

Metformin at the Interface of Chemistry, Pharmacokinetics, and Clinical Translation: Mechanistic Insights into Efficacy and Adverse Effects

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Abstract. Metformin is a key drug for the treatment of type 2 diabetes. Its efficacy and side effects depend on its chemical properties and how it is transported through transporters in the body. Research has shown that these factors influence metformin's function, individual variability, and potential safety issues. Metformin exerts its effects by inhibiting mitochondrial complex I and activating AMP-activated protein kinase (AMPK) during periods of energy deprivation. Furthermore, it alters glucose processing in the intestine and liver through the PEN2-ATP6AP1 signaling pathway. These effects also influence gut hormones and the composition of the gut microbiota. This process explains metformin's effects on blood glucose, gastrointestinal intolerance, vitamin B12 deficiency, and lactic acidosis. Understanding the underlying mechanisms of these effects opens new avenues for clinical application. Adjusting dose based on transporter differences can improve efficacy. Modifying the gut microbiota can alleviate gastric intolerance. Developing new drugs in this class could provide more effective treatment options. These results suggest that understanding metformin's function could lead to more personalized and effective treatments for type 2 diabetes.

Keywords: Metformin, Transporter-dependent pharmacokinetics, Mitochondrial complex I inhibition, Gut-liver axis, Precision medicine.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a major health problem worldwide, and metformin, a widely used oral medication, is the mainstay of treatment. Metformin has demonstrated excellent clinical efficacy over the years and offers significant advantages, such as excellent glucose-lowering activity, a favorable safety profile, and cost-effectiveness. Recent data suggest that metformin's potent pharmacological effects and associated side effects stem from its physicochemical properties, transporter-dependent pharmacokinetics, and tissue-specific molecular targets. Metformin is a hydrophilic cation with low passive permeability. Its absorption, hepatic uptake, and excretion are dependent on the transporters Organic Cation Transporter (OCT) 1 and 2, and multidrug and toxin extrusion transporter (MATE) 1 and 2-K, which correlates with its significant therapeutic effects [1]. At the molecular level, metformin's acute hypoglycemic effects have been shown to be mediated by inhibition of mitochondrial complex I and subsequent reduction of energy stress-induced gluconeogenesis [2]. Recently, a novel AMP-independent signaling pathway has been discovered through the lysosomal PEN2-ATP6AP1 pathway, suggesting that AMP-activated protein kinase (AMPK) has specific activation patterns and tissue-specific metabolic regulation [3, 4]. In addition to its hepatocyte effects, metformin also acts on the gut-liver axis by promoting intestinal glucose absorption, reshaping the microbiome, and increasing appetite through upregulating endocrine mediators such as Glucagon-Like Peptide-1 (GLP-1) and Growth Differentiation Factor 15 (GDF15), thereby regulating glycemic control at the systemic level [5]. However, the major side effects and complications of metformin, such as intestinal discomfort, vitamin B12 deficiency, and occasional complications of lactic acidosis, share similar chemical and pharmacological principles. In summary, this review positions metformin as one of the chemicals with both benefits and risks that has become the basis for precision drug delivery, microbiome-targeted therapy, and rational equivalent drug design to optimize the treatment of type 2 diabetes.

2. Properties and Pharmacokinetics

Metformin is a hydrophilic biguanide that exists as a positively charged cation at in body PH level. Due to its polarity, metformin has a relatively low passive membrane permeability. In humans, metformin absorption, distribution, and excretion are entirely dependent on active transport [6]. The pharmacokinetics of metformin are regulated by specific solute transporters. Notable examples include the OCTs and the MATEs. In the intestine, absorption is mediated by OCT1, OCT3, and the membrane monoamine transporter (PMAT). Hepatic absorption is crucial for pharmacological activity and is primarily dependent on OCT1. Renal excretion typically involves tubular uptake via basolateral OCT2 and urinary excretion via the apical MATE1/MATE2-K protein [7].

Metformin's distribution to the liver is crucial for its glucose-lowering effects. This is because metformin inhibits gluconeogenesis during hepatocyte uptake. Genetic mutations in the OCT1 gene (SLC22A1) have been shown to be important determinants of treatment response. Polymorphisms in the OCT1 gene (rs622342, rs628031, rs12208357, and others) are associated with altered pharmacokinetic parameters. These changes include increased area under the concentration-time curve (AUC) and maximum plasma concentration (C max), as well as decreased clinical efficacy, reflected in worsening glycemic control. A systematic review and meta-analysis published in 2023 demonstrated that the clinical significance of OCT1 gene polymorphisms (particularly rs622342 and rs628031) was strongly associated with reduced efficacy in lowering glycated hemoglobin (HbA1c) [1]. These findings highlight the important role that OCT1 gene mutations play in the variability of patient responses to metformin.

