JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
COMMENTARY

New mechanism of metformin action mediated by lysosomal presenilin enhancer 2

Metformin is a commonly prescribed oral hypoglycemic drug for type 2 diabetes, with its suppression of glucose production in the liver having been thought to be important for its hypoglycemic effect. Activation of 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) by an increase in the intracellular AMP/5′-adenosine triphosphate (ATP) concentration ratio resulting from inhibition of mitochondrial complex I in hepatocytes has been implicated in this suppression of glucose production by metformin. However, whereas the blood concentration of metformin after oral administration of the usual dose (1.5–2.0 g) is approximately10–30 μmol/L, the drug concentration required for inhibition of mitochondrial complex I and for AMP accumulation, as revealed by *in vitro* and *in vivo* experiments, is suprapharmacological.[1] The clinical relevance of metformin action mediated by AMP-dependent AMPK activation in the liver has thus been questioned. Further insight into the hypoglycemic action of metformin has therefore awaited elucidation of a possible AMP-independent mechanism of AMPK activation operative at pharmacological drug concentrations. Ma *et al.*[2] recently discovered such a mechanism of metformin action.

The researchers found that metformin at 5 μmol/L, a clinically relevant concentration, inhibited lysosomal vacuolar H^+-ATPase (v-ATPase) activity in primary hepatocytes. This group had previously shown that a low concentration of metformin promotes translocation of the scaffold protein AXIN and the liver kinase 1 (LKB1) to lysosomes independently of an increase in the intracellular concentration of AMP, with this recruitment resulting in formation of a Ragulator-v-ATPase-AXIN/LKB1-AMPK complex and activation of AMPK by LKB1.[3] As a follow up to this previous study, Ma *et al.*[2] attempted to identify metformin-binding proteins associated with isolated lysosomes by an affinity- and click chemistry-based approach with photoactive metformin probes. Gene silencing experiments targeting the identified candidate proteins then showed that depletion of presenilin enhancer 2 (PEN2), a subunit of the γ-secretase complex, blocked the activation of AMPK and inhibition of v-ATPase by metformin. Given that approximately 40% of PEN2 is localized to lysosomes in mouse embryonic fibroblasts and that targeting of the ectopic protein to other organelles in PEN2-null cell lines did not confer activation of AMPK by metformin, the researchers suggested that lysosome-associated PEN2 plays an important role in metformin-induced AMPK activation.

The authors also analyzed the biophysical nature of the interaction between metformin and PEN2. Isothermal calorimetry and surface plasmon resonance measurements showed the dissociation constant for the interaction to be 1.7 and 0.15 μmol/L, respectively, values that are consistent with intracellular metformin concentrations achieved after administration of the regular dose of the drug. Furthermore, *in silico* analysis implicated the amino acids phenylalanine-35 and glutamic acid-40 located on the cytosolic surface of PEN2 in binding to metformin. Ma *et al.*[2] next attempted to identify target proteins of PEN2 to shed light on the precise mechanism by which metformin binding to PEN2 inhibits v-ATPase activity. Immunoprecipitation analysis identified 123 PEN2 binding proteins whose affinity for PEN2 was altered by metformin. Among these proteins, the authors focused on ATPase H^+ transporting accessory protein 1 (ATP6AP1), a subunit of the v-ATPase complex. Binding experiments with various truncation mutants of ATP6AP1 implicated the transmembrane region comprising residues 420–440 in the interaction with PEN2. On the basis of these results and those of their previous study, the researchers proposed that formation of the PEN2-ATP6AP1 complex induced by the binding of metformin to PEN2 results in the inhibition of v-ATPase activity and in the recruitment of AXIN/LKB1 to lysosomes, which in turn results in the phosphorylation and activation of AMPK.

Ma *et al.*[2] also generated two mouse models – intestine-specific PEN2 knockout mice and liver-specific PEN2 knockout mice – to analyze the role of PEN2 and ATP6AP1 in the beneficial effects of metformin. In the intestine-specific knockout mice, the metformin-induced improvement in blood glucose level after a glucose load was disrupted, likely as a result of impairment of metformin-induced secretion of insulin and glucagon-like peptide-1. A similar phenotype was also apparent for gut-specific AMPK knockout mice, consistent with the notion that the blood glucose-lowering effect of metformin is mediated by activation of an intestinal PEN2-ATP6AP1-AMPK axis. In contrast, the

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Received 20 September 2022; revised 23 September 2022; accepted 26 September 2022

reduction in hepatic triglyceride content induced by metformin administration was found to be impaired in liver-specific PEN2 knockout mice, suggesting that this effect of metformin is mediated by PEN2-ATP6AP1-AMPK signaling associated with lysosomes in the liver.

Furthermore, studies with genetically modified *Caenorhabditis elegans* and mice showed that AMPK activation mediated by PEN2 and ATP6AP1 contributes to the lifespan extension induced by metformin in these species. The authors suggested that activation of a 'small pool of AMPK' is desirable for metformin efficacy, given that pronounced AMPK activation can give rise to heart disease.

