Chapter

Combined Effect of Metformin and Statin

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Abstract

Diabetes mellitus (DM) is considered a risk factor for the development of coronary artery disease (CAD). Metformin, an anti-diabetic drug, has been shown to lower the cardiovascular events in pre-clinical and clinical studies. Many research articles suggests that metformin has a protective effect on CAD beyond its hypoglycemic effects. Patients with diabetes type 2 have an increased risk for cardiovascular disease and commonly use combination therapy consisting of the anti-diabetic drug metformin and a cholesterol-lowering statin. Statins have been found to be a safe and effective approach to reduce serum low density lipoprotein cholesterol (LDL-C) levels, which is the cornerstone for primary and secondary prevention of atherosclerosis. However, regular statin monotherapy in some patients may not be sufficient to achieve a therapeutic LDL-C. It has been reported that statins increased the incidence of new-onset diabetes in a dose dependent manner especially in women, the elderly, or in the presence of a family history of type 2 diabetes (T2D) and Asian ethnicity. The molecular mechanisms contributed to antioxidation, anti-inflammation, and anti-apoptosis. In this chapter, we aimed to investigate whether the combined administration of metformin and atorvastatin could achieve superior protective effects on different disease treatment purpose and to elucidate its molecular mechanisms of the combinations.

Keywords: combination therapy, metformin, statins, diabetes mellitus, clinical studies

1. Introduction

World Health Organization (WHO) defines diabetes mellitus as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with alterations of carbohydrate absorption, fat and protein metabolism. DM is one of the four major non-communicable diseases along with cardiovascular disease (CVD), cancer and chronic respiratory diseases. Once a disease of affluence, it is now increasingly common among the poor countries [1]. The morbidity and mortality associated with DM arises from minor and macrovascular complications, ischemic heart disease (IHD) and peripheral vascular disease (PVD) [2]. Metformin acts by several mechanisms of action but the major mechanism is inhibiting hepatic gluconeogenesis [3]. The drug may antagonize the action of glucagon, and reduces fasting blood glucose (FBG) [4]. In addition, metformin increases insulin action at target sites, increases peripheral glucose uptake, enhances fatty acid oxidation and reduces glucose absorption from gastrointestinal tract [5]. Diabetes mellitus and statins have a complex association and are the attention of patient and healthcare

debate. Statins are widely used as a part of diabetes mellitus care due to that patients with DM have a greater CVD [6]. At the early stage, the heart only showed transcriptional and metabolic altercations, including enhanced inflammation, oxidative stress, depletion of antioxidant proteins, and changes in energy metabolism. Use of statins in diabetes is a controversial when compared with metformin. Although the potential detrimental effects of statin on muscle and liver have been known for a long time, new concerns have emerged regarding the risk of new onset diabetes (NOM). This often leads to discontinuation of statin, non-adherence to therapy, or concerns correlating with initiating statin therapy.

There are several CVD risk factors, including hypertension, dyslipidemia, diabetes mellitus (DM), smoking and obesity, as well as platelet dysfunction. Certain drugs are currently available for treating these risk factors, whereas drug combinations are frequently needed to achieve therapeutic goals especially in hypertension, DM and coronary heart disease (CHD). Based on these considerations our objectives were 1) to assess whether combination therapy shows clinical effectiveness for cognition and functional benefits in a well-characterized prospective cohort of patients with T2DM treated over years with metformin; 2) to determine the magnitude and duration of benefit; 3) to characterize the long-term treatment of patients who receive combination therapy compared to those who were never treated with statins and those who only received metformin as monotherapy; and 4) to use modeling methods to make predictions about the mechanism and clinical course in different treatment groups and dose levels.

Both metformin and statins thus act on glucose—as well as lipid metabolism which is why metformin–statin combination therapy is prescribed to many T2DM patients. Since both drugs act on glucose as well as lipid metabolism, it is important to understand in detail the interactions between metformin and statin mechanism of action on treatment design with different dose level and optimal safety/efficacy profiles. This chapter is therefore designed to provide insight in the mechanism of combined effect of statin/metformin not only on DM and CVD but also with different types of cancer and other diseases. This chapter also explain the interaction of both drugs on preclinical and clinical studies to determine an optimal dosing strategy of both drugs.

2. Metformin

Metformin is an oral antidiabetic drug, discovered in 1922, came on the market as late as 1979 [7]. The drug is belongs to the biguanide classification and derivative from guanidine found in *Galega officinalis*. It is available in different formulations based on its duration of action like immediate-release, extended release and delayed-release metformin [8, 9]. The latter two forms were developed to expand the absorption of metformin along the gut. Metformin administration in 30 min before a meal produced highest therapeutic efficacy in lowering postprandial hyperglycemia [10].

