Charles University in Prague

Faculty of Pharmacy in Hradec Kralove

Drug-drug interactions (DDI) and pharmacodynamic effects of metformin

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Diploma thesis

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I declare that this work is my original author work, which developed by myself. All literature and other sources, which I have used, all are given in the list of used literature and they are quoted in text regularly.

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Babalola Hakeem-Habeeb

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I would like to thank Doc. PharmDr. Petr Pávek Ph.D for the opportunity to work with him and all of his guidance and mentoring throughout my studies at the Faculty of Pharmacy in Hradec Králové, I would also like to thank all of his colleagues in the department of Pharmacology and toxicology.

Thank you,

Babalola Hakeem-Habeeb

ABSTRACT

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Metformin is a widely used medication in the treatment of type 2 diabetes and is being increasingly used in the treatment of other conditions. Metformin is concidered the 1st line treatment in type 2 diabetes due to its effectiveness, minimal drug-drug interactions and rare self limiting side effects.

Metformins full mechanism of action is still unknown. The molecular mechanism of metformin action has been proposed in the liver related to AMPK activation and up-regulation of SHP. SHP suppresses functions of several nuclear receptors and transcriptional factors involved in the regulation of hepatic metabolism including PXR, which is a major regulator of drug/xenobiotic metabolism.

Therefore it was hypothesized that SHP is an important link between hepatic drug/xenobiotic metabolism regulated by nuclear receptors such as PXR and intermediary metabolism controlled by AMPK pathway.

In the diploma thesis we summarize the recent finding regarded the proposed mechanism of metformin action and its putative role on PXR in regulation of cytochrome P450 enzymes. Metformin's various roles and the common drug-drug interactions, specifically with competitive cationic drugs will be summarized and the potentially lethal effect of co-administration with contrast dyes.

ABSTRAKT

Karlova Univerzita v Praze

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Katedra farmakologie a toxikologie

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Drug-drug interactions (DDI) and pharmacodynamic effects of metformin

Metformin je široce užívané léčivo v terapii 2. typu diabetu. Kromě toho je čím dál více používán v dalších terapeutických aplikacích. Metformin je léčivo první volby u diabetu 2 typu především z důvodu jeho účinnosti, minimálních lékových interakcí a nežádoucích účinků.

Mechanizmus účinku metforminu však není znám. Předpokládá se, že AMPK signální kaskáda a zvýšená exprese SHP mohou být předpokládaným mechanizmem metforminu. SHP inhibuje funkce několika nukleárních receptorů a transkripčních faktorů, které řídí expresi faktorů 1. a 2. fáze biotransformace i metabolismu endogenních látek. Neznámějším z těchto nukleárních receptorů je Pregnanový X receptor (PXR).

SHP byl proto navržen jako spojnice mezi biotransformačními enzymy (metabolismem xenobiotic) a intermediárním metabolismem.

Ve své diplomové práci shrnuji nejdůležitější poznatky týkající se mechanizmu účinku a role metforminu na metabolismu xenobiotik řízený PXR receptorem nebo metabolismus intermediární.

Kromě toho sumarizuji nejdůležitější lékové interakce metforminu.

ABBREVIATIONS LIST

ACC	acetyl-CoA carboxylase
Ad	adenovirus
AMP	adenosine monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	AMP- activate protein kinase
APC	antigen presenting cell
ATP	adenosine triphosphate
CAR	coxsackie and Adenovirus Receptor
CL_{NR}	non-renal clearance
CYP	cytochrome p450
FFA	free fatty acids
GLc-6-Pase	glucose 6-phosphatase
GLUT-1	glucose transporter 1
GLUT4	glucose transporter type 4
GR	glucocorticoid receptor
HbA_{1c}	glycosylated haemoglobin
HDL	high density lipoproteins
IRS-2	insulin receptor substrate 2
LDL	low density lipoproteins
mRNA	messenger ribonucleic acid
NAFDL	non-alcoholic fatty liver disease

PAI-1	plasminogen activator inhibitor-1
PCOS	polycystic ovary syndrome
PEPCK	phosphoenolpyruvate carboxykinase
PXR	pregnane x receptor
SHP	small heterodimer partner
siRNA	small interfering RNA
SR	slow release
SREBP-1	sterol response element binding protein-1
TOR	target of rapamycin
VDR	vitamin D receptor
XR	extended release

Contents

1. The aim

Metformin is considered to experts as the gold standard oral drug in the treatment of type 2 diabetes. Although metformin is one of the most widely used drug, it's mechanism of action is still not clear. Metformin is a safe and well tolerated drug, which is believed not to interfere with biotransformation enzymes of cytochrome P450 family.

The aim of the thesis is to comprehensively summarize and critically consider the latest information about metformin´s mechanism of action at the molecular level.

The second aim is to summarize clinical data that would indicate drug-drug interactions of metformin with other drugs through cytochrome P450 biotransformation enzymes.

For the purpose, PubMed database will be used.

2. Introduction

Type 2 diabetes is a polygenic disorder that results from the interaction between genetic predisposition and environmental factors. It is characterized by chronnic hyperglycemia, which is a consequence from defects in both insulin sensitivity and β-cell function (Strack T et al 2008). Most patients with type 2 diabetes manifest insulin resistance and reduced insulin response to glucose function (Strack T et al 2008). Globally in 2003 it was estimated that there were 150 million people with type 2 diabetes (Green A et al 2001), this incidence varies substantially in different parts of the world due to the environment and lifestyle factors (Immet P et al 2001).

Type 2 diabetes promotes the development of serious complications that are major causes of morbidity and mortality function (Strack T et al 2008). This chronic disease is associated with both macro-vascular complications, such as myocardial farction and stroke and micro-vascular complications, such as diabetic nephropathy, retinopathy and neuropathy function (Strack T et al 2008). Long-term consequences of diabetes include organ damage (kidneys, eyes, heart and nervous system) function (Strack T et al 2008).