PEN2, the metformin-binding protein identified by Ma *et al.*,[2] is a small membrane protein with a molecular size of approximately 12 kDa that contains an intramembrane domain (amino acids 18–36) and a transmembrane domain (amino acids 58–78). Cryo-electron microscopy of the γ-secretase complex previously showed that PEN2 binds to presenilin-1 within the membrane and to nicastrin at the luminal surface of organelles.[4] ATP6AP1 contains a transmembrane helix and a substantial luminal domain. The cryo-electron microscopy structure of the v-ATPase complex shows that the transmembrane domain of ATP6AP1 is present in the Vo portion of the complex and is located inside a bundle of transmembrane α-helices that is known as the c-ring (Figure 1).[5] ATP6AP1 is thought to serve as a structural hub for assembly of Vo by binding to the c-ring and lipids.

Ma *et al.*[2] concluded that the lysosomal PEN2-metformin complex is recruited to ATP6AP1 of the v-ATPase complex, and they propose a model in which the transmembrane domain of PEN2 binds to that of ATP6AP1. However, the cryo-electron microscopy structure of the v-ATPase complex suggests that it might be difficult for PEN2 to access the transmembrane region of ATP6AP1, because the c-ring might impose a barrier. The binding of PEN2 to ATP6AP1 might therefore trigger a large structural rearrangement of the v-ATPase within the lysosomal membrane. It will be of interest to determine whether such a rearrangement is thermodynamically possible and how it might be induced by the metformin-PEN2 complex. Structural studies will also be necessary to elucidate how the interaction of metformin-PEN2 with ATP6AP1 inhibits v-ATPase activity. PEN2 has been found to affect the stability of other γ-secretase subunits, and the expression of PEN2 itself is impaired in the absence of nicastrin, suggesting that the stability of PEN2 is dependent on complex formation.[6] It will be important to examine whether the stability of PEN2 or ATP6AP1 is affected by their interaction.

Ma *et al.*[2] found that metformin slightly increases γ-secretase activity. Given that γ-secretase contributes to the accumulation of amyloid-β protein, they point out that the effect of chronic metformin administration on the risk of Alzheimer's disease development warrants further investigation. However, the authors have provided important insight into how metformin achieves its clinical effects. Their findings have not only identified a new mechanism of action of metformin, but also deepened our understanding of the relationship between

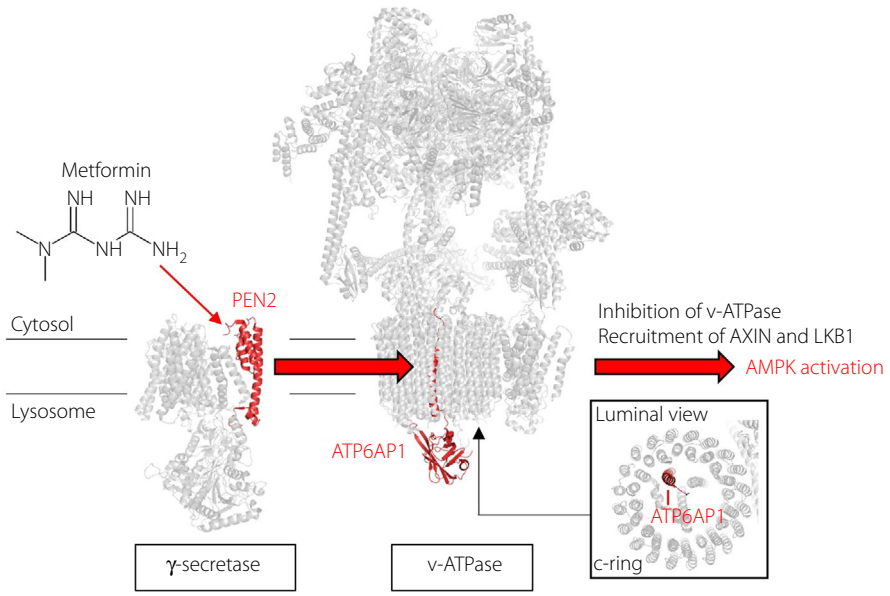

Figure 1 | Proposed mechanism for metformin action in a manner dependent on a metformin-presenilin enhancer 2 (PEN2)-ATPase H^+ transporting accessory protein 1 (ATP6AP1) axis. The overall structures of γ-secretase (PDB: 6IYC) and v-ATPase (PDB: 6WM2) are shown in gray, with PEN2 and ATP6AP1 being shown in red. AMPK, 5′-adenosine monophosphate-activated protein kinase.

lysosomes and glucose metabolism, and of the importance of the intestine in the regulation of glucose metabolism. Furthermore, the discovery of the metformin-PEN2-ATP6AP1-AMPK pathway should pave the way for the development of new therapeutic agents for type 2 diabetes.

ACKNOWLEDGMENTS

WO has received research support from Astellas, Dainippon-Sumitomo Pharma, Daiichi Sankyo, Novartis and Takeda Pharmaceutical, as well as lecture fees from Astellas, Dainippon-Sumitomo Pharma, Novartis and Takeda Pharmaceutical. KS declares no conflict of interest.

DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: N/A
Informed Consent: N/A
Approval date of Registry and the Registration
No. of the study/trial: N/A
Animal Studies: N/A

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Doi: 10.1111/jdi.13925