2.1 Metformin absorption and distribution

Oral administration of metformin transported into the small intestine across the apical membrane into the enterocytes via several transporter proteins. The main proteins are the plasma monoamine transporter (PMAT; SLC29A4), organic cation transporter 1 (OCT1; SLC22A1) and serotonin transporter protein (SERT; SLC6A4) [11].

Metformin accumulated majorly in the intestine, and in the stomach, liver, kidney and lesser extent in muscle. The accumulation of metformin in intestine

and stomach is because of these organs are most exposed to high concentrations of metformin after oral administration. A recent study confirmed the high metformin levels are accumulated in these organs [12]. These concentrations are tenfold higher than metformin concentrations in the liver, indicating that the intestine is probably an important site of action. In fact, the metformin effects in the intestine may be rather different than the effects in the liver. The concentration of metformin in human jejunum has been shown to be 30 to 300 fold greater than in plasma, and earlier studies demonstrating accumulation of metformin in the intestinal mucosa. Metformin navigates to the liver via the portal vein and is taken up predominantly by organic cation transporter (OCT1) as well as by Thiamine transporter (THTR-2). In this chapter, the effects of metformin on the lipid metabolism are highlighted, thereby creating a special focus on the effects on lipids related to the activation of AMPK by metformin (**Figure 1**) [13].

Metformin is transported into hepatocytes mainly via OCT1, and inhibited the mitochondrial respiratory chain (complex I) through a currently unknown mechanism(s). The deficit in energy production is balanced by reducing gluconeogenesis in the liver. This is mediated in two main ways. First, a decrease in ATP and a concomitantly increase in AMP concentration. Second, increased AMP levels function as a key signaling mediator to (1) allosterically inhibit cAMP–PKA signaling by suppression of adenylatecyclase, (2) allosterically inhibit FBPase, (3) activates AMPK. This leads to inhibition of gluconeogenesis (1 and 2) and lipid/cholesterol synthesis (3).

Metformin is present for over 99% in the mono protonated form in all tissues of the body except in the stomach. The sparse data showed, that metformin is mostly distributed in the cytosolic fraction (~ 70%) of rat hepatic cells compared to mixed membranes (12%), nucleus (~ 5%), and mitochondrial and lysosomal fractions (8%). A low binding affinity of metformin to mitochondrial membranes was seen, and this may be because of the two methyl groups present in metformin structure [14]. Previous study concludes that, the mitochondrial membrane

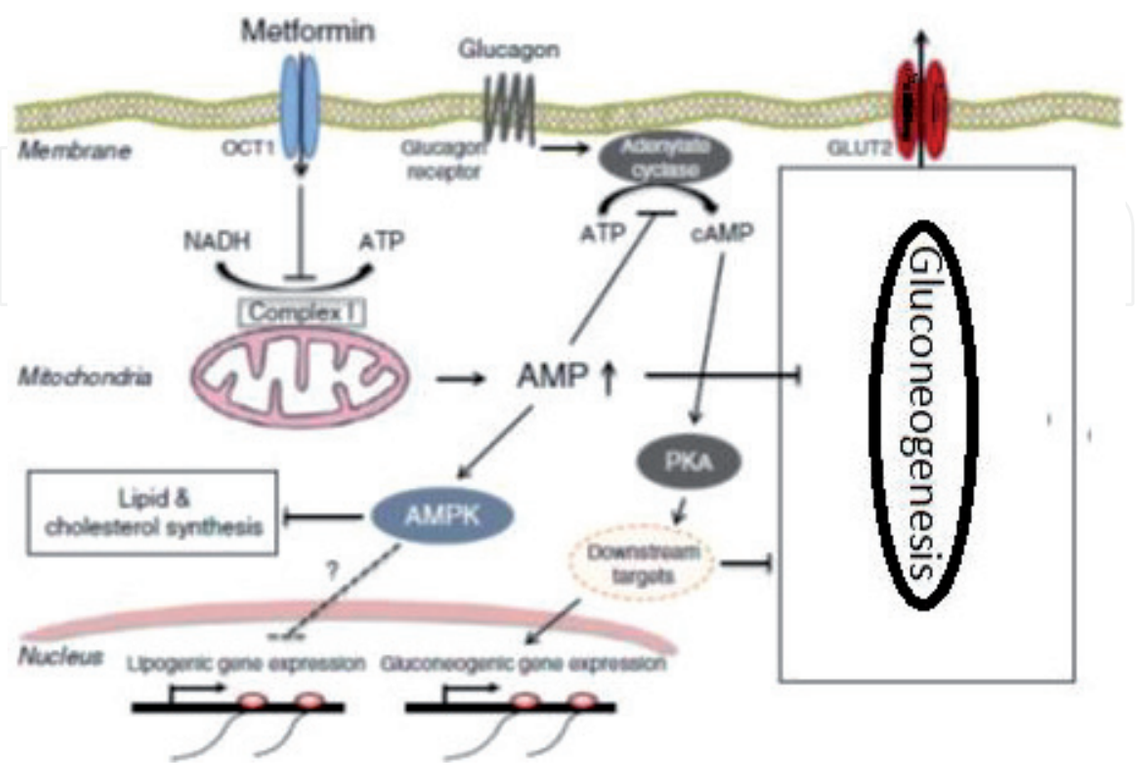

Figure 1.
Schematic diagram of the anti-hyperglycaemic action of metformin on the liver cell.

potential may promote entry of metformin (positively charged) [15], which will then concentrate inside the mitochondria (negatively charged) [16]. Molecular modeling of the metformin distribution and validation study confirmed the presence of high concentrations of the drug in the endoplasmic reticulum (ER) and in the mitochondria, based on its membrane potential [17].