Metformin is an oral anti-diabetic drug in the biguanide class. It is the first-line drug choice for the treatment of non-insulin-dependant diabetes, in particular , in overweight and obese people & those with normal kidney function (IDB 2005, National collaborating 2002, American diabetes association 2009)

Metformin was first synthesized and found to reduce blood sugar in the 1920s; metformin was forgotten for the next two decades as research shifted to insulin and other anti-diabetic drugs. Interest in metformin was rekindled in the late 1940s after several reports that it could reduce blood sugar levels in people. It was introduced to the United Kingdom in 1958, Canada in 1972, and the United States in 1995. Metformin is now believed to be the most widely prescribed anti-diabetic drug in the world (Bailey CJ 2004)

3. Metformin - Structure & properties

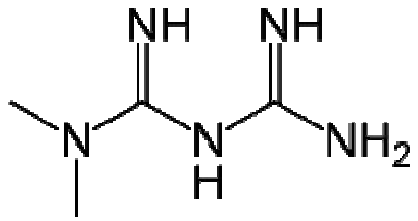

Figure1. Skeletal formula of metformin

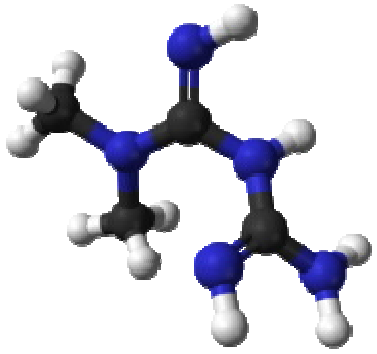

Figure 2. Ball and stick model of metformin

Synonyms: 1,1-dimethylbiguanide

Sytematic (IUPAC) name: *N*,*N*-dimethylimidodicarbonimidic diamide

Formula: C4H11N5

Mol. Mass: 129.164 g/mol (free)

165.63 g/mol (HCL)

Bioavailabity: 50 to 60% under fasting conditions

Half-life: 6.2 hours

Excretion: Active renal tubular excretion

Routes: Oral

3.1. Synthesis of metformin

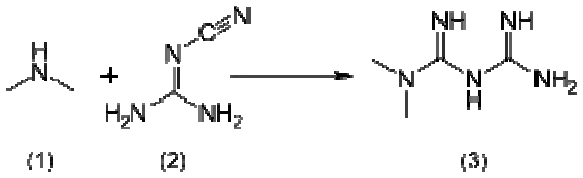

Figure 3. The usual synthesis of metformin, originally described in 1922 and reproduced in multiple later patents and publications, involves the reaction of dimethylamine hydrochloride and 2-cyanoguanidine (dicyandiamide) with heating.

Later patents and publications, involves the reaction of dimethylamine hydrochloride and 2-cyanoguanidine (dicyandiamide) with heating (Werner E et al 1922).

The procedure described in 1975 *Aron patent and the Pharmaceutical Manufacturing Encyclopedia*, demonstrated that equimolar amounts of dimethylamine and 2-cyanoguanidine are dissolved in toluene with cooling to make a concentrated solution and equimolar amounts of hydrogen chloride is slowly added. The mixture begins to boil on its own and after cooling, metformin hydrochloride precipitates with 96% yield (William A et al 2007).

3.2. Formulations of metformin

Metformin is known under several trade names, including *Glucophage XR, Riomet, Fortamet, Glumetza, Obimet, Dianben, Diabex and Diaformin*

Metformin immediate release is available in 500 mg, 850 mg, and 1000 mg tablets; all are now generic in the US.

Metformin SR or XR was introduced in 2004, in 500 mg and 750 mg strengths, mainly to counteract the most common gastrointestinal side effects, as well as to increase patient compliance by reducing pill burden. There is no difference in effectiveness between the two preparations.

Metformin is often prescribed to type 2 diabetes patients in combination with other drugs. Several are available as fixed-dose combinations, also with the purpose of reducing pill burden and making administration simpler and more convenient (Bailey CJ et al 2009).

As of 2009, the most popular brand-name combination was metformin with rosiglitazone, sold as *Avandamet* by GlaxoSmithKline since 2002 (GSK 2010) . Rosiglitazone actively makes cells more sensitive to insulin, complementing the action of the metformin. In the United States, metformin is also available in combination with pioglitazone (trade name *Actoplus Met*), the sulphonylureas glipized (trade name *Metaglip*) and glibenclamide (known as glyburide in the United States, trade name *Glucovance*), the dipetidyl peptidase-4 inhibtor sitagliptin (trade name *Jamumet*) and the megltinide repaglinide (*PrandiMet*). Generic formulations of metformin/glipizde and metformin/glibenclamide are available (the latter being more pouplar) (Institute for healthcare 2010). A generic formulation of metformin/rosiglitazone from Teva has received tentative approval from the FDA, and is expected to reach the market in early 2012 (Reuters, Teva 2009).

3.3. Metformin pharmacokinetics

Metformin has an oral bioavailability of 50–60% under fasting conditions, and is absorbed slowly (Bristol-Myers Squibb, FDA Glucophage 2008, Heller JB 2007). Peak plasma concentrations (C_{max}) are reached within one to three hours of taking immediate-release metformin and four to eight hours with extended-release.

Metformin is not metabolized by the body and is cleared from the body by tubular secretion then excreted unchanged in the urine, metformin is undetectable in blood plasma within 24 hours of a single oral. The average elimination half-life in plasma is 6.2 hours. Metformin is distributed to and appears to accumulate in red blood cells, with a much longer elimination half-life: 17.6 hours (reported as ranging from 18.5 to 31.5 hours in a single-dose study of non-diabetic people) dose (Robert F et al 2003).

3.4. Clinical effects

Type 2 diabetes is associated with the development and progression of vascular complications. Glycemic control, hypertension and dyslipidaemia management reduces micro and macrovascular diseases, including cardiovascular events. Glycemic control is a crucial factor in the reduction of microangiopathy, cardiovascular morbidity and mortality. Metformin is one of the most effective antihyperglycemic agents, possessing the capability to lower glycosylated haemoglobin A1c (HbA1c) levels. Several studies and randomized trials indicate that metformin therapy reduces cardiovascular morbidity and mortality in diabetic patients. It has been reported that metformin treatment counteracts insulin resistance, reduces hyperinsulinemia, reduces body mass index and improves lipid profile, especially by reducing triglycerides and LDL cholesterol levels and increasing HDL cholesterol levels. *The United Kingdom Prospective Diabetes Study* showed that intensive treatment with metformin decreases macrovascular events and mortality in overweight diabetic patients, when compared with intensive treatment with exogenous insulin or sulphonylurea derivatives. A recent clinical research study also showed that metformin treatment in people at risk for diabetes improves weight, lipid profiles, and insulin resistance, and reduces new-onset diabetes by 40% compared with placebo or no treatment (Strack T et al 2008).

3.4.1. Role of metformin in vascular protection

The pathogenesis of type 2 diabetes markedly increases the incidence of vascular complications (Strack T et al 2008). This disorder is associated with an increased prevalence of vascular risk factors, including hyperglycemia, hypertension and dyslipidemia, insulin resistance and hyperinsulinemia, present in type 2 diabetes, contribute for impaired fibrinolysis, inflammation and oxidative stress. Hyperglycemia is responsible for the induction of cellular damage in endothelial cells. The structural and functional integrity of the endothelium plays a critical role in vascular homeostasis. Therefore, endothelial dysfunction, a hallmark of type 2 diabetes, is intimately involved in the onset of diabetic vascular complications. Metformin improves vascular functions and dramatically reduces the incidence of vascular complications. The improvement of glycemic control in type 2 diabetes could be beneficial to prevent diabetes related vascular complications. Long term control of blood glucose levels in type 2 diabetic patients may decrease the incidence and retard the development of diabetic retinopathy, nephropathy and neuropathy (Strack T et al 2008).

