Metformin use and the hepatic and renal toxicity

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Abstract

Objectives

Metformin is an oral anti-diabetic that is commonly used in the treatment of type 2 diabetes mellitus. Metformin has been rarely associated with hepatic and renal toxicity and it is suggested that the hepatic toxicity is caused by the upregulation of hydrogen sulfide (H2S) as a result of changing the mitochondrial metabolism of H2S due to the inhibition of complex I by metformin. The renal toxicity is thought to be caused indirectly by the development of metformin associated lactic acidosis due to a pre-existing renal impairment.

This essay will include our findings on the molecular mechanism behind metformin's toxic and beneficial effects on the liver and kidneys. Furthermore, the following questions are aimed to be answered:

Can the upregulation of H2S and lactate be the cause of the hepatic and renal injury, respectively, and how does this happen? What effects does the pre-existing renal impairment have on damage development? How does metformin exert its tissue-protective effects?

Results

From the data, we have found that metformin use is rarely associated with hepatic toxicity by which patients treated with metformin started to show signs of mixed type hepatocellular and cholestatic hepatic injury within two months after starting metformin treatment. Furthermore, the renal injury was found to occur in patients suffering from pre-existing renal impairments due to the development of lactic acidosis. On the other hand, we have found that metformin can be beneficial in some cases of liver and kidney impairments due to its pleiotropic actions.

Conclusion

Metformin use can cause hepatic and renal toxicity, but these conditions occur rarely which made it challenging to fully understand the injury. However, we have found from animal studies that the upregulation of both H2S and lactate resulting from metformin use can give us insights for future research on human tissues.

Contents

- Introduction

Metformin is an oral antihyperglycemic agent and is the most prescribed drug for the treatment of type 2 diabetes mellitus; metformin and its related drug phenformin (withdrawn from most countries because of lactic acidosis side effects) are biguanides that were synthesized from galegine [1]. Galegine is a guanidine derivative found in *Galega officinalis* (French lilac) used in herbal medicine in medieval Europe as a glucose-lowering plant in the 1920s but was found to be too toxic and was withdrawn as a consequence of its toxicity [2].

Metformin and phenformin were tested at about the same time of galegine discovery, but they were introduced for clinical use in the 1950s [1]. Nevertheless, metformin was also withdrawn from the market because of lactic acidosis side effects concerns. However, it was proven safe and effective in reducing glucose levels, and it was reintroduced in 1995. [3].

Apart from this, it was found that metformin plays a role in improving conditions such as cardiovascular diseases and cancer, and it also helps eliminate nicotine withdrawal syndrome [4 - 9]. On the other hand, it is also associated with side effects, such as diarrhea, gastrointestinal upset, abdominal pain, nausea, metallic taste, weakness, headache, dizziness, and rash. Rare but potentially severe adverse events include lactic acidosis, hypoglycemia, dehydration, and hypersensitivity reactions [10, 11].

Although rare, liver injury cases have been reported in less than 1% of patients treated with metformin, and its mechanism of liver injury is not entirely understood so far [12 - 28]. Still, it is thought that it is closely related to the upregulation of hydrogen sulfide (H2S) in the hepatic region [29 - 31].

Metformin has shown signs of improving the renal function in patients suffering from renal damage by preventing or weakening acute kidney injury (AKI) and mild to moderate chronic kidney disease (CKD) [37]. However, it has also been associated with renal injury, and it has been suggested that the damage is caused by lactate upregulation, which may lead to lactic acidosis [32].

The development of metformin-associated hepatic and renal damage is affected by other diseases that might increase the risk of injury, such as pre-existing renal impairment which increases the risk of metformin-associated lactic acidosis as a consequence of renal insufficiency [33 - 35].

Additionally, metformin was known in several studies as a hepatic and renal protective drug, which might seem controversial; however, it is suggested that metformin protection is related to its pleiotropic actions [36 - 58].

Therefore, in this essay, we will study the molecular mechanism behind metformin's toxic and beneficial effects on the liver and kidneys. Furthermore, the following questions are aimed to be answered:

Can the upregulation of H2S and lactate be the cause of the hepatic and renal injury, respectively, and how does this happen? What effects does the pre-existing renal impairment have on damage development? How does metformin exert its tissue-protective effects?