2.2 Metformin mechanisms on glucose and lipid metabolism

The main mechanisms of metformin involved in decreasing the endogenous glucose production and plasma glucose have all been extensively reviewed and critically discussed in earlier studies [18]. Metformin shows beneficial effects on the glucose and lipid metabolism, even though the pathways are not fully understood [19]. In patient studies, the variations of metformin efficacy may be due to the presence of responders and non-responders to the drug treatment [20], racial and ethnic background [21], and personal variation in the adaptation of metformin treatment. Sonne et al., [22] proposed a pathway inducing reduction of LDL cholesterol by the. Inhibition of the intestinal absorption of bile acids is caused by metformin. It causes an increased synthesis of bile acids in the liver, and cholesterol is used for this process [23], thereby causing a decreased amount of cholesterol in the hepatic cells. Upregulation of the LDL-C receptor may increase the uptake of lipoproteins, to restore a sufficient level of cholesterol in the liver. Hence, metformin indirectly decrease the LDL-C concentration and plasma total cholesterol concentrations.

2.3 (In)-direct effects of metformin on β cells

A decreased β cell mass is an important factor in the development of T2DM. High glucose and FFA induce damaging effects on β cells (e.g. decreased insulin secretion and β cell mass) [24]. It is therefore of interest to consider possible beneficial effects of metformin on β cell function. Lipase and amylase are secreted by the pancreas and are often measured to monitor the condition of the pancreas. There were no changes observed in the enzyme levels, and the pancreas volume when metformin (1950 mg/day) was given to T2DM patients for 24 weeks. This works suggesting that metformin does not repair damaged β cells [25].

3. Statin

Statins, block an enzyme called HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase) that is involved in the synthesis of mevalonate, a naturally occurring substance that is then used by the body to make cholesterol. By inhibiting this enzyme, LDL-cholesterol and cholesterol production is decreased. Statins also increase the number of LDL receptors on liver cells, which increases the uptake and breakdown of LDL-cholesterol. Most of the effects of statins, including the blocking of the HMG-CoA reductase enzyme occur in the liver. Many research have shown that elevated levels of total cholesterol, LDL-cholesterol, triglycerides, and apolipoprotein B increase a person's risk of developing heart disease or having a stroke.

3.1 Classification of statins and its general source

Statins are classified based on different criteria, including: 1) how they are obtained, 2) liver metabolism, 3) physicochemical properties, and 4) specific activity. Some of the statins are obtained after fungal fermentation: lovastatin,

pravastatin and simvastatin, others by synthesis: fluvastatin, atorvastatin, and cerivastatin. Only five statins are, at this moment, in clinical use: lovastatin, simvastatin, pravastatin, atorvastatin and fluvastatin. Pravastatin is extremely hydrophilic, fluvastatin has intermediate characteristics, lovastatin, simvastatin, atorvastatin and cerivastatin are hydrophobic.

3.2 General uses of statins

- Statins differ in their potency at lowering total cholesterol, triglycerides, LDL-cholesterol, or increasing HDL-cholesterol; their propensity for drug interactions; and their reported safety in people with kidney disease.
- Reduce a person's risk of having a heart attack or stroke or developing angina
- Reduce the risk of further heart disease in people with type 2 diabetes or coronary artery disease.
- Simvastatin and atorvastatin produce the greatest percentage change in LDL cholesterol levels. Fluvastatin and atorvastatin are also preferred in hypocholesteremic patients with kidney disease.
- Pravastatin and fluvastatin have a lower risk of drug interactions because they are not metabolized by cytochrome p450 3A4.
- Pitavastatin has a similar effectiveness to atorvastatin but reportedly produces greater increases in HDL-cholesterol that are sustained over the long-term. It is effective at low dosages and has minimal drug interactions.