3.4.2 Role of metformin in polycystic ovary syndrome

Polycystic ovary syndrome is one of the most common female endocrine disorders affecting approximately 5%-10% of women of reproductive age (12–45 years old) and is considered to be one of the leading causes of female subfertility (Goldenberg N et al 2008, Boomsma CM et al 2008, Azziz R et al 2004).

The principle features are obesity, anovulation which results in an irregular menstruation cycle or amenorrhea, excessive amounts of androgenic hormones. The symptoms and severity of the syndrome vary greatly among women. The causes are unknown but studies show insulin resistance, diabetes and obesity are all strongly correlated with polycystic ovary syndrome. About 50% of women with this syndrome are overweight and one third become diabetic at some time in their lives. In general, as weight is increased with PCOS the symptoms get worse and the risk of diabetes rises.

Conventional treatment for PCOS aims to suppress ovarian testosterone production using the combined oral contraceptive pill. In some women oral contraception is not an appropriate treatment due the side effects of the pill such as the increased risk of forming a blood clot (thrombosis) and this risk exaggerated in obese women. Also, in some women the use of oral contraceptive drugs causes weight gain and this might make the symptoms of PCOS worse in long term use. For these reasons alternative treatments are increasingly being tested.

Raised insulin concentrations have a side effect in the body of stimulating the ovary to produce more testosterone. Altered diet, weight loss or pharmacological treatment results in a lowering of testosterone and a reduction in the severity of symptoms caused by PCOS. The only drug currently available in the UK which reliably reduces insulin concentrations is Metformin.

Several studies have recorded the use of metformin in women with PCOS. Metformin is effective in reducing testosterone levels and in stabilising the menstrual cycle. While Metformin starts to improve the prospects for fertility in a few weeks, a reduction in unwanted hair growth would be expected to take some months and be slower than conventional treatment. Women can find weight loss easier when taking Metformin even though it is not a traditional weight reducing agent. One placebo-controlled trail has shown that Metformin is better than placebo in inducing ovulation in women with PCOS. The effectiveness of Metformin has been best demonstrated in obese women and it is likely that women of normal weight would benefit very little from this drug (Gerard C et al 2000).

3.4.3 Role of metformin in gestational diabetes

Gestational diabetes is a condition in which women without previous history of diabetes exhibit high blood glucose levels during pregnancy, usually occurring during the third trimester of pregnancy. During pregnancy some women will not secrete the excess insulin required during pregnancy leading to increased blood glucose levels.

Several complications can lead to gestational diabetes; babies are at risk of being large for gestational age also known as big baby syndrome, which may lead to delivery complications, low blood glucose levels and jaundice. Women are at increased risk of developing type 2 diabetes as well as a higher incidence of pre-eclampsia. Offspring are also prone to developing childhood obesity with type 2 diabetes later in life.

Metformin in combination with insulin or as an alternative has shown good effectiveness in the treatment of gestation diabetes. (Rowan JA et al 2008)

3.4.4 Role of metformin in non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease is one cause of fatty liver changes, when fat is deposited in the liver which can lead to no or few symptoms such as fatigue, malaise, abdominal discomfort and rarely mild jaundice. NAFLD is associated with insulin resistance, obesity, combine hyperlipidemia and high blood pressure. This condition responds metformin in the control of insulin resistance and lipid level control (Adams LA et al 2006).

3.5. Mechanism of action

The antihyperglycemic effect of metformin is achieved by its ability to supress gluconeogenesis, enhance glucose uptake and increase insulin sensitivity in peripheral tissues (Strack T et al 2008).Therefore, Metformin is capable of reducing plasma glucose levels, which are the crucial factors in the development of type 2 diabetes and associated complications (Radziuk J et al 997).

A report found a decreased gluconeogenesis in perfused livers, essentially through inhibition of lactate uptake, by metformin. Furthermore, in vitro studies using isolated rat hepatocytes showed that metformin lowers intracellular levels of ATP, an inhibitor of pyruvate kinase (Strack T et al 2008).

Metformin also inhibits pyruvate carboxylase-phosphoenol-pyruvate carboxykinase (PEPCK) activity and activates the pyruvate to alanine (Strack T et al 2008). Despite the mechanism of action of metformin in hepatocytes remains uncertain, the primary site of action of metformin appears to be the mitochondria. Metformin inhibits the mitochondrial respiratory chain, particularly at the complex I level, impairing mitochondrial function and consequently cell function (Strack T et al 2008).

The inhibition of cellular respiration decreases gluconeogenesis and enhances the expression of glucose transporters, stimulating glucose uptake (Strack T et al 2008). The insulin receptor and the glucose transporters seem to be the potential sites of action of metformin. A study performed in human hepatocytes demonstrated that metformin quickly increases insulin receptor activation and signalling, essentially through insulin- receptor substrate-2 (IRS- 2), and improves glucose transport through increased GLUT- 1 translocation. Besides the effect of metformin in gluconeogenesis, some studies also indicate that metformin reduces glycogenolysis. Evidence from the literature also demonstrates that metformin enhances

insulin-mediated glucose uptake & in vitro studies demonstrated the ability of metformin to increase glucose uptake in skeletal (Strack T et al 2008).

Elevated plasma free fatty acids play an important role in the establishment of insulin resistance. Chronic elevation in plasma FFA levels is commonly associated with impaired insulin-mediated glucose uptake in skeletal muscle, which coexists with obesity and type 2 diabetes (Strack T et al 2008). Furthermore, increased plasma FFA concentration exerts a lipotoxic effect on the β-cell. It has been attributed a reduction in FFA oxidation to metformin treatment. In type 2 diabetic patients, metformin leads to the suppression of FFA and lipid oxidation, it has also been reported that chronic metformin treatment results in the lowering of lipids in human skeletal muscle. Metformin treatment is frequently associated witha reduction in circulating triglycerides as a consequence of decreased synthesis and increased clearance of VLDL. A study in human pancreatic islets demonstrated that metformin exerts a protective effect against lipotoxicity. So, a reduction in the concentration of plasma FFA can contribute to the improvement in insulin action and may also help to correct impaired insulin secretion by β –cells (Strack T et al 2008). It has also been reported that metformin has a significant effect on the digestive tract by inducing a decrease in intestinal absorption of glucose, which could reduce postprandial blood glucose levels. It has been hypothesized that increased glucose consumption in the small intestine of metformin-treated patients may prevent further glucose transport to the hepatic circulation (Strack T et al 2008).

In summary, metformin ameliorates hyperglycemia and insulin resistance through the suppression of gluconeogenesis, glycogenolysis and intestinal glucose absorption, reduction of FFA and by the improvement in glucose (Strack T et al 2008).

Table 1. The potential metabolic changes resulting from the therapy of metformin.