- Pharmacology of metformin

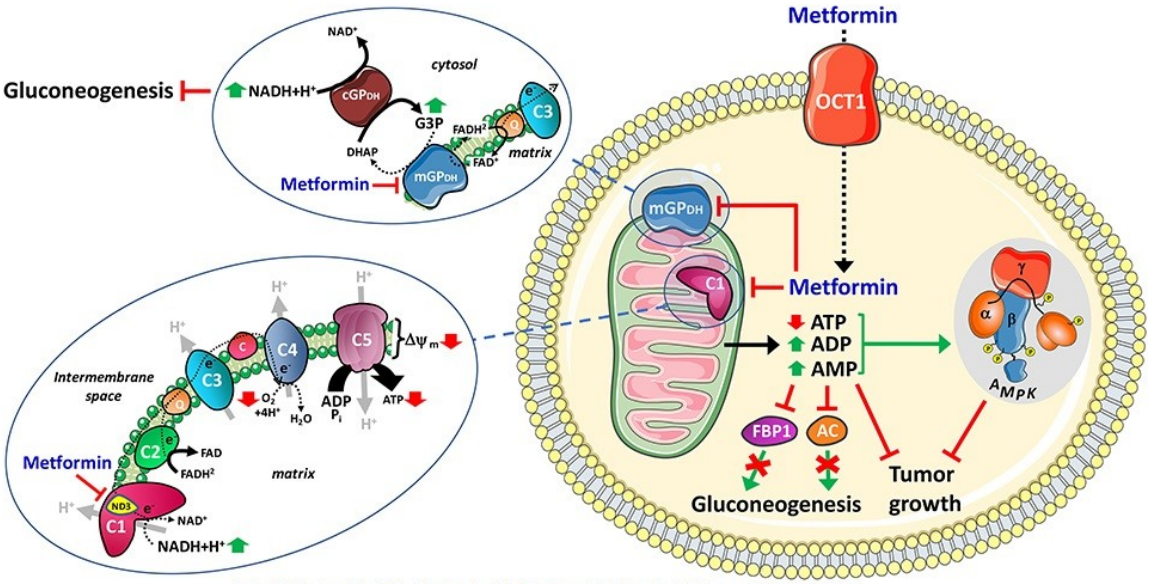
Mechanism of pharmaceutical action

Metformin is a drug with pleiotropic effects, and the exact mechanism of its glucose-lowering effect is still not entirely clear. It is generally accepted that its major mode of action the is inhibition of

hepatic gluconeogenesis by blocking the mitochondrial redox shuttle. Furthermore, metformin is an insulin sensitizer that improves the sensitivity of cells to the metabolic effects of insulin, and it acts in the gut lumen to reduce glucose levels [59].

Mitochondrial gluconeogenesis inhibition

Gluconeogenesis in the liver is controlled by the mitochondria, which is an energy-intensive process that requires six ATP (adenosine triphosphate) equivalents per molecule of glucose synthesized. The six ATP used are provided from mitochondria then balanced by the hepatocytes. Metformin is positively charged, and the membrane potentials across the plasma membrane and the positively charged mitochondrial inner membrane outer layer allow the passage of metformin inside the cell, and it slowly accumulates in mitochondria to concentrations up to 1000-fold higher than in the extracellular medium to reach the thermodynamic equilibrium according to the Nernst equation which represent an approximately 200-fold accumulation of metformin in organs [60, 61]. Inside the mitochondrion, metformin inhibits the respiratory chain Complex I resulting in a decreased NADH oxidation, reduced proton gradient across the inner mitochondrial membrane, reduced oxygen consumption rate, and the suppression of ATP production (Fig 1) [60, 62].



Role of Mitochondria in the Mechanism(s) of Action of Metformin. Guillaume Vial, et al. (2019)

Fig 1 Mitochondrial gluconeogenesis inhibition. After hepatocellular uptake through OCT1, metformin targets the mitochondria and inhibits the respiratory chain complex I by interacting with the ND3 core subunit and mitochondrial glycerophosphate dehydrogenase (mGPDH). The inhibition of complex I decrease the oxidation of NADH, oxygen consumption rate, and proton pumping across the inner mitochondrial membrane that results in a lower proton gradient (1%) and suppressed ATP synthesis from ADP and inorganic phosphate (Pi). mGPDH inhibition modulates cytosolic and mitochondrial redox state resulting in increased cytosolic NADH. FBP1 (fructose-1,6-bisphosphatase-1). AC (adenylate cyclase).

