

Mechanism of Action of Metformin

Introduction

Metformin is a biguanide antihyperglycemic agent that has been used for decades as a first-line pharmacotherapy in the management of type 2 diabetes. Unlike most modern drugs, metformin was not designed to target a specific pathway or disease mechanism. Instead, it is derived from galegine, a natural product from the plant *Galega officinalis*, which was used in herbal medicine in medieval Europe. Metformin and the related drug phenformin (withdrawn in most countries due to side effects of lactic acidosis) are synthetic derivatives of galegine. Chemically, metformin (dimethylbiguanide) contains two coupled molecules of guanidine with additional substitutions.

Despite its clinical use for over 60 years, the molecular mechanisms of action of metformin remain complex and not fully understood. This review synthesizes current evidence on how metformin works at physiological, cellular, and molecular levels.

Pharmacokinetics and Distribution

Following oral administration of immediate-release metformin, approximately 70% of the dose is absorbed from the small intestine, with the remainder passing into the colon before being excreted in feces. Metformin is excreted unchanged in urine, with no reported metabolites. Plasma concentrations in humans typically range from 8-24 $\mu\text{mol/L}$, but concentrations in the intestines can be 30-300 times higher.

A positron emission tomography (PET) study using $[^{11}\text{C}]$ metformin demonstrated that oral metformin becomes highly concentrated in the intestines, liver, kidneys, and bladder, with only slow accumulation in muscle. The hepatic tissue to systemic blood activity ratio is approximately 5 following oral dosing, suggesting hepatic concentrations of about 50-100 $\mu\text{mol/L}$. In rats, metformin also accumulates in the pancreas and adipose tissue at approximately half the concentration seen in the liver, though the human relevance of this finding is unclear.

The absolute bioavailability of a 500 mg metformin tablet administered in the fasting state is about 50-60%. At usual clinical doses, steady-state plasma concentrations are achieved within 24-48 hours and are normally less than 1 $\mu\text{g/mL}$. Food reduces metformin absorption, as demonstrated by approximately 40% lower peak plasma concentration, 25% lower area under the plasma concentration curve, and a 35-minute prolongation of time to peak plasma concentration.

Hepatic Mechanisms

Metformin has traditionally been thought to act primarily on the liver to improve blood glucose levels. Several lines of evidence support this:

1. In mice lacking the organic cation transporter 1 (OCT1), which is responsible for metformin uptake into the liver, the drug was ineffective at improving blood glucose after high-fat feeding.
2. Tracer studies in humans show that metformin lowers hepatic glucose production, with minimal impact on peripheral insulin-mediated glucose uptake.
3. Multiple studies in mouse hepatocytes and transgenic mice provide evidence for metformin's role in reducing hepatic gluconeogenesis and/or improving insulin sensitivity.

At the molecular level, metformin accumulates within mitochondria to concentrations up to 1000-fold higher than in the extracellular medium. This occurs because metformin carries a positive charge, and the membrane potentials across the plasma membrane and mitochondrial inner membrane drive metformin into the cell and subsequently into the mitochondria.

The most intensively studied mitochondrial action of metformin is the inhibition of Complex I of the respiratory chain, which suppresses ATP production. While early studies required high extracellular concentrations (mmol/L) to observe rapid effects, lower concentrations (50-100 $\mu\text{mol/L}$) do inhibit Complex I in rat hepatoma cells after several hours, attributed to the slow uptake of metformin by mitochondria.

Gluconeogenesis is an energy-intensive process, consuming six ATP equivalents per molecule of glucose synthesized. By inhibiting Complex I, metformin reduces ATP production, thereby limiting the energy available for gluconeogenesis. Other consequences of respiratory chain inhibition, such as changes in the $\text{NAD}^+:\text{NADH}$ ratio, may also contribute to metformin's effects on gluconeogenesis.

AMPK-Dependent and Independent Mechanisms

Metformin has been shown to act via both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms. AMPK is a key enzyme that plays an important role in the regulation of glucose metabolism.

The inhibition of mitochondrial ATP production by metformin leads to increased cytoplasmic ADP:ATP and AMP:ATP ratios, which activate AMPK. Activated AMPK phosphorylates two isoforms of acetyl-CoA carboxylase enzyme, thereby inhibiting fat

synthesis and leading to fat oxidation, reducing hepatic lipid stores and increasing liver sensitivity to insulin.

Increases in the AMP:ATP ratio also inhibit the fructose-1,6-bisphosphatase enzyme, resulting in the inhibition of gluconeogenesis, while also inhibiting adenylate cyclase and decreasing the production of cyclic adenosine monophosphate (cAMP), a derivative of ATP used for cell signaling.

In addition to the AMPK-dependent pathway, metformin may also inhibit mitochondrial glycerophosphate dehydrogenase, and there is evidence for a mechanism involving the lysosome in AMPK activation.

Intestinal Mechanisms

In recent years, there has been renewed interest in the gut as a major site of action for metformin. Three lines of evidence highlight that the liver may not be as important for metformin action in individuals with type 2 diabetes as commonly assumed:

1. The glucose-lowering effect of metformin can only partially be explained by a reduction in endogenous glucose production (EGP), suggesting other glucose-lowering mechanisms.
2. Genetic studies in humans have established that loss-of-function variants in SLC22A1 (the gene encoding OCT1), which reduce hepatic uptake of metformin, do not impact the efficacy of metformin to lower HbA1c in individuals with type 2 diabetes.
3. A delayed-release metformin formulation that is largely retained in the gut, with minimal systemic absorption, is as effective at lowering blood glucose as the standard immediate-release formulation in individuals with type 2 diabetes.

Metformin increases anaerobic glucose metabolism in enterocytes (intestinal cells), resulting in reduced net glucose uptake and increased lactate delivery to the liver. This effect is apparent in PET imaging, where metformin-treated patients show considerable intestinal fluorodeoxyglucose (FDG) uptake, especially in the colon.

Metformin may also impact glucose metabolism by increasing glucagon-like peptide-1 (GLP-1) secretion, an effect described for both immediate-release and delayed-release metformin. GLP-1 is an incretin hormone that stimulates insulin secretion and suppresses glucagon secretion, thereby lowering blood glucose levels.

Another intriguing gut-mediated mechanism for metformin action was identified in rats and involves a pathway linking duodenal metformin exposure to suppression of hepatic

glucose production via the nucleus tractus solitarius and vagal efferents, through AMPK and GLP-1 receptor activation (gut-brain-liver crosstalk).

Summary of Mechanisms

In summary, metformin's mechanisms of action are complex and multifaceted:

1. It decreases hepatic glucose production (gluconeogenesis) through:
 2. Inhibition of mitochondrial Complex I, reducing ATP production
 3. Activation of AMPK, leading to reduced expression of gluconeogenic enzymes
 4. Inhibition of mitochondrial glycerophosphate dehydrogenase
5. Alterations in the NAD⁺:NADH ratio
6. It enhances peripheral glucose uptake and utilization through:
 7. Increased insulin sensitivity in peripheral tissues
 8. Reduced hepatic lipid stores, improving insulin signaling
9. It decreases intestinal absorption of glucose through:
 10. Increased anaerobic glucose metabolism in enterocytes
 11. Enhanced GLP-1 secretion
 12. Gut-brain-liver signaling pathways
13. It may also have effects on the gut microbiome, inflammation, and aging, though these mechanisms are still being investigated.

In the last decade, our understanding of metformin has evolved from a simple picture—that it improves glycemia by acting on the liver via AMPK activation—to a much more complex picture reflecting its multiple modes of action. More research is required to fully understand how this drug works in its target population: individuals with type 2 diabetes.

References

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