Reported effects	Range	%
Glucose metabolism		
Fasting blood glucose	↓2-4 mmol/l	↓20-30
Postprandial blood glucose	↓3-6 mmol/l	↓30-40
Glycosylated hemoglobin	↓1-2 %	↓10-25
Fasting plasma insulin	↓0-3.5 uU/ml	↓0-20
Serum lipids		
TG	↓0-1.67 mmol/l	↓0-50
TC	↓0-0.35 mmol/l	↓0-10
LDL-C	↓0-1.00 mmol/l	↓0-25
VLDL-C	↓0-0.60 mmol/l	↓0-39
HDL-C	↓0-0.16 mmol/l	↓0-17
FFA	↓0-0.15 mmol/l	↓0-14
Vascular and hematologic indices		
Blood pressure	No change	
PAI-I antigen	↓10-15ng/ml	↓10-45
Peripheral blood flow	↑0-1.0 mL/100 mL Tissue per minute	↑0-25
Body weight	↓0-4kg	↓0-6

(Stepehen M.S et al 2003)

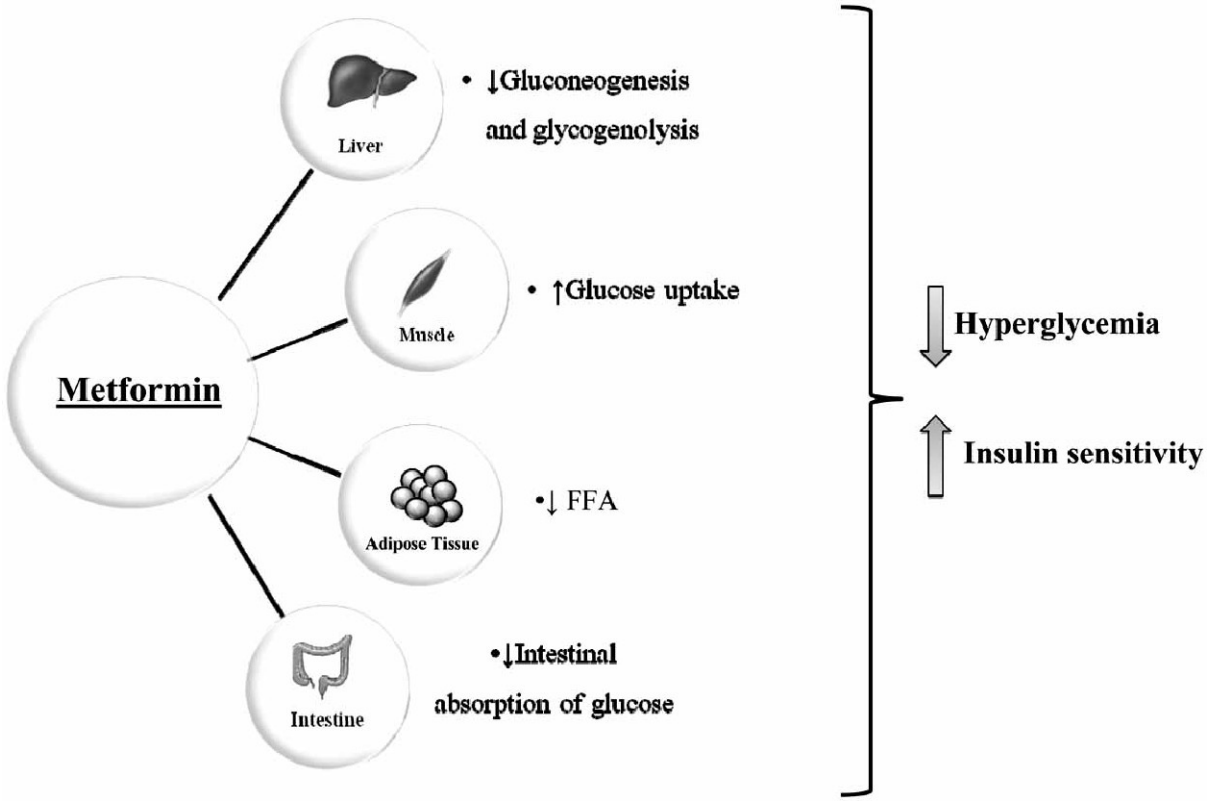

Figure 4. General overview of the antihyperglycemic action of metformin

3.5.1. Potential molecular mechanisms of metformin action

Although the molecular mechanism underlying metformin action remains unclear, it has been suggested that this drug activates AMPK, a major regulator of cell and body energy homeostasis, by increasing its phosphorylation state but without any changes in AMP/ ATP ratio (Figure. 5).

Recent studies suggest that activation of the AMP-activated protein kinase (AMPK) may play a central role in metformin actions (Zhou G et al 2001). AMPK is activated via ATP depletion due to factors such as ischemia, hypoxia, exercise and glucose deprivation. This causes AMPK to switch off ATP utilization (inhibition of *de novo* glucose synthesis and cell growth) while facilitating ATP production (via fatty acid oxidation, increased muscle glucose uptake and glycolysis) (Forgarty S et al 2010, Khan BB et al 2008, Owen MR et al 2000). Metformin does not act as a direct allosteric stimulator of AMPK; however, it may act, in part, by inhibiting mitochondrial respiratory chain complex-1, hence suppressing mitochondrial ATP synthesis and activating the AMPK pathway (Own MR et al 2000). Downstream targets of AMPK include transcription factors such as. HNF4α, nuclear receptors co-regulators e.g. SRC1 and core proteins involved in gene transcription (Leff T et al 2003).