Furthermore, the decrease in ATP and the subsequent rise in intracellular ADP (adenosine diphosphate) and AMP (adenosine monophosphate) levels leads to an increase in the AMP-activated protein kinase (AMPK) activity in hepatocytes. AMPK is a protein kinase that acts as an energy gauge to sense the cellular energy status by monitoring AMP, ADP, and ATP levels [63 - 65].

After that, AMPK inhibits the consuming anabolic processes and stimulates the generating catabolic pathways of ATP by a large number of downstream effectors direct phosphorylation involved in the regulation of various metabolic processes to restore the cellular energy balance [64].

In general, the activation of AMPK alone is not sufficient for inducing acute inhibition of gluconeogenesis. Therefore, studies demonstrated that metformin could inhibit gluconeogenesis through other mechanisms like the AMP-mediated inhibition of adenylate cyclase and subsequent reduction in glucagon increased cyclic adenosine monophosphate (cAMP) levels [66], and gluconeogenesis inhibition through the modulation of cytosolic redox state via direct inhibition of the mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) (Fig 1) [67].

Altogether, the decrease in ATP and the subsequent rise in intracellular ADP and AMP levels resulting from the weak and reversible metformin respiratory chain complex I inhibition is essential for inhibiting hepatic gluconeogenesis. On the other hand, complex I inhibition by metformin and the decreased ATP production might cause an undesired effect that might lead to damage to organs like the liver and kidneys by the upregulation of H2S and lactate, respectively [68, 69].

Pharmacokinetic properties

Metformin is a hydrophilic base that exists as a cationic species at physiological pH (>99.9%). The drug has a limited passive diffusion ability through the cell membranes. Its mean ±SD fractional oral bioavailability (F) is 55 ± 16%. Metformin is mostly absorbed from the small intestine. It is excreted in the urine unchanged, and its elimination half-life (t½) is approximately 5 hours during multiple dosages in patients with normal renal function [1]. From previous studies [70], metformin mean renal clearance (CLR) of the population and the apparent total clearance after oral administration (CL/F) were estimated to be 510 ± 130mL/min in healthy people and 1140 ± 330mL/min in diabetic patients with normal kidney function. In a population with a variety of renal function, mean values of CLR and CL/F of metformin are 4.3 ± 1.5 times greater in healthy people and 10.7 ± 3.5 times greater in diabetic patients as the clearance of creatinine (CLCR). The decrease of CLR and CL/F is proportion to CLCR, so the dosage of metformin in patients with impaired kidney function should be adjusted according to the levels of CLCR and the GFR to avoid renal damage by the high concentrations of metformin and the development of lactic acidosis (Table 1) (Fig 2) [1].

Table 1 Metformin maximum recommended doses

Renal function (creatinine clearance)	**Maximum daily metformin dose**
15 - 30 mL/min	500 mg
30 - 60 mL/min	1000 mg
60 - 120 mL/min	2000 mg

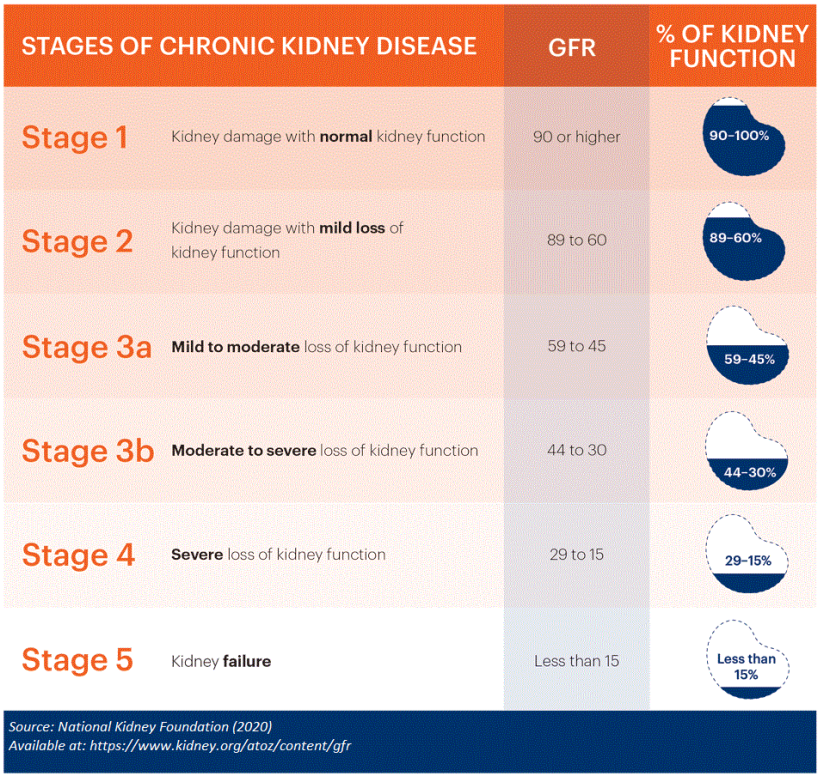


Fig 2 Five stages of chronic kidney disease based on kidney function (%) and eGFR (mL/min per 1.73)

