptual advance in understanding of metformin mechanism in humans but also the lower gut as a promising target site for future metformin research.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Buse JB, DeFronzo RA, Rosenstock J, et al. The primary glucose-lowering effect of metformin resides in the gut, not the circulation: results from short-term pharmacokinetic and 12-week dose-ranging studies. Diabetes Care 2016;39:198–205
2. Panzram G. Mortality and survival in type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1987;30:123–131
3. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. Cell Metab 2014;20:953–966
4. Pollak MN. Investigating metformin for cancer prevention and treatment: the end of the beginning. Cancer Discov 2012;2:778–790
5. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012;122:253–270
6. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 1998;352:854–865
7. Graham GG, Punt J, Arora M, et al. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011;50:81–98
8. He L, Wondisford FE. Metformin action: concentrations matter. Cell Metab 2015;21:159–162
9. Shaw RJ, Lamia KA, Vasquez D, et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 2005;310:1642–1646
10. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167–1174
11. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 2013;494:256–260
12. El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem 2000;275:223–228
13. Madiraju AK, Erion DM, Rahimi Y, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 2014;510:542–546
14. Bailey CJ, Wilcock C, Scarpello JH. Metformin and the intestine. Diabetologia 2008;51:1552–1553
15. Bailey CJ, Turner RC. Metformin. N Engl J Med 1996;334:574–579
16. Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. Endocrinology 2011;152:4610–4619
17. Lien F, Berthier A, Bouchaert E, et al. Metformin interferes with bile acid homeostasis through AMPK-FXR crosstalk. J Clin Invest 2014;124:1037–1051
18. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. Appl Environ Microbiol 2014;80:5935–5943
19. Cabreiro F, Au C, Leung KY, et al. Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. Cell 2013;153:228–239