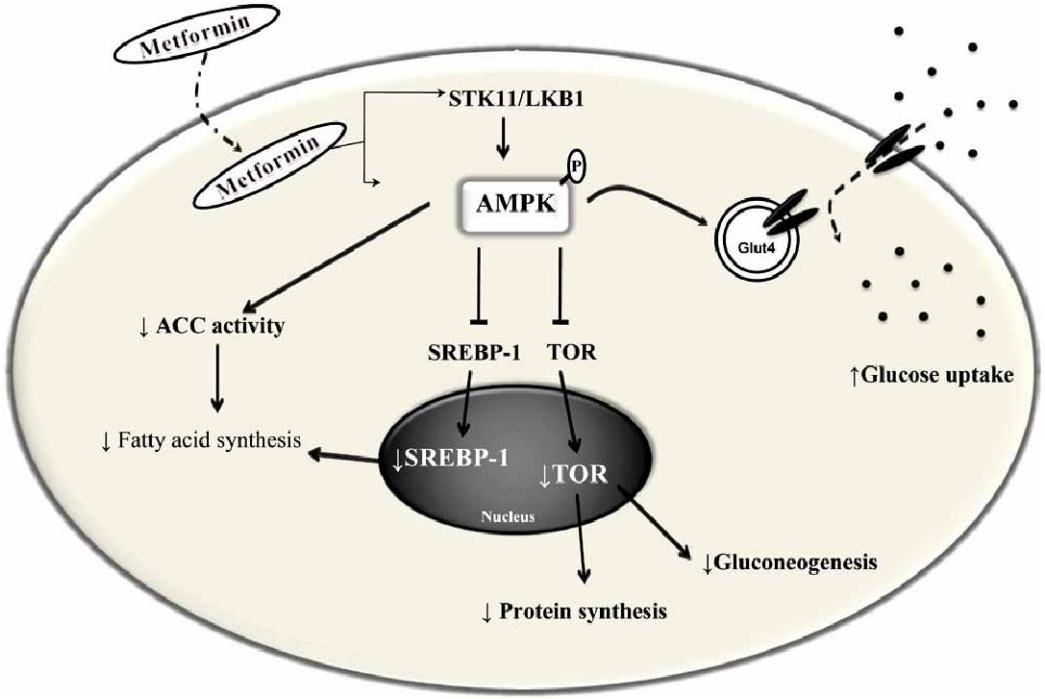

Figure 5. **Potential molecular mechanisms of metformin action.** Metformin activates AMP-activated protein kinase (AMPK) by increasing its phosphorylation state and serine-threonine kinase 11. Increased AMPK activity is associated with the translocation of glucose transporter (GLUT4) to the membrane and with the stimulation of glucose uptake. AMPK inactivates acetyl-CoA carboxylase (ACC), decreasing fatty acid synthesis. Activation of AMPK by metformin also reduces the expression of sterol response element binding protein-1 (SREBP-1), a transcription factor that induces the expression of lipogenic genes, favouring the inhibition of fatty acid synthesis. Additionally, the activation of AMPK also promotes the inhibition of target of rapamycin (TOR) pathway and, consequently, suppresses gluconeogenesis and protein synthesis.

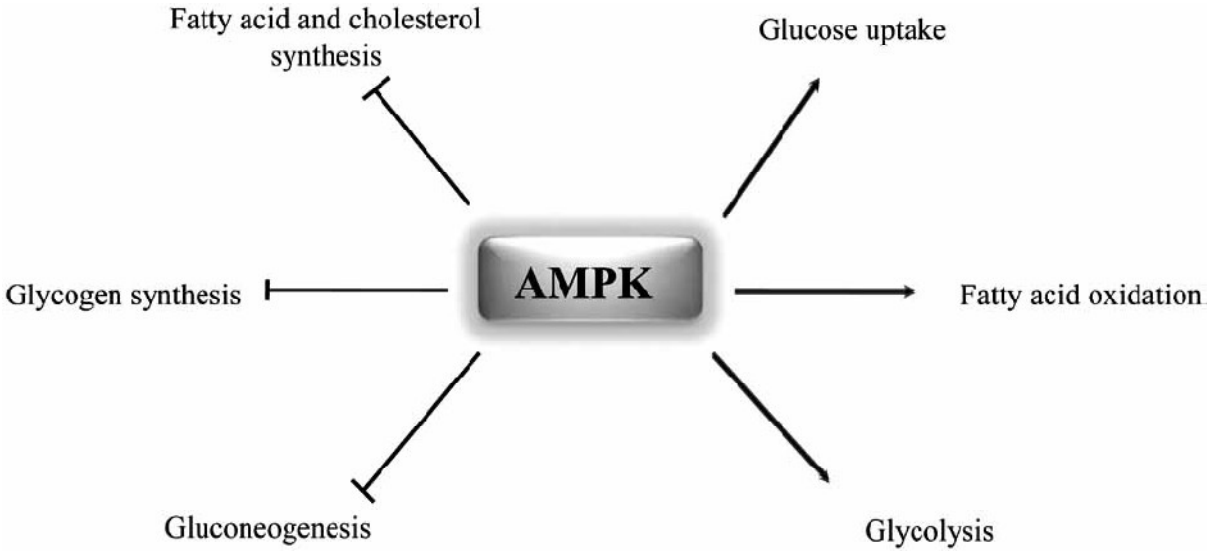

Figure 6. **Regulation of energy metabolism by AMP-activated protein kinase** (AMPK). Activation of AMPK inhibits fatty acid and cholesterol synthesis, gluconeogenesis and glycogen synthesis. AMPK stimulates glucose uptake, glycolysis and fatty acid oxidation.

3.5.2 SHP Regulation

SHP is a protein that is encoded by NR0B2 gene in humans. SHP is a member of the nuclear receptor family of intracellular transcription factors, it lacks DNA binding domain, so is therefore technically it is neither a transcription factor nor nuclear receptor (Lee HK et al. 1998). SHP function appears to be repression of other nuclear receptors through association to produce a non-productive heterodimer (Bavner A et al. 2005).

There is debate to whether SHP is influenced by metformin.

A study was carried out to determine whether metformin regulates hepatic gluconeogenesis through the orphan nuclear receptor SHP. The regulation of hepatic SHP gene expression was assessed by Northern blot analysis with metformin and adenovirus containing a constitutive form of AMPK (Ad-AMPK) and evaluated SHP, PEPCK and G6Pase promoter activities via transient transfection assays in hepatocytes. The results showed that hepatic SHP gene expression was induced by metformin, ALCAR and Ad-AMPK. Metformin-induced SHP gene expression was abolished by adenovirus containing the dominant negative form of AMPK, as well as by compound C. Metformin inhibited hepatocyte nuclear factor-4α-or FoxA2-mediated promoter activity of PEPCK and G6Pase. Inhibition was blocked with siRNA SHP. Furthermore oral administration of metformin increased SHP mRNA levels in mice and overexpression of SHP by Ad-SHP decreased blood glucose levels and hepatic gluconeogenic expression in mice (Yong Deuk et al. 2007).

It was concluded that metformin inhibits hepatic gluconeogenesis through AMPK-dependent regulation of SHP.

Another theory by the Department of Pharmacology, Faculty of Pharmacy in Hradec Kralove states that SHP expression is not significantly influenced by metformin in primary human hepatocytes. The mRNA and protein expression of SHP in multiple cells was analysed, in the presence and absence of metformin. A slight induction as well as SHP mRNA down-regulation after treatment with metformin depending on hepatocyte preparation, was observed. Regression analysis did not indicate statistically significant concentration-dependent effect on metformin on SHP mRNA after 24, 48 or 60 hour treatments in hepatocyte preparations from five different sources. At the protein level, no significant effect of metformin on SHP protein expression in two primary human preparations was observed.

Concluded, SHP mRNA was not significantly affected by metformin in concentration-dependent manner, but apparent down-regulation of SHP mRNA by 2mM metformin in cells was observed (Krausova L et al in preparation).