Furthermore, a study in 2018 was performed in type 2 diabetes mellitus patients treated with metformin while having any CKD stage (stages 1-5), they were given different doses of metformin according to their CKD stage (Table 2), and after 4 months, metformin concentrations remained stable and within the generally accepted upper limit (5.0 mg/L) while hyperlactatemia was absent. The study determined that metformin can be safe and pharmacologically effective in moderate-to-severe CKD provided that the dose is adjusted according to the kidney function [71].

Table 2 Metformin dosage in patients with CKD

CKD stage	**Dose and dosing regimen**
CKD3A	1500 mg (500 mg in the morning & 1000 mg in the evening)
CKD3B	1000 mg (500 mg in the morning & 500 mg in the evening)
CKD4	500 mg (once in the morning)

- Mechanisms of metformin toxicity

Mechanism of hepatotoxicity

Metformin is not metabolized but excreted unchanged in the urine and its use may induce hepatotoxicity as seen from previous case reports (Table 3) which amounts to less than 1% of patients treated with metformin due to its rarity. The toxicity appears as a mixed type hepatocellular and cholestatic hepatic injury that is associated with the increase in serum alanine (ALT), aspartate (AST) aminotransferases, and serum alkaline phosphatase (ALP) levels (hepatocellular injuries are characterized by elevations in serum ALT and AST while cholestasis is associated with elevated

serum ALP levels). The enzymes levels usually return to normal after the discontinuation of metformin treatment. However, the injury could lead to liver failure and might be fatal if not handled properly [19].

The exact mechanism of liver injury is still not entirely known, but it was suggested using a female mice model (BALB/c nude mice) aging about 5 to 6 weeks under standard care, that metformin use can cause an increase of H2S in the hepatic region by an unknown mechanism which might cause the injury [72, 73].

H2S is synthesized endogenously during the metabolism of cysteine in the liver and vasculature by cystathionine γ-lyase (CSE) [74], and it is associated with several physiological and pathological processes [69, 74]. The toxic effects of H2S are dose-related, and since the liver is the most crucial regulatory site of H2S, it is subject to high levels of H2S from endogenous generation and exogenous sources [69, 74]. It is thought that the inhibition of complex I by metformin could affect H2S levels by changing its mitochondrial metabolism, and the mitochondrial oxidation of H2S can cause periods of tissue hypoxia to the liver, which results in the increase of ROS generation that causes the hepatic injury. Therefore, metformin may cause a significant increase in the concentration of H2S, and that may cause severe hepatic tissue hypoxia, which leads to liver injury [29, 69, 75].

Table 3 Case reports of metformin-associated hepatotoxicity between 1998 and 2019

Year	Medications	Description	Laboratory results*	Survival	Reference
1998	Metformin	A 75-year-old man with type 2 diabetes mellitus developed hepatotoxicity two months after treatment with metformin 500mg twice daily.	AST 322 U/L. ALT 413 U/L. ALP 684 U/L.	Metformin stopped, and enzyme levels became normal. No re-challenge.	Swislocki ALM, et al. [12]
2006	Metformin, nateglinide and pioglitazone	A 73-year-old woman developed hepatotoxicity after three weeks of receiving 500 mg/day metformin for 2-DM.	ALT 772 U/L. AST 689 U/L. ALP 635 U/L. Total bilirubin 6.5 mg/dL.	Metformin stopped, and enzyme levels became normal after 7 weeks.	Kutoh E. [13]
2009	Metformin, acenocoumarol, digoxin, torasemide, irbesartan, atenolol and simvastatin	An 83-year-old man developed liver injury 10 days after treatment with 850mg every 12 hours of metformin for 2-DM.	AST 36 U/L. ALT 47 U/L. GGT 740 U/L. Total bilirubin 2.3 mg/dL. ALP 586 U/L.	Metformin stopped, and enzyme levels became normal after 4 months.	de la Poza G´omez G, et al. [14]
2010	Metformin, niacin and simvastatin	A 61-year-old man developed hepatotoxicity two weeks after treatment with metformin 500mg twice daily.	High AST, ALT, ALP, and total bilirubin.	Metformin stopped, and enzyme levels became normal after 2 months.	Cone CJ, et al. [15]

