Therapy: Metformin takes a new route to clinical efficacy.

Marc Foretz, Benoit Viollet

Therapy

Metformin takes a new route to clinical efficacy

Marc Foretz and Benoit Viollet

Refers to Duca, F. A. *et al.* Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nat. Med.* http://dx.doi.org/10.1038/nm.3787

Metformin is currently the first-line treatment option for patients with type 2 diabetes mellitus, yet its mechanism of action remains uncertain. A new study reveals the important role for the activation of a duodenal AMPK-dependent neuronal pathway in the acute antihyperglycaemic effect of metformin and the inhibition of hepatic glucose production.

The prevalence of type 2 diabetes mellitus (T2DM) has reached epidemic proportions worldwide and represents an important threat to global public health systems. On the basis of available clinical evidence, the American Diabetes Association (ADA) reaffirmed metformin as the optimal first-line therapy for patients with T2DM, unless contraindications or intolerance are present.[1] This recommendation was made not only because metformin is easily accessible and affordable, but also because of the long-standing evidence base for its efficacy, safety (low risk of hypoglycaemic episodes) and potential benefits on multiple cardiovascular risk factors.[1,2] However, although metformin has been prescribed for >50 years in Europe (it was only approved by the FDA in December 1994), its mechanism of action is still disputed. The action of metformin is probably dependent on a combination of distinct activities, as the potential mechanisms by which it has its beneficial effects include various organs such as the liver and the small intestine.

In the past few years, much of the basic research on metformin has focused on its ability to suppress hepatic glucose production (HGP). Multiple underlying mechanisms with the mitochondria as the primary target have been described.[2] For a decade, metformin-induced inhibition of hepatic gluconeogenesis has been ascribed to the activation of the serine/threonine kinase 11/ Liver kinase B1 (STK11/LKB1)–5'-AMP-activated protein kinase (AMPK) signalling pathway, which senses cellular energy levels and regulates the balance between catabolic and anabolic processes. However, this mechanism of action has been

seriously challenged by the use of liver-specific STK11/LKB1 and AMPK knockout mouse models.[3,4]

Findings published in 2010 further established that metformin acts through AMPK-independent mechanisms to lower HGP.[3] Indeed, metformin-dependent inhibition of complex I of the mitochondrial electron transport triggers a decrease in the cellular ATP:AMP ratio, which results in low levels of ATP that are insufficient to drive energy-consuming hepatic gluconeogenesis. In addition, a metformin-induced increase in AMP levels leads to the inhibition of adenylate cyclase and of the induction of gluconeogenesis by glucagon.[4] AMP is also a potent allosteric inhibitor of fructose 1,6-bisphosphatase, a key enzyme in gluconeogenesis. Furthermore, inhibition of glycerol-3-phosphate dehydrogenase in the mitochondria results in an altered cellular redox state and limits the contribution of lactate and glycerol to hepatic gluconeogenesis.[5] The intestine also contributes to the overall glucose-lowering effect of metformin and might be an important site of action of this drug.[6] Increased intestinal use of glucose, which enhances anaerobic metabolism of glucose to lactate, increases glucose turnover and supports the antihyperglycaemic action of the drug. In addition, it should be noted that oral dosing produces a stronger and longer glucose-lowering response than metformin administered intravenously and metformin accumulates in the intestinal mucosa to a greater extent than it does in the liver, which supports the notion that the gastrointestinal tract is an important target organ for metformin.[6]

The recent study reported by Duca *et al.*[7] proposes a new paradigm for the diverse modes of action of metformin. Previous findings had highlighted the importance of nutrient sensing in the gut for triggering a neuronal negative-feedback system for glucose homeostasis. Duca and co-workers went on to show that preabsorptive metformin lowers HGP through a gut–brain–liver neuronal network.[7] This effect was observed when metformin (50 mg kg^{-1} or 200 mg kg^{-1}) was infused directly into the duodenal lumen of insulin-resistant rats, but not when it was delivered via the portal vein. By using a combination of pharmacological approaches, Duca and colleagues uncover the cellular mechanisms underlying the action of metformin on intestinal mucosa and subsequent inhibition of HGP.

Duca and colleagues attribute the effect of duodenal infusion of metformin to the release of glucagon-like peptide 1 (GLP-1), probably from the enteroendocrine L cells, and activation of the GLP-1 receptor (GLP1R) on the afferent vagus nerve innervating the small intestine to

trigger a gut–brain–liver axis that regulates HGP (Figure 1). They hypothesize that metformin-induced activation of AMPK in the intestinal mucosa could stimulate the release of GLP-1. This theory is consistent with other findings that metformin and the AMPK agonist 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) are GLP-1 secretagogues.[8] Duca *et al.* infected the duodenum of insulin-resistant rats with an adenovirus encoding a dominant-negative mutated form of AMPK, which showed that duodenal AMPK is required for the preabsorptive action of metformin. However, it was not possible to determine the specific contribution of cells that produce GLP-1 and/or other cell types in the therapeutic benefit of metformin-induced activation of AMPK. In addition, it remains unclear whether locally produced GLP-1 (acting directly on close afferent neurons) and/or enhanced circulating levels of GLP-1 (for distant hormonal actions) are a prerequisite for the acute therapeutic effect of metformin. Unfortunately, the study lacks information on plasma levels of GLP-1 during and after intraduodenal treatment.