3.3 Statins mechanism on glucose and lipid lowering metabolism

Statins are a major class of drugs that decrease plasma cholesterol levels and are prescribed as first choice to patients suffering from CVD [26]. Simvastatin and atorvastatin are often given as a first choice to patients with cardiovascular risk factors/cardiovascular disease. In earlier studies reported that low dose (20 mg/day) of atorvastatin given to patients with myocardial infarction showed improved lipid, adipokine, and pro-inflammatory markers and decreased insulin resistance. Higher dose (40 mg/day) of atorvastatin showed hyperglycemia, increased leptin levels and ghrelin deficiency [27, 28] in diabetic patient. It was also discovered that the reduction in LDL-C by statins is an important indicator of increased T2DM risk [29]. Genetic factors and/orange-related factors could as well lead to the development of T2DM during statin treatment.

Several mechanisms possibly involved in the effect of statins on glucose metabolism are summarized in **Figure 2**. Statin signaling pathway that stimulates endogenous glucose production (EGP) by activation of gluconeogenic genes in human liver cells. Statin activates the pregnaneX receptor (PXR) in the cytoplasm. Many functions are exerts by PXR, such as the stimulation of the expression of proteins involved in regulation of hepatic glucose and removal of xenobiotics, and lipid metabolism [17].

3.4 Effects of statins in the β cell of pancreas

Statin mechanism may contribute to a decreased insulin secretion in the β cell, possibly contributing to the progress of T2DM. The upregulation of LDL-C receptor seen upon inhibition of HMG-CoA reductase are one of the directly

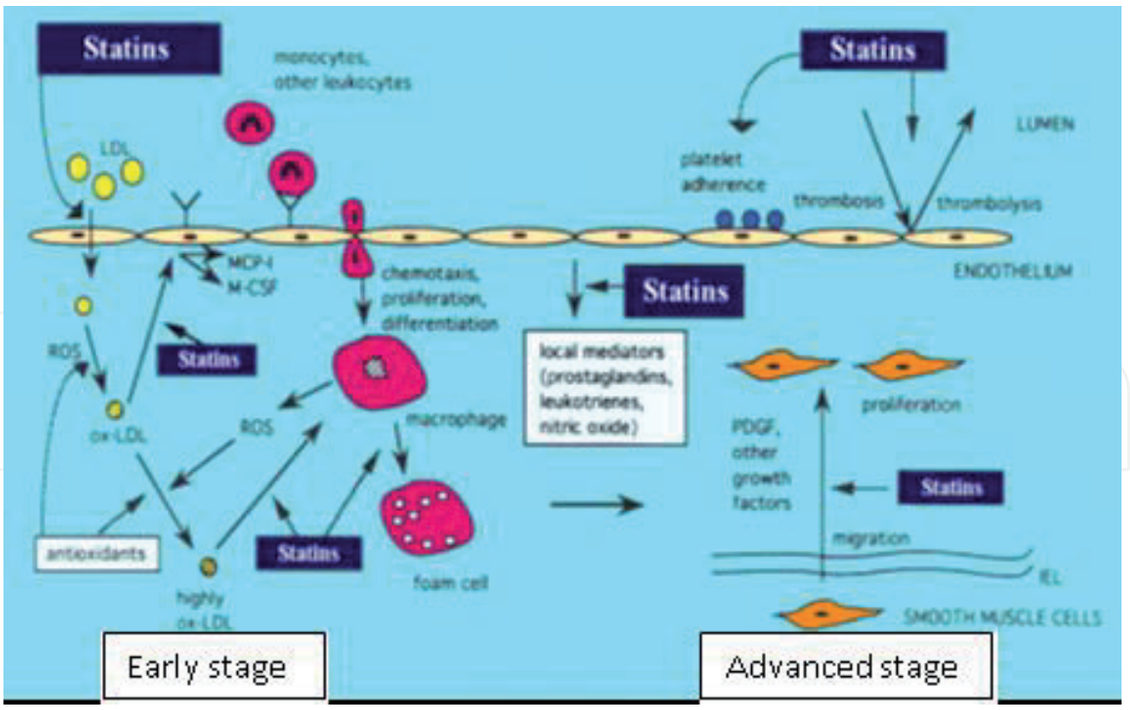

Figure 2.
Hypercholesterolemia enhance the entry of LDL particles into sub endothelial space at lesion-prone arterial sites. Monocyte chemotactic protein-1 (MCP-1) and oxidized-LDL act as chemoattractants to direct accumulation of monocytes and their migration to the subendothelial space, where monocytes undergo phenotypic transformation into macrophages. Oxygen free radicals concurrently modify LDL. Oxidatively modified LDL is taken up by nondownregulating macrophage receptors to form lipid-rich foam cells. The foam cells develop into fatty streaks that is the, precursor of atherosclerotic plaques. Statins exhibit pleiotropic effects on many components of atherosclerosis that accompany hypercholesterolemia, abnormal endothelial function and including platelet coagulation abnormalities, and determinants of plaque thrombogenicity such as plaque inflammation and proliferation.