2010	Metformin	A 73-year-old woman with 2-DM developed hepatotoxicity, lactic acidosis, and renal failure two weeks after treatment with metformin 850mg three times daily.	Elevated aminotransferases. Anemia. Leucocytosis with neutrophilia and thrombocytopenia. INR 7.43. Prothrombin activity 9%. APTT 41.7 seconds.	Admitted to the ICU for intensive treatment, then discharged home while receiving heart and kidney treatment, and her aminotransferase, urea, and creatinine levels were within normal limits.	Olivera-Gonzalez S, et al. [16]
2012	Metformin	A 44-year-old woman presented with new-onset diabetes had an increase in ALT after one month that peaked after five months since starting metformin 500mg twice daily, which is a sign of hepatotoxicity.	Peak ALT 738 U/L. Peak GGT 42 IU/L.	Metformin stopped, and enzyme levels became normal after a month.	Hashmi T. [17]
2012	Metformin	A 61-year-old man developed hepatotoxicity with painless jaundice, acolia, and coluria after six weeks of receiving 1,700 mg/day metformin for 2-DM.	Cholestasis pattern.	Six weeks after discharge, there was an accidental re-challenge, but the outcome was not stated.	Miralles-Linares F, et al. [18]
2012	Metformin and simvastatin	A 48-year-old obese and diabetic man developed hepatotoxicity after two weeks from starting metformin 500mg twice daily.	ALT 3165 U/L. AST 1833 U/L. ALP 208 U/L. Total bilirubin 3.3 mg/dL. Creatinine 0.5 mg/dL. INR 1.1.	Metformin and simvastatin stopped, and enzyme levels became normal after 3 weeks except ALT (73 U/L).	Mallari A, et al. [19]
2013	Metformin and antiretrovirals	A 44-year-old HIV-positive man developed acute liver failure with lactic acidosis while receiving stavudine, didanosine, and metformin. The duration of exposure to antiretroviral therapy was 9.1 years.	Clinical manifestations included splenomegaly, encephalopathy, ascites, and esophageal varices.	The outcome was not stated.	Kovari H, et al. [20]
2013	Metformin, amoxicillin/clavulanic acid for acute parotitis, aspirin,	A 78-year-old man developed acute mixed hepatocellular and	Total bilirubin 22.2 mg/dL. ALT 1050 U/L.	Metformin and pravastatin stopped, and enzyme levels	Saadi T, et al. [21]

	pravastatin, amlodipine, atenolol, candesartan cilexetil and hydrochlorothiazide	cholestatic hepatic injury two weeks after treatment with metformin 850 mg/day.	AST 496 U/L. ALP 1001 U/L. GGT 1264 U/L.	improved after a week and became normal after 2 months.	
2015	Metformin, linagliptin, and sitagliptin	A 79-year-old woman with asymptomatic hepatitis C virus infection and long-term type 2 diabetes mellitus developed hepatotoxicity during treatment with linagliptin and then subsequently five weeks after treatment with sitagliptin and metformin.	ALT 242 U/L. GGT 260 U/L.	Linagliptin was discontinued, and after three months, the woman's liver enzymes normalized. Ten months later, she started receiving sitagliptin/metformin, and tests revealed cytolysis and sitagliptin were discontinued. Two months later, cytolysis had resolved.	Patier De La Pena JL, et al. [22]
2016	Metformin	A 70-year-old woman developed liver injury after five weeks of receiving 500 mg twice a day metformin for type 2 diabetes mellitus.	Total bilirubin 2.2 mg/dL. AST 1152 U/L. ALT 1093 U/L. ALP 176 U/L.	Metformin stopped, and enzyme levels became normal after 10 days.	Zheng L. [23]
2016	Metformin	A 56-year-old man with type 2 diabetes mellitus developed hepatotoxicity one month after treatment with metformin. And a 61-year-old man with type 2 diabetes mellitus developed hepatotoxicity two months after treatment with metformin	The 56-year-old man: AST 4422 U/L. ALT 4701 U/L. Total bilirubin 20.7 mg/dL. ALP 192 U/L. The 61-year-old man: Total bilirubin 25.6 mg/dL. ALP 916 U/L. AST 916 U/L. ALT 1269 U/L.	Metformin was withdrawn, and the conditions improved then the liver enzymes normalized.	Dayanand S, et al. [24]
2017	Metformin	A 56-year-old man developed metformin toxicity followed by lactic acidosis, decreased level of consciousness, abdominal pain, hypoglycemia, fatal cardiac arrest, and a 72-year-old man developed metformin toxicity followed by lactic acidosis, stupor,	The 56-year-old man: Hypoglycemia and severe lactic acidosis. pH 6.91. Bicarbonate 4.8 mmol/L. Lactate 13.84 mmol/L. Urea 219 mg/dL. Creatinine 11.89 mg/dL.	The 56-year-old man developed cardiac arrest (asystole) and died after hospital admission. The 72-year-old man received intensive treatment and was discharged from the hospital after 30 days of admission.	Ortiz-Lasa M, et al. [25]