3.6. Adverse effects

The most common adverse effect of metformin is gastrointestinal upset, including diarrhea, cramps, nausea, vomiting and increased flatulence; metformin is more commonly associated with gastrointestinal side effects than most other anti-diabetic drugs (Bolen et al 2007).

The most serious potential side effect of metformin use is lactic acidosis, this complication is very rare and the vast majority of these cases seem to be related to comorbid conditions, such as impaired liver or kidney function, rather than directly to metformin itself (Khurana R, 2010).

Metformin has also been reported to decrease the blood levels of thyroid-stimulating hormone in patients with hypothyroidism and in men, luteinising hormone and testosterone. The clinical significance of these changes is still unknown (Vigersky RA et al 2006).

Metformin reduces pyruvate dehydrogenase activity and mitochondrial transport of reducing agents enhancing anaerobic metabolism. This shift to anaerobic metabolism, in the presence of reduced insulin, increases production of precursors for the Krebs cycle. The inhibition of pyruvate dehydrogenase results in a decreased ability to utilize these precursors into aerobic metabolism which results in increased metabolism of pyruvate to lactate and increases the net lactic acid production. In healthy individuals, this slight excess is simply cleared by other mechanisms including uptake by the kidneys, when their function is unimpaired and no significant elevation in blood levels of lactate occurs (Maharani U et al 2010).

When there is impaired renal function, clearance of metformin and lactate is reduced, leading to increased levels of both, and possibly causing lactic acidosis due to a build-up of lactic acid. Because metformin decreases liver uptake of lactate, any condition that may precipitate lactic acidosis is a contraindication to its use.

Common causes of increased lactic acid production include alcoholism, heart failure, and respiratory disease due to inadequate oxygenation of tissues, the most common cause of impaired lactic acid excretion is kidney disease (Lippincott et al 2005).

Metformin has also been suggested to increase production of lactate in the small intestine, this could potentially contribute to lactic acidosis in patients with risk factors (Brunton L et al 2006). However, the clinical significance of this is unknown and the risk of metformin associated lactic acidosis is most commonly attributed to decreased hepatic uptake, rather than increased intestinal production (Maharani et al 2010, Lippincott et al 2005, Brunton L, et al 2006).

Metformin has also been implicated with increased homocysteine levels and vitamin B12 deficiency (Strack T et al 2008). In fact, it has been demonstrated that 10-30% of diabetic patients that received long-term metformin therapy develop vitamin B12 malabsorption, indicated by reduced concentrations of total vitamin B12 and its bioavailable form. Chronic use of this anti-diabetic agent may predispose to hepatotoxic injury.

3.7. Drug-drug Interactions

Metformin drug interactions with other medications such as certain decongestants, calcium channel blockers, and diuretics can potentially lead to problems. Some of these drug interactions can make metformin less effective, increasing the chance of high blood sugar, or can increase the level of metformin in the blood, increasing the risk of side effects.

3.7.1. Drug-drug interactions with common drugs

Contrast medium induced nephrotoxicity is considered an important cause of hospital acquired renal failure. This is not surprising, since diagnostic and interventional procedures requiring the use of contrast media are performed with increasing frequency. In addition, the patient population subjected to these procedures is progressively older with more co-morbid conditions. Even a small decrease in renal function due to contrast medium nephrotoxicity may greatly exacerbate morbidity caused by co-existing conditions. Sepsis, bleeding, coma and respiratory failure are frequently observed in patients with acute renal failure. (Thomsen HS et al 1999).

Diatrizoate, Iodamide, Iodipamide, Iodixanol, Iohexol, Iopamidol, Iopromide, Iothalamate, Ioversol, Ioxaglate, Ioxilan – Contrast mediums

The use of contrast media in patients receiving metformin is the most serious interaction and should be carried out with care. Approximately 90% of metformin is eliminated via the kidneys in 24 hours. Renal insufficiency will lead to retention of biguanides in the tissues and the potential for the development of fatal lactic acidosis (Thomsen HS et al 1999).

Contrast media can induce a reduction in renal function, which occurs after the contrast medium has reached the kidney, leading to retention of metformin that may induce lactic acidosis.

Cephalexin – 1st generation β-lactam antibiotic from the class of Cephalosporins

Renal clearance of metformin is reduced in a time-dependent manner in the presence of cephalexin. Cephalexin inhibits the renal tubular secretion of metformin resulting in higher circulating serum concentrations (Jayasagar G et al 2002).

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Gatifloxacin – 4th generation fluoroquinlone antibiotic

Gatifloxacin may interfere with the therapeutic effects of oral anti-diabetic agents and insulin. The use of various quinolones has been associated with disturbances in blood glucose homeostasis possibly stemming from effects on pancreatic beta cell ATP-sensitive potassium channels that regulate insulin secretion. Hypoglycemia and hyperglycemia have been reported more frequently with gatifloxacin than with other quinolones. Gatifloxacin-induced hypoglycemic episodes have generally occurred within the first 3 days of therapy and sometimes even after the first dose, while hyperglycemia usually occurred 4 to 10 days after initiation of therapy. Death has been reported in severe cases. Co-administration of gatifloxacin with sulphonylureas (most often glyburide) and/or other oral hypoglycemic agents has resulted in severe, refractory hypoglycemia and hypoglycemic coma. Elderly patients and patients with reduced renal function are particularly susceptible. (Micromedex 2011).

Furosemide – loop henle diuretic

A single-dose metformin and furosemide drug interaction study in healthy subjects demonstrated, that pharmacokinetic parameters of both compounds were affected by co-administration. Furosemide increased the metformin plasma and blood C_{max} by 22% and blood AUC by 15%, without any significant change in metformin renal clearance. When administered with metformin, the C_{max} and AUC of furosemide were 31% and 12% smaller, respectively, than when administered alone and the terminal half-life was decreased by 32%, without any significant change in furosemide renal clearance (Bristol-Myers Squibb U.S. Food and Drug Administration Glucophage 2008).

Nifedipine – dihydropyridine Ca^{+} channel blocker

A single-dose, metformin and nifedipine drug interaction study in normal healthy volunteers demonstrated that co-administration of nifedipine increased plasma metformin C_{max} and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. Tmax and half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine clearance (Bristol-Myers Squibb U.S. Food and Drug Administration Glucophage 2008).

Cationic drugs - (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Such interaction between metformin and oral cimetidine has been observed in normal healthy volunteers in both single and multiple-dose, metformin-cimetidine drug interaction studies, with a 60% increase in peak metformin plasma and whole blood concentrations and a 40% increase in plasma and whole blood metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics. Although such interactions remain theoretical (except for cimetidine), careful patient monitoring and dose adjustment of metformin or metformin extended release and/or the interfering drug is recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system clearance (Bristol-Myers Squibb U.S. Food and Drug Administration Glucophage 2008).