affected processes, which results in increased uptake of plasma LDL-C into the β cell [30]. The increased amount of cholesterol within the cell causes interference with translocation of glucokinase, to the mitochondria [31]. A decreased glucose transporter (GLUT2) expression level was observed in simvastatin treated mouse MIN6 cells which resulted in a reduction of ATP levels. This may be the mechanism of inhibition of the KATP channel closure, membrane depolarization and calcium channel opening all leading to reduced insulin secretion [32]. Inhibition of the ATP-dependent potassium channel, depolarization and the decreased influx of intracellular calcium, and calcium concentrations were observed and were related to a decreased insulin secretion. In an ex vivo study, intracellular calcium levels were not affected even though intact with single-islets were treated with simvastatin [33]. Statin treatment may cause inactivation of Ras and Rho molecules, hence the activation and membrane translocalization of GLUT-4 is inhibited. Experiments with atorvastatin treatment in mouse adipocytes confirmed that GLUT-4 located on the plasma membrane moved to the cytosol during treatment and this may result in an increased insulin resistance [34].

3.5 Statins on cancer

Since 1959, evidence from many studies had revealed that there was an association between T2DM and cancer, and patients who had T2DM were more likely to be diagnosed with cancer than patients who had not [35, 36]. A lot of evidence has also shown its beneficial effects in cancers, including prostate, breast, lung, and colorectal cancers [37]. Experimental results in vitro have suggested the effect of statins on

growth, migration, apoptosis, and autophagy of cancer cells [38, 39]. The data from in vivo cell culture studies, statins may act as a preventive drug for hepatocellular carcinoma, malignant glioma and bladder cancer [40]. However, the role of statins on the incidence of cancer in patients with T2DM has not been well documented. Fei et al., [41] performed a meta-analysis to evaluate the impact of different types of statins on the risk of cancers with T2DM.The study was systematically searched with the Cochrane Library, PubMed, Embase, and Wanfang databases from January 1999 to March 2017. A pairwise meta-analysis used to estimate the pooled ratios (ORs) and 95% confidence intervals (CIs). NMA was performed to compare different types of statins. In pairwise meta-analysis result showed that, the incidence of cancer in T2DM patients was reduced when simvastatin, atorvastatin, pravastatin, fluvastatin, lovastatin, rosuvastatin, and pitavastatin were used. The analyses suggest that rosuvastatin may be more effective than others.

4. Combination therapy of metformin and atorvastatin

4.1 On antidiabetic activity-preclinical studies

Previous studies on diabetic rats (200–220 g) reported that after 2 weeks of metformin–atorvastatin combination therapy (500 mg metformin and 20 mg atorvastatin per 70 kg body weight), reduced blood glucose, lipid-lowering effects, and reduced in elevated oxidative stress, and positive effects on cardiovascular hypertrophy occurred [42]. The reduction of oxidative stress and liver protection (blood analysis and liver histology studies, e.g. CRP, TNF-α, IL-6, protein carbonyl levels) was also seen in T2DM rats treated with metformin and atorvastatin [43].

Statins consistently showed a protective role in the setting of diabetes cardiomyopathy (DCM) due to their roles of anti-inflammation, anti-oxidation, and antiapoptosis effects [44]. In previous animal experiments, statins could prevent DCM by all evicting left ventricular dysfunction and inhibiting myocardial fibrosis through anti-apoptosis and anti-inflammation pathways. It seems that statins may facilitate the onset of diabetes by impacting peripheral insulin sensitivity and islet b-cell function, while statins can effectively modify the promotive factors and promoting DCM, including inflammation and oxidative stress, thereby protecting the heart against diabetic conditions [45].

4.2 On Antiatherosclerogenic activity-preclinical studies

An animal study was designed to evaluate the effectiveness safety and mechanism of an atorvastatin/metformin combination therapy in a rabbit atherosclerosis model induced by a high-cholesterol diet. At the end of the experiment, all rabbits were sacrificed by injection of an overdose of sodium pentobarbital solution and the aortas were separated from the surrounding tissues. From the initiation of the aortic arch, 0.5 cm sections were excised for paraffin treatment [46] and the remaining aortas were soaked in 4%paraformaldehyde and then stained with Oil Red O solution, to evaluate the atherosclerotic lesion area of the aorta by image-processing software (ImageJ). One portion stained with hematoxylin and eosin (H&E) before quantification using ImageJ software. In an animal study 12-week high-cholesterol diet induced a significant increase in atherosclerotic lesion area in rabbits in the control (Ctrl) group; after 10 weeks of atorvastatin or metformin treatment, the atherosclerotic lesion area was significantly reduced by 51% and 35%, respectively.