		lividity, cardiac arrest, and shock.	GFR 4. The 72-year-old man: Cardiac arrest with electromechanical dissociation. pH 6.96, Bicarbonate 7.1 mmol/L, Lactate 11.57 mmol/L. Urea 213 mg/dL. Creatinine 10.10 mg/dL. GFR 5.		
2017	Metformin	A patient of unstated age and a 55-year-old man had developed diarrhea, nausea, vomiting, and anorexia; and increased blood lactic acid, respectively, during treatment with metformin for 2-DM 1500 mg/day and 2250 mg/day.	The results were not stated.	The outcome was not stated.	Odawara M, et al. [26]
2018	Metformin/saxagliptin, empagliflozin, gliclazide, and fenofibrate	A 33-year-old man developed drug-induced liver injury one week after starting the treatment with saxagliptin/metformin (5mg/1000mg) for type 2 diabetes mellitus.	ALT 307 IU/L. GGT 808 IU/L.	Saxagliptin/metformin therapy was discontinued. Metformin monotherapy was re-initiated, and empagliflozin was continued. Liver function tests were improved in the third week and the seventh week following saxagliptin/metformin discontinuation.	Thalha AM, et al. [27]
2019	Metformin/spironolactone	A female patient developed hepatotoxicity during treatment with metformin for insulin resistance from metabolic syndrome and spironolactone.	The results were not stated.	The outcome was not stated.	Singh P, et al. [28]

**Normal laboratory results: AST (7–38 U/L), ALT (4–36 U/L), ALP (115–359 U/L), Total bilirubin (0.2–1.0 mg/dL), Gamma glutamyl transferase GGT (9–48 U/L), International Normalized Ratio (INR) (1.1 or below), Activated partial thromboplastin time (APTT) (30 to 40 seconds), Creatinine (0.84 to 1.21 mg/dL), Bicarbonate (23 to 30 mmol/L), Lactate (0.5-1 mmol/L), Urea (7 to 20 mg/dL) and Glomerular filtration rate (GFR) (90 to 120 mL/min/1.73 m2)*

A study on mice showed that metformin concentrations were high in the liver after giving the mice an overdose of 2 mg/day per mouse dissolved in a saline solution for 5 consecutive days compared to the control group of mice receiving the same volume of intraperitoneal injection of physiological saline and using an NR-NO2 probe (designed by adopting the strong electron-withdrawing group dinitrophenyl ether as the recognition moiety for H2S and as the fluorescence quencher as well) to detect the levels of H2S and it was found that H2S levels were elevated and liver injury was evident by using the multispectral optoacoustic tomography (MSOT) to detect the injury which was probably caused as a result of hypoxia and ROS formation [72].

Another study conducted on rats suggests that the rapid oxidation of H2S by the liver maintains low systemic circulating levels of H2S [76]. However, production of exogenous H2S from microflora is likely to increase H2S levels in the hepatic portal blood during infections, or endogenous H2S may increase as a result of metformin use and the excess H2S is removed from the circulation by the liver, and the increase of H2S oxidation may cause tissue hypoxia which reduces the hepatic O2 levels, then H2S metabolism capacity is decreased leading to an increase of H2S in the circulation. Therefore, tissue hypoxia increases ROS generation and causes hepatic injury [76]. Additionally, Hypoxia-inducible factor 1-alpha (HIF-1-alpha), a hypoxia-dependent signaling molecule that is released as a response of hypoxia, is pro-inflammatory and may contribute to the hepatic injury [76].