The authors showed that the signal to release GLP-1 that is induced by metformin is relayed by the activation of GLP1R on vagal afferent neurons through effects on a neuronal network. This action was demonstrated by co-infusion with the GLP1R antagonist exendin-9, chemical denervation and vagotomy that completely eliminated the effects of metformin in the duodenum on HGP. Curiously, this result contrasts with a previous report demonstrating that the acute glucoregulatory actions of metformin are independent of GLP1R.[8] Further elucidation of the mechanisms underlying the action of metformin in the duodenum will benefit from animal models that target AMPK and intestinal signalling molecules.

One intriguing question raised by this work is the contribution of the acute action of metformin in the duodenum in clinical settings, in which patients are usually exposed to metformin for long periods of time. Interestingly, an increasing amount of evidence suggests that metformin ‘shapes’ the metabolic plasticity of the gastrointestinal tract by inducing alteration in the recirculation of bile acids and the composition of the gut microbiota, which results in enhanced GLP-1 secretion in patients with T2DM.[9] Thus, acute and chronic glucose-lowering effects of metformin might be largely mediated by a combination of intestinal and direct hepatic mechanisms.

Collectively, these results provide compelling evidence that metformin targets a duodenal AMPK-dependent neuronal network to regulate HGP. A major implication of these findings

is that AMPK could be a primary therapeutic target within the intestine to restore impaired glucose homeostasis in T2DM. Interestingly, Duca and co-workers showed that intraduodenal infusion of the small molecule AMPK activator A-769662 mimicked the effect of intraduodenal metformin infusion.[7] Of note, activation of duodenal AMPK with the polyphenol resveratrol also initiates a gut–brain–liver axis to lower HGP.[10] Whether these new insights could lead to therapeutic strategies in humans remains to be determined.

INSERM U1016, CNRS UMR8104 and Université Paris Descartes, 24 rue du Faubourg Saint Jacques 75014 Paris, France (M.F., B.V.).

Correspondence to: B.V.
benoit.viollet@inserm.fr

Acknowledgements

The authors acknowledge funding from INSERM, CNRS, Université Paris Descartes, Agence Nationale de la Recherche, Société Francophone du Diabète, Région Ile de France, Association pour la Recherche sur le Diabète and the Département Hospitalo-Universitaire (DHU) AUToimmune and HORmonal diseaseS.

Competing interests

The authors declare no competing interests.

References

1. American Diabetes Association. Standards of medical care in diabetes--2014. *Diabetes Care* **37 Suppl 1**, S14-80 (2014).
2. Foretz, M., Guigas, B., Bertrand, L., Pollak, M. & Viollet, B. Metformin: from mechanisms of action to therapies. *Cell Metab* **20**, 953-966 (2014).
3. Foretz, M., *et al.* Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest* **120**, 2355-2369 (2010).
4. Miller, R.A., *et al.* Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature* **494**, 256-260 (2013).
5. Madiraju, A.K., *et al.* Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* **510**, 542-546 (2014).
6. Bailey, C.J., Wilcock, C. & Scarpello, J.H. Metformin and the intestine. *Diabetologia* **51**, 1552-1553 (2008).
7. Duca, F.A., *et al.* Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nat Med* (2015).

8. Maida, A., Lamont, B.J., Cao, X. & Drucker, D.J. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-alpha in mice. *Diabetologia* **54**, 339-349 (2011).
9. Napolitano, A., *et al.* Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus. *PLoS One* **9**, e100778 (2014).
10. Cote, C.D., *et al.* Resveratrol activates duodenal Sirt1 to reverse insulin resistance in rats through a neuronal network. *Nat Med* (2015).

Figure legend

Figure 1: Metformin lowers hepatic glucose production through a gut–brain–liver axis. Metformin activates duodenal AMPK, induces the enteroendocrine L cells to release GLP-1 and triggers a gut–brain–liver neuronal network to regulate hepatic glucose production. Abbreviations: AMPK, AMP-activated protein kinase; GLP-1, glucagon-like peptide 1; GLP1R, GLP-1 receptor; NTS, nucleus tractus solitarius.

Pullquotes

...metformin-induced activation of AMPK in the intestinal mucosa could stimulate the release of GLP-1

Author biographies

Benoit Viollet is junior director of research at the French National Institute of Health and Medical Research (Inserm) and is working at the Cochin Institute, Paris. His main interests include the pathophysiology and treatment of obesity and type 2 diabetes mellitus with a special interest in the contribution of AMPK.

Marc Foretz obtained his PhD in 2000 from Paris Diderot University, France. Since 2006, he is senior research fellow at CNRS in the Cochin Institute, Paris. His research is focused on the role of AMPK-related kinases in energy metabolism control and diabetes, and the mechanism of action of metformin.