Atorvastatin/metformin combination therapy resulted in an 80% reduction of atherosclerotic plaques compared with the control group. The combination

therapy showed which was more effectively than each monotherapy. Compared with control group, the treatment of atorvastatin or metformin significantly reduced the lesion size by 68% and 42%, respectively, while atorvastatin/metformin combination therapy further reduced atherosclerotic lesion size by 86%. It was reported that large HDL is inversely associated with cardiovascular disease [47]. The results suggest that atorvastatin and metformin combination therapy is superior to atorvastatin monotherapy for the treatment of atherosclerosis and the underlying mechanisms might be associated with cholesterol efflux in macrophages. The study results demonstrated that atorvastatin/metformin combination therapy did not show a better lipid-lowering effect than atorvastatin, which is similar with the recent clinical and preclinical data [48]. The CAMERA study revealed that metformin did not affect the lipid profile in statin-treated patients [49]. Forouzandeh *et al*. confirmed the plasma cholesterol in apoE−/− mice fed a high-fat diet did not affect and found that metformin markedly reduced atherosclerotic plaques [50]. Earlier studies also suggest that an additional anti-atherosclerotic mechanism of metformin when added to atorvastatin, which is independent of the lipid-lowering effect. Study report is the first, to demonstrate that atorvastatin/metformin combination therapy increases the percentage of large HDL sub fraction. Goldberg *et al*. [51] found that metformin could raise the concentrations of large HDL in a clinical trial. The research article also suggested an inverse association of large HDL sub fraction with coronary artery disease, which may involve reverse cholesterol transport (RCT).

4.3 On antidiabetic activity-clinical studies

In a clinical study a great number of patients are selected and treated with metformin–atorvastatin combination tablet administered as a single daily dose [52]. There is only a minor chance for toxic drug interactions when treated with metformin and statin together because metformin is not metabolized and is the mechanism for most statins are via the cytochrome P450 system [53]. Since metformin shows beneficial effects on both dyslipidemia and glycemic control and has been shown to reduce CVD risk while statins may have an added beneficial effect on CVD risk. Hence the combined treatment with both drugs seems a good option. Clinical studies on the effects of metformin and statin combination therapy have been carried out but for different diabetic complications [54–56]. Each of these studies had different objectives and included different patients groups, i.e. either with T2DM, dyslipidemia, treated (different doses), untreated, or newly diagnosed T2DM. This criteria were compared in these studies to arrive at overall results of metformin statin combination therapy. The lowest dose of metformin (500 mg) and atorvastatin (10 mg) once daily resulted in the highest reduction of fasting plasma glucose (−35%). Atorvastatin 20 mg showed to attenuate the glucose and HbA1c-lowering effect in combination with 1000 and 2000 mg metformin.

In another clinical trial, a total of 50 newly diagnosed patients with T2DM with age range of 47.8 ± 7.4 years and prescribed 850 mg/day of metformin (sustained release), with dietary restriction, were enrolled in open-label multi center pilot study. WHO criteria was followed for the selection of newly diagnosed patients [57] and underwent a physical examination and information about their medical history, demographic parameters, and medication history were obtained by questionnaire. The patients received a constant dose regimen of metformin during the 90-day study period. In that study, the use of metformin in newly diagnosed T2DM patients, improves body weight and glycemic control; however, the addition of

low-dose atorvastatin did not improve these conditions. Metformin, in a long-term study, reduces the risk of macrovascular disease after a follow-up period of 4 years [58], and this beneficial effect supports to continue metformin treatment with T2DM patients unless contraindicated. The result of this study is consistent with that reported in an experimental animal model, which indicates that the combination of atorvastatin with metformin did not produce a better lipid-lowering effect than atorvastatin [59]. In addition, the study indicated that 10 mg/day did not increase the HbA1c and serum glucose levels, but there was no additional significant improvement in the studied markers when compared with the metformin-treated group.

4.4 On lipid metabolism -clinical studies

The effects of metformin on lipid homeostasis discussed earlier in this chapter, indicate that lipid metabolism is positively affected in the intestine and liver leading to decreased plasma triglycerides, LDL-C, and total cholesterol. Metformin effects on lipid metabolism seem to be localized to the intestine. Statins mainly act on plasma cholesterol via activation of the LDL-receptor suggesting that combination therapy should show an additional effect on plasma lipids. Combination therapy with statins and metformin demonstrated beneficial effects in patients with other disease(s)/disorder(s) than T2DM and dyslipidemia [60].

In earlier studies, the effect of metformin alone on the lipid profile was studied, and the result analysis showed that only TG levels and LDL/HDL ratio were significantly improved. Whereas these effects were not significantly different compared with its combination with atorvastatin that improves all lipid profile components. These results indicated that the addition of atorvastatin with metformin did not influence the lipid-lowering effects of monotherapy in newly diagnosed T2DM patients with metformin. In previous studies, although metformin moderately improves the lipid profile, there were inconsistencies in its effects on the lipid parameters [61]. Accordingly, the addition of atorvastatin to metformin treatment in newly diagnosed T2DM patients showed relatively normal lipid profile may be irrational and cost ineffective and the emergence of adverse effects may be highly expected with long-term use.