Mechanism of renal toxicity

Metformin is mainly eliminated by the kidneys through active tubular secretion which is known to be the principal route for metformin elimination. Metformin causes toxicity by its accumulation in the plasma due to renal insufficiency, which leads to systemic lactic acidosis as a result of the increase in plasma lactate concentration (>1.5 mmol/L), and a possible mechanism by which metformin increases the concentration of plasma lactate is that it inhibits mitochondrial respiration in tissues, including the liver and muscles responsible for lactate removal and this results in both accelerated lactate production and reduced lactate metabolism [68].

Both the liver and kidney are major lactate metabolizing organs, accounting for about 60% and 30% of lactate removal, respectively [77]. In the liver, after being converted in the muscles from glucose and carried to the liver by the blood, lactate is converted to pyruvate and used for gluconeogenesis then the resulting glucose is released and travels back to the muscles, that is known as the Cori cycle [91]. Most of lactate removal by the renal cortex in the kidneys is through lactate metabolism rather than excretion [77], and impaired kidneys may decrease its ability to metabolize the increase in lactate levels. A previous study showed that metformin use in people with a renal function of less than 60% and an eGFR of less than 60 mL/min/1.73 m2 are at a higher risk of developing lactic acidosis [68]. Lactic acidosis associated with metformin may worsen the condition of patients with a pre-existing renal impairment, which causes damage by tissue hypoxia, and that might lead to a decrease in eGFR [78].

From previous studies, metformin clearance is reduced in patients with mild to severe CKD, but drug levels remained within a safe range (Table 2) [71, 79]. In those single-dose studies, mild CKD (creatinine clearance, 60-90 mL/min) was associated with 23% to 33% reductions in medication clearance and moderate CKD (30-60 mL/min) with 74% to 78% reductions. Yet, metformin levels remained in the therapeutic range of 0.47 - 2.5 mg/L [79].

On the other hand, a 6-month study included 616 diabetic patients (484 continued metformin treatment and 132 discontinued metformin treatment) showed that metformin therapy was associated with a decline in renal function in type 2 diabetes mellitus patients with moderate CKD associated with an increase in the concentration of plasma lactate (unstandardized coefficient β, -2.072; 95% confidence interval, -3.268 - -0.876, p-value = 0.0007 ($p < 0.05$ is statistically significant)) [68]. Furthermore, renal function may deteriorate and progress to severe CKD (creatinine clearance, less than 30 mL/min), leading to the kidneys being unable to lose the excess metformin and its accumulation resulting in the development of lactic acidosis and that is because lactate removal in the kidneys is through lactate metabolism rather than excretion and the impaired renal function may decrease the ability of the kidneys to metabolize an increase in lactate caused by metformin [68].

- Metformin tissue-protective effects

Metformin use has shown potential for preventive and therapeutic effects for several conditions, including liver and kidney diseases. Metformin has some impacts of unknown mechanisms that might suggest benefits in treating CKD and liver diseases, especially in the context of insulin resistance and inflammation, such as non-alcoholic steatohepatitis (NASH), which is known as a condition in which the liver is inflamed, has cell damage, and excess fat, and non-alcoholic fatty liver disease (NAFLD) which is known as a condition in which excess fat is stored in the liver [80]. A meta-analysis concluded that metformin might be a good option in prediabetes or diabetic patients because of the evidence of insulin resistance improvements associated with NAFLD [81]. However, metformin cannot be recommended for NASH treatment because it did not demonstrate any significant improvement in liver histology in randomized controlled studies [82]. Metformin seems to be safe and increases the survival of diabetic patients with liver cirrhosis, but it does not offer any therapeutic potential once hepatic malignancies are already established [83].

Human and animal studies suggested that the alterations in the intestinal microbiota, barrier function, and the resulting elevation in the bacterial endotoxin levels are critical in the development of NAFLD, while the beneficial effects of metformin have been related to these alterations by which it increases glucagon-like peptide-1 (GLP-1) secretion and activates AMPK [84, 85].

Another animal study suggests that the protection against the onset and the progression towards more severe stages of NAFLD were related to the weakening of the loss of tight junction proteins in the proximal small intestine but not in the distal small intestine, and of the induction of MMP13 (Matrix Metallopeptidase 13) in the proximal small intestine which is suggested to be critical in the degradation of tight junction proteins [86, 87].