Cimetidine – H_2-receptor antagonist

Cimetidine causes an increase in the plasma concentration of metformin, by reducing clearance of metformin by the kidneys, Metformin and cimetidine are both cleared from the body by tubular secretion, and both, particularly the cationic (positively charged) form of cimetidine, may compete for the same transport mechanism.

To determine whether cimetidine altered the renal handling of metformin, seven subjects took 0.25 g metformin daily with and without cimetidine 0.4 g twice daily. Blood and urine samples were collected and assayed for metformin, cimetidine and creatinine by HPLC, Cimetidine significantly increased the area under the plasma metformin concentration-time curve by an average of 50% and reduced its renal clearance over 24 hours by 27%. There was no alteration in the total urinary recovery of metformin when cimetidine was co-administered. The effect of cimetidine on the renal clearance of metformin was time dependent, being significantly reduced up to 6 hours following cimetidine. These results appeared to be consistent with competitive inhibition of renal tubular secretion. Cimetidine had no effect on the renal clearance of creatinine, but time-dependent variations in both metformin and creatinine renal clearance was observed. Metformin had no effect on cimetidine disposition. It is concluded that cimetidine inhibits the renal tubular secretion of metformin, thus resulting in higher circulating plasma concentrations. Because of its propensity for causing dose and concentration-dependent adverse effects, the dose of metformin should be reduced when cimetidine is co-prescribed (Somogyi A et al 1987).

Metformin is negligibly bound to plasma proteins and is, therefore, less likely to interact with highly protein-bound drugs such as salicylates, sulphonamides, chloramphenicol, and probenecid, as compared to the sulfonylureas, which are extensively bound to serum protein (Bristol-Myers Squibb U.S. Food and Drug Administration Glucophage 2008).

3.7.2. Interaction of metformin with biotransformation enzymes

Cytochromes P450 enzymes constitute a large superfamily of haem-thiolate proteins involved in the metabolism of a wide variety of both exogenous and endogenous compounds. CYPs are the major enzymes involved in drug metabolism, accounting for 75% of the total metabolism. Most drugs undergo deactivation by CYPs, either directly or by facilitated excretion from the body. Also, many substances are bio-activated by CYPs to form their active compounds.

Many drugs may increase or decrease the activity of various CYP isozymes, either by inducing the biosynthesis of an isozyme (enzyme induction) or by directly inhibiting the activity of the CYP (enzyme inhibition). This is a major source of adverse drug interactions, since changes in CYP enzyme activity may affect the metabolism and clearance of various drugs. For example, if one drug inhibits the CYP-mediated metabolism of another drug, the second drug may accumulate within the body to toxic levels.

A series of experiments using various inducers and inhibitors of CYP isozymes was conducted to find out what types of CYP isozymes are involved in the metabolism of metformin in rats (ChoiYH et al 2006).

Metformin at a dose of 100 mg kg^{-1} was administered intravenously to rats. The rats were pretreated with CYP inducers such as 3-methylcholanthrene, orphenadrine, isoniazid, and dexamethasone (major inducers of CYP1A1/2, 2B1/2, 2E1 and 3A1/2 respectively in rats) or CYP inhibitors such as SKF-525 (a non-specific inhibitor of CYP isozymes), and sulfaphenazole, quinine, and troleandomycin (major inhibitors of CYP2C11, 2D1, and 3A1/2, respectively in rats). The time-averaged non-renal clearance (CL_{NR}) of metformin was compared with that of controls (Choi YH et al 2006).

In rats pretreated with dexamethasone, the CL_{NR} was significantly faster (57% increase) than for the controls. In rats pretreated with SKF-525-A, sulfaphenazole, quinine, and troleandomycin, the CL_{NR} was significantly slower (24.3, 62.9, 77.6, and 78.7% decrease, respectively) than for the controls. However, the CL_{NR} values did not significantly different in the rats pretreated with 3-methylencholanthrene, orphenadrine, and isoniazid compared with the controls (Yong DK et al 2008).

The results concluded that metformin is metabolized primarily via hepatic microsomal cytochrome P450 of the specific family from CYP2; CYP2C11, CYP2D1 and CYP3A1/2 in rat cells (Yong DK et al 2008).

Further, experimental studies have suggested that metformin dramatically supresses the expression of CYP3A4 mRNA, both basal and ligand-induced, after treatment with prototype ligands of all the tested nuclear receptors in primary human hepatocytes (Krausova L et al in preparation).

There is evidence that regulation of hepatic CYPs are affected in diabetes and regulation of some CYP genes depend on nutritional status of cells. In fasted mice liver, CYP2B10 and Car genes are induced (Rencurel F et al 2006). It has been shown that AMPK activation and inhibition of mitochondrial function are connected with the effect of the prototype inducer phenobarbital on hepatic CYP genes (Rencurel F et al 2006). AMPK cascade activation is unlikely to be involved in suppression of CYP3A4 induction by metformin (Krausova L et al in preparation).

Putative role of SHP in cytochrome P450 enzymes regulation by metformin

Several studies by a Korean group claim that SHP is involved in metformin-mediated activation of AMPK. AMPK activation was proposed to induce SHP, which inhibits gluconeogenesis by down-regulating phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) genes (Kim YD et al 2008, Lee JM et al 2010). SHP suppresses transcriptional activity of pregnane X nuclear receptor (PXR) in vitro and in vivo (Li T et al 2005, Ourlin JC et al 2003, Wang L et al 2002). In a positive feedback loop, PXR inhibits SHP gene transcription (Li T et al 2005). Importantly, other nuclear receptors (NRs), such as constitutive androstane receptor (CAR) (Seol W et al 2005, Bae Y et al 2004, Baes M et al 1994) and glucocorticoid receptor (GR) (Borgius LJ et al 2002) also interact with SHP being a corepressor of the nuclear receptors (Bavner et al 2005). Significantly, these NRs are key regulators of xenobiotic-metabolizing enzymes of the cytochrome P450 superfamily (CYP) (Pavek P et al 2008). PXR is the most important nuclear receptor and it is referred to as a "master regulator" or "xenosensor" for metabolism and clearance of diverse endogenous and exogenous compounds (Pavek P et al 2008, Tirona RG et al 2005, Biswas A et al 2009). When ligand tethered (by any of a large number of structurally diverse xenobiotics and some endobiotics and bile acids) (Pavek P et al 2008, Tirona RG et al 2005, Orans J et al 2007), target genes are up-regulated through transcriptional induction. CYP3A4 is the most important and well studied target gene of PXR, which is the crucial drug/xenobiotic enzyme that metabolizes about 50% of drugs and xenobiotics (Martinez-J CP et al 2007). In addition to CYP3A4, PXR regulates the expression of several other important drug/xenobiotic-metabolizing enzymes such as CYP2C9, CYP2B6, CYP2C19, CYP3A5, UGT1A1 and SULT2A1; as well as drug transporters such as MDR1(P-glycoprotein) and MRP2 (Pavek P et al 2008).