Renal excretion is another important factor contributing to the variability in metformin pharmacokinetics. OCT2 is responsible for metformin penetration into proximal tubular cells. MATE1 and MATE2-K transport the drug across the apical membrane into the urinary filtrate. The OCT2 rs316019 mutation has yielded mixed results. Some studies suggest minimal effects on glycemic responses, while others suggest it may lead to population-specific differences in clearance [8]. In contrast, the MATE1 promoter polymorphism (g-66T→C) is more consistently associated with better glycemic control. This is thought to be due to reduced transporter expression, thereby improving metformin retention and efficacy [9].

Drug-drug interaction studies further demonstrate the clinical relevance of transporter-dependent pharmacokinetics. Currently, the application of pharmacokinetic (PBPK) models for metformin is limited. However, recent experiments have shown that inhibition of OCT2 and MATEs alters systemic exposure. For example, the OCT2 and MATE1 inhibitor cimetidine reduces renal clearance and increases metformin plasma concentrations in healthy subjects. Importantly, the magnitude of this interaction alteration correlates with OCT2 genetic polymorphisms (e.g., 808 G>T). This suggests that genetic factors and drug-drug inhibition interactions can influence pharmacokinetics [7].

In summary, recent research findings from 2021 to 2025 highlight the importance of transporter-dependent pharmacodynamics in metformin metabolism. Mutations in the OCT1 gene are strongly associated with differences in metformin pharmacokinetics and reduced efficacy. Polymorphisms in the OCT2 and MATE genes, while variable, can significantly impact elimination and therapeutic response. Furthermore, drug-drug interactions via transporters can alter systemic exposure, primarily with coadministration of cimetidine. These findings highlight the importance of understanding transporter-dependent mechanisms. This will not only help optimize metformin dosing strategies but also predict treatment outcomes for different individuals. Ultimately, this knowledge will lay the foundation for advances in precision medicine for type 2 diabetes.

3. Mechanism of Action

The anti-hyperglycemic effects of metformin are mediated by complex mechanisms acting at the liver, intestinal, and systemic levels. Accumulating evidence suggests that inhibition of mitochondrial complex I is the primary acute mechanism of metformin's pharmacological activity. Metformin reduces oxidative phosphorylation, in part by inhibiting respiratory chain activity in hepatocytes. This

leads to metformin-induced depletion of intracellular ATP, an increase in the AMP:ATP ratio, and inhibition of gluconeogenesis via energy stress signaling pathways. The importance of this mechanism has been preliminarily demonstrated in a transgenic mouse model expressing the metformin-insensitive mitochondrial NADH dehydrogenase NDI1. This model significantly enhanced the hypoglycemic response, indicating that complex I is a key pharmacological node [2].

In addition to inhibiting Complex I, metformin activates AMPK through both known and novel pathways. The known mechanism involves ATP depletion and AMP accumulation. Activation of AMPK inhibits gluconeogenesis, suppresses lipogenesis, and promotes fatty acid oxidation. However, recent studies have uncovered an alternative, AMP-independent pathway. Metformin directly interacts with the presenilin-activating protein 2 (PEN2), a subunit of γ -secretase. This complex binds to ATP6AP1, a component of the lysosomal v-ATPase. This activates AMPK through lysosomal signaling nodes rather than energy stress. Functional data indicate that liver-specific depletion of PEN2 enhances the lipid-lowering effects of metformin. Intestinal ablation attenuates the glucose-lowering effects, demonstrating the tissue specificity and physiological importance of this pathway [3, 4].

Meanwhile, growing evidence supports the crucial role of the gut and liver in metformin's systemic effects. Positron emission tomography-computed tomography (PET-CT) studies in humans and animal models have demonstrated that metformin promotes basolateral intestinal glucose uptake (BIGU). This stimulates local glucose utilization and generates environmentally sensitive signals that inhibit hepatic gluconeogenesis independent of direct hepatic metabolism. Furthermore, metformin modulates endocrine signaling by increasing circulating levels of GLP-1 and GDF15. Both regulate appetite, gastric emptying, and overall glucose homeostasis. Knockout models identifying the gut, possibly the kidney, are major sources of metformin-induced GDF15. Intact GDF15-GFRAL signaling is essential for its glucose and energy regulatory effects. Furthermore, metformin significantly alters the composition of the gut microbiota, generally increasing the abundance of black-bellied fruit bats (*Acamanthia melanogaster*) and altering short-chain fatty acid metabolism. These changes may enhance incretin signaling, further improving glycemic control, leading to increased complexity in gut-liver interactions.