4.5 On prostrate cancer-clinical studies

Diabetic patients receiving metformin have been shown to have a reduced cancer incidence and a decrease in cancer-specific mortality [62]. Statin use was also found to be associated with a reduction in the risk of biochemical recurrence in patients with prostate cancer and a decreased risk of cancer mortality [63, 64]. Based on epidemiologic evidence and the preclinical data for metformin and atorvastatin individually in prostate cancer, the author concluded the beneficial effects of metformin and atorvastatin alone or in combination on SCID mice and cultured prostate cancer cells. Metformin and atorvastatin in combination exhibited potent inhibitory effect on the growth of prostate cancer cells *in vivo* and *in vitro*. The drug combination stimulated apoptosis in prostate cancer cells compared with individual treatment. Mariel concluded that, coupled with epidemiological studies, provide a strong rationale for clinically evaluating the combination of metformin and atorvastatin in prostate cancer patients [17].Recent studies showed that metformin in combination with simvastatin induced G1-phase cell cycle arrest, and Ripk1- and Ripk3-dependent necrosis in prostate cancer cells [65, 66]. The combination of metformin and simvastatin was found to decrease the levels of phospho-Akt and phospho-AMPKα1/α2 [67].

4.6 Combination therapy on other diseases

In T2DM patients with non-alcoholic fatty liver disease (NAFLD) the combination therapy was found to be benefited. Whereas, statin therapy associates negatively with non-alcoholic steatohepatitis and found to be significant fibrosis while a safe use of metformin in patients with T2DM and NAFLD was demonstrated [68]. Combination therapy consisting of metformin and statin treatment is frequently prescribed to women with polycystic ovary syndrome (PCOS). This syndrome increases the risk of T2DM and cardiovascular morbidity as it is associated with abnormal increased lipid levels, insulin resistance, endothelial dysfunction and systemic inflammation [69]. Meta-analysis showed that combined therapy in women with PCOS resulted in improved inflammation and lipid markers but it did not improve insulin sensitivity [70].

Treatments using statins, and combined statins and metformin can effectively improve IR, fasting insulin (F-INS), insulin sensitivity index, hyperandrogenemia, acne, hirsutism, testosterone and decreasing C reactive protein (CRP) [71–73]. Pre-treatment with atorvastatin for 3 months followed by metformin in patients with PCOS improves insulin and homeostasis model assessment of IR (HOMA-IR) indices and reduces CRP level but does not improve the lipid profile compared with placebo treatment. Hence, atorvastatin pre-treatment enhances the effects of metformin in improving IR, whereas inflammatory markers are not affected by decreased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) after cessation of atorvastatin [74].

The lipid-lowering effect of statins administered with or without metformin in PCOS patients remains ambiguous. This finding is also supported with the meta-analysis performed by Gao et al. [75]. A clinical trial demonstrated that insulin secretion was found to be increased after 6 weeks of statin therapy in women with PCOS [76]. The meta-analysis found that statins fail to improve F-INS and HOMA-IR in single or in combination with metformin. This finding may be due to the following reasons. First, statins may damage endothelial function through loss of the protective anti-proliferative and anti-angiogenic effects of adiponectin, resulting in impaired insulin sensitivity [77]. Second, statins decrease the levels of cholesterol mediated by the farnesoid X receptor (FXR), the deficiency of which is related to IR [78]. The activation of FXR can lower the levels of glucose-6-phosphatase, reduce phosphoenol pyruvate carboxykinase in gluconeogenesis, and increase glycogen synthesis [79]. Hence, induced IR caused by statin therapy may be related to the low expression of FXR [80]. Third, statins (lipophilic) are possibly absorbed by extra-hepatic cells; these statins can deregulate cholesterol metabolism, thus deteriorating IR and attenuating β-cell function [81].

Combination therapy could also be considered for T2DM patients with diabetic retinopathy. Diabetic retinopathy (DR) is a microvascular complication of diabetes caused by hyperglycemia and hyperosmolarity. In T2DM patients and pre-existing DR patients, the use of statin showed a protective effect against development of diabetic macular edema [82]. In T2DM patients receiving statin therapy in combination with increased levels of cholesterol remnants and triglycerides were associated with slight decreased in left ventricular systolic function. Targeting cholesterol remnants might be beneficial for finding cardiac function in T2DM patients receiving statins [83].

5. Combination therapy of metformin and simvastatin- clinical studies

A high daily dose of metformin (3000 mg) and simvastatin (40 mg) resulted in an improved insulin resistance, but fasting plasma glucose decreased only by 5%,

and observed minor changes on lipid metabolism parameters. This may probably due to the fact that metformin was given on top of simvastatin treatment. The patients involved in these studies had an impaired fasting glucose, dyslipidemia, newly diagnosed T2DM and/or dyslipidemia. However, it could be used for hypothesis-generation rather than making rigid decisions, considering the lack of multiple dose dependent combination studies.