Since SHP suppresses PXR, the effect of SHP up-regulation by metformin on PXR transcriptional regulation of its target genes can be proposed (Krausova L et al submitted).

Dr. Pavek's group studied the hypothesis and found that metformin represses its induction via major xenobiotic- and hormone-dependent nuclear receptors PXR, CAR, VDR and GR. Data shows that in the case of PXR the phenomenon is not due to metformin on SHP expression hepatocytes (Krausova L et al submitted). The theory is that metformin disrupts co-activation of PXR and SRC1 independently on PXR ligand binding domain, this resulting in inhibition of PXR transcriptional activity and suppression of its target genes expression. SHP is thought to be an important link between drug/xenobiotic metabolism regulated by nuclear receptors such as PXR and intermediary metabolism controlled by AMPK pathway. Hepatocytes (Krausova L et al submitted). However, SHP was not consistently up-regulated by metformin in their experiments from five primary human hepatocyte preparations. Similarly it was observed that there was no consistent induction of SHP mRNA in either MZ-Hep1 or LS174T cells. Additionally, silencing of SHP or over expression of SRC1 co-activator had no effect on metformin-mediated suppression of induced CYP3A4 expression; this showed that SHP up-regulation is not involved in the phenomenon and raises debate to whether metformin indeed induces SHP in normal human hepatocytes (Krausova L et al submitted).

There is evidence that regulation of hepatic CYPs is affected in diabetes and that the regulation of some CYP genes depends on nutritional status of cells (Maglich JM et al 2004)

Dr Pavek's group showed that AMPK cascade activation is unlikely to be involved in suppression of CYP3A4 induction by metformin. (Krausova L et al submitted).

This conclusion was supported by the fact AICAR does not display the same effects as does metformin on either CYP3A4 transactivation in gene reporter assay or non CYP3A4 mRNA expression (Krausova L et al submitted). It was found that inhibition of AMPK cascade by BML275 regulated significant reduction of both basal CYP3A4 transactivation and was concluded from the data that activation of AMPK does not supress CYP3A4 expression and that activated AMPK cascade is necessary of transactivation of CYP3A4 (Krausova L et al submitted).

4. Discussion

Many theories for the mechanism of action of metformin have been hypothesized but there are still many unknown explanations of the mechanism of metformin. The activation of AMPK, a liver enzyme which plays an important role in insulin signalling, whole body energy balance and the metabolism of glucose and fats, has been widely discussed and is considered to be the principle feature in metformin action. Activation of AMPK is required for metformin's inhibitory effect on the production of hepatic glucose production and recent research demonstrates that AMPK activation is required for an increase in the expression of Small heterodimer partner (SHP), which in turn inhibits the expression of hepatic gluconeogenic genes PEPCK and Glc-6-Pase.

The mechanism by which metformin increases the activity of AMPK remains unknown and research suggests that metformin increases the amount of cytosolic AMP as opposed to a change in the total AMP or total AMP/ATP.

SHP is a protein that is encoded by NR0B2 gene in humans. SHP is a member of the nuclear receptor family of intracellular transcription factors, it lacks DNA binding domain, so is therefore technically it is neither a transcription factor nor nuclear receptor (Lee HK et al. 1998). SHP function appears to be repression of other nuclear receptors through association to produce a non-productive heterodimer (Bavner A et al. 2005).

There is debate to whether SHP is influenced by metformin.

A study was carried out to determine whether metformin regulates hepatic gluconeogenesis through the orphan nuclear receptor SHP. The results showed that hepatic SHP gene expression was induced by metformin, AICAR and Ad-AMPK. In this way, metformin inhibited hepatocyte nuclear factor-4α-or FoxA2-mediated promoter activity of PEPCK and G6Pase. Furthermore oral administration of metformin increased SHP mRNA levels in mice and overexpression of SHP by Ad-SHP decreased blood glucose levels and hepatic gluconeogenic expression in mice (Yong Deuk et al. 2007). It was thus concluded that metformin inhibits hepatic gluconeogenesis through AMPK-dependent regulation of SHP.

However, another theory by the Department of Pharmacology, Faculty of Pharmacy in Hradec Kralove states that SHP expression is not significantly influenced by metformin in primary human hepatocytes. The mRNA and protein expression of SHP in multiple cells was analysed, in the presence and absence of metformin. A slight induction as well as SHP mRNA down-regulation after treatment with metformin depending on hepatocyte preparation, was observed. Regression analysis did not indicate statistically significant concentration-dependent effect on metformin on SHP mRNA after 24, 48 or 60 hour treatments in hepatocyte preparations from five different sources. At the protein level, no significant effect of metformin on SHP protein expression in two primary human preparations was observed. Authors concluded, SHP mRNA was not significantly affected by metformin in concentration-dependent manner, but apparent down-regulation of SHP mRNA by 2mM metformin in cells was observed (Krausova L et al submitted).

This discrepancy needs further investigation into the study of SHP regulation on metformin.

In addition, since SHP is a critical corepressor of numerous nuclear receptors involved in biotransformation enzymes regulation, comprehensive understanding of the role of SHP could evaluate potential drug-drug interactions of metformin.

Summary

Metformin is the first-line drug choice for the treatment of type 2 diabetes and has extra beneficial effects in overweight and obese people with normal kidney function. It is capable of effectively reducing HbA_{1c} values, having a positive effect on lipid profiles by reducing levels of LDL particles, total cholesterol, PAI-1 and on the other hand enhances fibrinolysis, vascular reactivity, blood flow and HDL cholesterol levels decreasing the risk of vascular complications and having a direct effect on controlling blood glucose levels by the suppression of gluconeogenesis and increasing skeletal uptake of glucose.

Metformin is considered a safe drug with uncommon and rare side effects with a low number of drug-drug interactions. The adverse effects of metformin are generally tolerable and self-limiting. The most important and potentially life-threatening adverse effect of metformin is lactic acidosis, which is extremely rare due to continuous studies and the market withdrawal of the more prone to risk of lactic acidosis from the biguanide phenformin. Metformin has a low number of drug-drug interactions where the most serious is the interaction with contrast dyes.

Metformin is increasingly being used in polycystic ovary syndrome, non-alcoholic fatty liver disease and premature puberty; all of these conditions feature insulin resistance. These indications are still considered experimental but randomized control trials have found significant improvement in the use of metformin with these conditions.

Metformins full mechanism of action is still unknown. The molecular mechanism of metformin action has been proposed in the liver related to AMPK activation and up-regulation of SHP. Therefore it was hypothesized that SHP is an important link between hepatic drug/xenobiotic metabolism regulated by nuclear receptors such as PXR and intermediary metabolism controlled by AMPK pathway.

In the diploma thesis we summarize the recent finding regarded the proposed mechanism of metformin action and its putative role on nuclear receptors in regulation of cytochrome P450 enzymes.

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