Taken together, these results reveal a hierarchical pharmacological model of metformin's effects. In the acute phase, mitochondrial complex I inhibition and AMP-dependent AMPK activation play key roles in suppressing hepatic gluconeogenesis. Under chronic or low-dose exposure, lysosomal PEN2-ATP6AP1-v-ATPase signaling becomes a key regulator of AMPK activation, functioning independently of energy stress. At a systemic level, gut-derived pathways (such as BIGU, incretins, and GDF15 secretion) as well as microbiome remodeling contribute to the regulation of gastrointestinal balance and glucose homeostasis. This multidimensional framework incorporates the highly dependent, tissue-specific, and inter-individual variability of metformin responses. From a clinical perspective, these findings may lead to the development of innovative therapies. For example, strategies include optimizing mitochondrial interactions to balance metformin efficacy and tolerability, targeting lysosomal AMPK signaling to improve low-dose efficacy, and exploiting intestinal mechanisms by modulating incretin trafficking or targeting the microbiome. Future research should prioritize the identification of quantitative biomarkers of gut-liver signaling pathways and the elucidation of the structure of the PEN2-ATP6AP1-v-ATPase complex. This will accelerate the development of precision medicine approaches and pave the way for next-generation therapies.

4. Chemical Basis of Metformin Side-Effects

The efficacy of metformin is closely related to its chemical properties and efflux transporters, but these properties also influence its adverse effects. Multiple properties of metformin may contribute to its adverse effects. For example, metformin's polarity, positive charge at body fluid pH, and dependence on specific transporters such as OCTs and MATEs have been associated with the development of metformin gastrointestinal intolerance, vitamin B12 deficiency, and lactic acidosis.

Therefore, analyzing metformin's chemical and pharmacological pathways of action can help better understand its adverse effects.

4.1. Gastrointestinal Intolerance: Local Accumulation and Microbiome Remodeling

Gastrointestinal intolerance is the most common adverse drug reaction, characterized by symptoms such as diarrhea, bloating, and abdominal discomfort. From a pharmacokinetic perspective, metformin's low lipophilicity and transporter-dependent properties may lead to elevated intestinal drug concentrations, especially when OCT and PMAT absorption approaches saturation [10]. Local intestinal accumulation impairs epithelial absorption and increases direct mucosal exposure of the drug. Beyond transport kinetics, metformin also induces microbiome remodeling, leading to significant growth of bacterial species such as *Akkermansia* and impairing bile acid metabolism [11]. These microbial changes can lower blood glucose by enhancing incretin signaling, but they can also impair intestinal motility and fermentation, exacerbating gastrointestinal discomfort. Therefore, the enterohepatic system, responsible for regulating blood glucose levels, also creates a chemical environment in the intestine that promotes intolerance

4.2. Vitamin B12 Deficiency: Altered Competition and Transport Mechanisms

Long-term treatment has been associated with vitamin B12 deficiency. This effect is explained by inhibition of the absorption pathway rather than direct metabolic toxicity. Biochemical data suggest that metformin inhibits calcium-dependent endocytosis of the vitamin B12-intrinsic factor complex in the ileum. This may involve direct competitive transport or indirect manipulation of epithelial transporter activity. Alterations in bile acid signaling and the gut microbiota may also inhibit intrinsic factor interactions, thereby reducing systemic bioavailability of cobalamin. Clinical manifestations include decreased serum vitamin B12 concentrations and, in severe cases, megaloblastic anemia and neurologic impairment. Notably, these mechanisms share similarities with transporter-based pharmacokinetics. Just as OCT gene polymorphisms can affect the liver, subtle perturbations of the intestinal absorption system can also be a root cause of adverse food reactions.

4.3. Lactic Acidosis: Impaired Mitochondrial Inhibition and Clearance

A serious condition called lactic acidosis can occur after metformin administration, suggesting that metformin's molecular mechanism of action may be pathological in some cases. Partial therapeutic inhibition of mitochondrial complex I in hepatocytes reduces ATP levels and inhibits gluconeogenesis [2]. However, excessive accumulation, particularly in the setting of renal failure, exacerbates this effect by impairing the clearance capacity of OCT2/MATE, leading to widespread mitochondrial dysfunction. The resulting energy deficit shifts cellular metabolism toward anaerobic glycolysis, increasing lactate production and inhibiting hepatic lactate clearance [12]. Genetic or pharmacological alterations of transporters that limit intracellular metformin exposure increase this risk. Although the incidence remains low, this chemical mechanism highlights why monitoring renal function and transporter-drug interactions is so important for clinical safety.