The combination of metformin with insulin may be a better therapeutic option for patients with DM whose hyperglycemia is poorly controlled on insulin treatment. Aviles et al. [84] stated that increased frequency of dosage of insulin causes more improvement in glycemic control and significantly reduce HbA1c which was compared with a combination therapy of insulin and metformin. Furthermore, unchanged FBG and PPBG and HbA1c in patients on metformin and insulin compared to combination of metformin, insulin and simvastatin treated patients. The HbA1c of diabetic patients on simvastatin showed a slight elevation as compared to other groups. Previous studies reported that statin use is associated with a rise of FPG in patients with and without DM [85]. Sattar et al., have identified deterioration in glucose homoeostasis in patients treated with statins and this depends on lipid solubility of statins. Simvastatin can enter easily extra hepatic cells because of its high lipid solubility and may suppress isoprenoid protein synthesis, thus attenuating the action of insulin. The abnormal level of FBG may translate into clinical syndrome of DM with rise in HbA1c is not excluded. The combination of metformin and insulin may be an attractive therapeutic option for patients with DM whose hyperglycemia is poorly controlled on insulin [86].

6. Conclusion

The mechanism of metformin is a controversial along with the use of statins in diabetes. Although the potential detrimental effects of statin therapy on muscle and liver have been known for a long time, new concerns have emerged regarding the risk of new onset diabetes (NOM) that often leads to discontinuation of statin, concerns correlating with initiating statin therapy or non-adherence to therapy.

Metformin is generally to exert its beneficial effects on glucose metabolism mainly in the liver. In line with recent research articles on the topic we conclude that the drug acts primarily in the intestine. This is due to the at least one order of magnitude higher concentrations of metformin in the intestine than in the liver. The drug present in the liver and its effects may be localized to this organ most probably via its effects on gluconeogenesis. A newly diagnosed patient with T2DM who show inadequate response to metformin may need better treatment approaches to lower atherogenic lipids. Supplementation with niacin or high-dose omega-3 fatty acid could be used in newly diagnosed T2DM patients with borderline values of lipid profile, secondary to lifestyle modifications before using a potent statin such as atorvastatin as the first treatment priority.

The effects of metformin on lipid metabolism as discussed in this chapter indicate that lipid level is positively affected in the intestine and liver leading to decreased LDL-C, plasma triglycerides and total cholesterol. Metformin effects on lipid metabolism seem to be localized to the intestine. Statins mainly act on plasma cholesterol levels via activation of the LDL-receptor suggesting that combination therapy should show an additional effect on plasma lipids. This may influence glucose homeostasis primarily by inhibition of insulin secretion in pancreatic β cells. T2DM patients receiving statin therapy in combination, with increased levels of cholesterol remnants and triglycerides were associated with slight decreased in left ventricular systolic function. Targeting cholesterol remnants in addition

to T2DM patients receiving statins might be shown beneficial effect on patient's cardiac function. To treat T2DM and its secondary complications, the combination therapy of metformin with statins seems well placed and may act as a double-sided sword particularly in the case of statins. Whereas, statins alone increases the risk on T2DM particularly in pre-diabetic subjects, and co-treatment with metformin might reduce this risk.

We have concluded that, previous studies investigated possible sites of interaction of metformin and statins and they act on largely parallel pathways. Many studies suggested that the benefits of statin therapy for diabetes far outweigh any real or perceived risks, not suggested/recommended for discontinuation of statins for diabetic patients. In conclusion, both metformin and atorvastatin can protect DCM via the mechanism of anti-inflammation and anti-apoptosis activities. The combined administration of metformin and atorvastatin resulted in superior protective effects on DCM than a single drug treatment. In this chapter, we have compiled the possible sites of interaction of metformin and statins and conclude that they act on largely parallel pathways.

Conflict of interest

The authors declare no conflict of interest among themselves.

Abbreviations

BA	bile acids
BMI	body mass index
CVD	cardiovascular disease
DCM	Diabetes cardiomyopathy
DI	disposition indices
DR	diabetic retinopathy
DM	Diabetes mellitus
EGP	endogenous glucose
FBG	Fasting blood glucose
FAS	fatty acid synthase
FFA	free fatty acid
GLUT	glucose transporter
HbA1c	glycated hemoglobin
HDL-C	high-density lipoprotein cholesterol
HMGCR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HMGCS	HMG-CoA synthase
IFG	impaired fasting glucose
IHD	Ischemic heart disease
LDL-C	low-density lipoprotein
NOM	Non onset diabetes
OCT	organic cation transporter
PDX	insulin promoter factor
PMAT	Plasma monoamine transporter
PXR	pregnane X receptor
RCT	Reverse cholesterol transport
RXR	retinoid X receptor
SERT	sodium-dependent serotonin transporter
TNF	Tumor necrosis factor

T2DM	type 2 diabetes mellitus
TG	triglycerides
THTR	thiamine transporter

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