Additionally, metformin has reno-protective effects that prevents or attenuates AKI and mild to moderate CKD, but the mechanism by which it can cause this effect is still not clear, and it is thought that metformin protects the kidney via pleiotropic actions against different aspects of the pathophysiology of renal diseases [37 - 60]. Furthermore, metformin has actions that cause AMPK activation, increase in adenosine formation, and the opening prevention of the mitochondrial permeability transition pore at reperfusion which were found to protect the heart and kidneys from ischemia–reperfusion injury (IRI) [89, 90].

Furthermore, the pleiotropic actions of metformin in a rat model can lessen gentamicin nephrotoxicity and improve mitochondrial homeostasis by decreasing the effects of gentamicin of depleting the respiratory components such as cytochrome c and NADH, due to the opening of mitochondrial transition pores [49].

- Pre-existing renal impairment and injury risk

Pre-existing renal impairment can increase the risk of developing metformin-associated hepatotoxicity because metformin is excreted unchanged in the urine. The impairment might cause its accumulation, which might lead to the upregulation of H2S liver damage [76]. Metformin high concentrations might also increase the chances of lactic acidosis by lactate upregulation, which causes metabolic acidosis in patients with moderate CKD, and that causes a deleterious effect on renal function leading to a decline in eGFR and progression of CKD, which leads eventually to severe CKD and the decrease in renal function caused by metabolic acidosis is associated with several factors, including ammonia-induced complement activation and acidosis-induced increased production of endothelin and aldosterone that might cause interstitial tubule injury and mediate a decline in eGFR [78].

- Discussion

Some animal studies suggested that metformin-induced hepatotoxicity is possibly caused by H2S upregulation. We have seen that metformin-induced inhibition of complex I can change the mitochondrial metabolism of H2S might lead to ROS formation and liver damage [72]. However, it is difficult for this mechanism to be considered the main cause of the hepatic injury due to the lack of human studies, the rarity of metformin-induced hepatotoxicity, and species differences. Therefore, further research on human tissues is still needed to ascertain the credibility of this mechanism of injury.

Metformin was also associated with mixed effects on the renal function, and we can see that it can indirectly affect the kidneys through lactic acidosis; in more details, metformin is excreted unchanged in the urine, and usually, it does not negatively affect the kidneys and can be beneficial to its function, but it was found in a retrospective cohort study [68], that when there is a pre-existing renal impairment (moderate CKD in this study), metformin concentrations may increase due to the kidneys being unable to discard the excess of metformin which might lead lactic acidosis which is responsible for causing the damage and worsening the condition of the kidneys.

Due to that, the FDA revised their warning in April 2016 regarding metformin use in patients with impaired kidney function, defining the renal impairment according to estimated glomerular filtration rate (eGFR). The guidelines stated that metformin use is absolutely contraindicated in patients with severe chronic kidney disease (CKD) (eGFR < 30 ml/min/1.73 m2) [88]. However, in other studies [71], we can see that metformin use is safe in some patients with severe CKD in controlled doses, which concludes that the injury might also be related to other factors that may increase the damage risk. However, they are not fully understood, and further research is still needed. These factors might be related to co-medication, advanced age, or other diseases. Therefore, we can see that the development of metformin-induced renal damage depends on the renal function in addition to other misunderstood factors.

Metformin is also known for its protective effects on the hepatic and renal systems. It is suggested that metformin can protect the liver by reducing fat accumulation and the kidneys by preventing or attenuating acute kidney injuries (AKI) and mild to moderate chronic kidney diseases (CKD), but its protection mechanisms are still not fully known; however, it might be due to the pleiotropic actions of metformin [36 - 58].

In conclusion, metformin use can cause damage. However, due to the rarity of metformin-associated hepatic and renal toxicity, the conditions of developing these injuries are not fully understood. However, we can see that the metformin-induced upregulation of H2S might give us insights for

determining the cause of the hepatic injury in future research on human tissues. As for metformin-induced renal damage, we can see that the damage occurs in patients who are already suffering from renal impairments, but other unknown conditions might also influence the injury development because studies showed that metformin could be beneficial in some renal injuries.

Therefore, the use of metformin should be closely monitored in patients with declining or impaired renal function, and precautions should be taken to avoid possible injuries. At the same time, further studies should be considered to ascertain the exact toxicity mechanisms and factors that might increase the risk.

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