4.4. Integrative Perspective

In summary, metformin's side effect profile reflects limitations in its therapeutic efficacy. Intestinal accumulation and microbiome modulation can lead to hypoglycemia and gastrointestinal side effects. Interactions with transporters may explain decreased liver function and vitamin B12 malabsorption. Mitochondrial inhibition underlies therapeutic suppression of gluconeogenesis and pathological lactic acidosis. Further research is needed. Transporter polymorphisms, interactions with the microbiome, and mitochondrial involvement are crucial for minimizing adverse drug interactions. Furthermore, maintaining efficacy and precise dosing will contribute to the future development of biguanides.

5. Clinical Implication and Future Directions

Elucidating the chemical mechanisms underlying metformin's adverse effects is crucial for linking these mechanisms to improved clinical treatment. Some of these adverse effects, such as gastrointestinal intolerance, vitamin B12 deficiency, and lactic acidosis, are mediated by the same transporter- and mitochondrial-mediated mechanisms. Therefore, clinical strategies must balance metformin's efficacy and safety. Three key approaches will help achieve this goal: precision drug delivery based on transporter function, targeted microbiome interventions, and rational development of analogs.

5.1. Transporter-Guided Precision Medicine

Genetic variants in organic cation transporters (OCT1, OCT2) and toxin excretion proteins (MATE1, MATE2-K) impair metformin absorption and clearance. For example, disruption of the allergen OCT1 reduces hepatic absorption and efficacy. Furthermore, reduced clearance via MATEs increases the risk of systemic accumulation and adverse reactions, particularly lactic acidosis [1]. From a clinical perspective, this supports the need for a shift toward transporter-based prescribing. Incorporating PBPK modeling into routine practice could enable various treatment strategies, including personalized daily dose adjustments, dose reductions, or the use of extended-release formulations for high-risk genotypes [13]. This approach aligns with the principles of precision medicine and holds the promise of achieving high efficacy with low risk.

5.2. Microbiome-Targeted Mitigation of Gastrointestinal Intolerance

Genetic Intestinal intolerance is a major cause of treatment discontinuation. It is associated with elevated intestinal concentrations, transporter-mediated accumulation, and microbiome remodeling. Increased Akkermansia and metabolic support for bile acid conversion lower blood glucose levels, but these changes stimulate intestinal motility and fermentation, exacerbating diarrhea and bloating [11]. Recent interventional studies have demonstrated that probiotics, prebiotics, and even postbiotics can alleviate gastrointestinal side effects and improve treatment adherence without compromising glycemic control [14]. Clinical implications include routine application of microbiome-targeted therapies or the use of microbial and fecal metabolic biomarkers for patient stratification. Future research should elucidate the most effective strains, formulations, and dosing regimens for reducing intolerance.

5.3. Rational Design of Next-Generation Biguanides

The discovery of metformin's chemical interaction mechanism has driven the development of less toxic alternatives. Between 2023 and 2025, a variety of preclinical models were explored, including prodrugs, derivatives that selectively interact with transporters, and sustained-release systems. These products are designed to minimize systemic accumulation and off-target mitochondrial inhibition [12]. For example, novel compounds that selectively target beneficial pathways such as PEN2-AMPK lysosomal signaling, gut-liver axis regulation, mitochondrial stress, and renal accumulation have been used. These approaches can reduce the risk of lactic acidosis and vitamin B12 deficiency. More translational strategies are needed. For example, integrating PBPK models into adaptive early clinical trials could accelerate the development and clinical application of metformin.

5.4. Integrated Roadmap of Clinical Translation

By combining these approaches, it shows how metformin's chemical mechanism of action can be applied to clinical treatment. Precision drug delivery based on transporter function can control interpatient variability. Interventions targeting the microbiome can address the most common causes of treatment discontinuation. Rational design of alternative drugs can pave the way for safer long-term treatment. The integration of pharmacogenomics, systems biology, and clinical pharmacology

holds a bright future for metformin. Furthermore, not only metformin itself, but also subsequent treatment can serve not only as a universal drug but also as a personalized treatment.

6. Conclusion

Metformin is a drug whose efficacy and side effects are closely related to its chemical properties and in vivo mechanisms of action. Due to its hydrophilicity, its transport in the body relies on transporters, reaching specific tissues via mitochondria, AMPK, and the enterohepatic pathway. These properties explain its hypoglycemic mechanism of action and its side effects. Gastrointestinal disturbances, vitamin B12 deficiency, and lactic acidosis have all been implicated in this mechanism of action. These connections explain why treatment regimens must be tailored to each patient. For example, transporter-based drug delivery, microbiome-targeted therapies, and the rational design of next-generation analogs have important clinical implications. Research and clinical practice provide opportunities to improve metformin use, reduce side effects, and provide more precise treatment for patients with type 2 diabetes.

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