Can combination therapy with insulin and metformin improve metabolic function of the liver, in type I diabetic patients? An animal model study on CYP2D1 activity

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Received: 7 June 2020 / Revised: 8 October 2020 / Accepted: 28 October 2020 / Published online: 3 November 2020
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Abstract

Introduction Changes in hepatic clearance and CYP2D1 activity after combination therapy with insulin and metformin in type-1 diabetes and insulin administration in type-2 diabetes was assessed in an animal model.

Methods Ten male Wistar rats were divided into two groups. Seven days after induction of diabetes, in treatment groups, type-1 diabetic rats received insulin plus metformin, and type-2 diabetic rats received insulin daily for 14 days. On day 21, rats were subjected to liver perfusion using Krebs-Henseleit buffer containing dextromethorphan as a CYP2D1 probe. Perfusate samples were analyzed by HPLC-FL.

Results The average metabolic rate of dextromethorphan and hepatic clearance changed from 0.012 ± 0.004 and 6.3 ± 0.1 ml/min in the control group to 0.006 ± 0.001 and 5.2 ± 0.2 ml/min in the untreated type-1 diabetic group, and 0.008 ± 0.003 and 5 ± 0.6 ml/min in the untreated type-2 diabetic rats [1]. In the present study, metabolic rate and hepatic clearance changed to 0.0112 ± 0.0008 and 6.2 ± 0.1 ml/min in the type-1 diabetic group treated with insulin plus metformin, and 0.0149 ± 0.0012 and 6.03 ± 0.06 ml/min in the insulin-receiving type-2 diabetic rats.

Conclusions Administration of insulin plus metformin in type-1 diabetes could modulate the function of CYP2D1 to the observed levels in the control group and made it clearer to predict the fate of drugs that are metabolized by this enzyme. Moreover, good glycemic control with insulin administration has a significant effect on the balance between hepatic clearance and CYP2D1 activity in type-2 diabetes.

Keywords CYP2D1 Phenoconversion · Type-1 Diabetes · Metformin · Isolated Perfused Liver (IPL)

Introduction

The expression and the activity of cytochrome P450s (CYP450) could be altered by many environmental factors, including medications, gender, smoking, and so on. One of the most important factors receiving special consideration is pathological conditions, such as diabetes, cancer, and hypertension [1–3]. On the other hand, the key role of inflammatory and oxidative stress processes in developing the secondary problems caused by diabetes has been shown in many studies [4–6]. Despite the essential role of the inflammatory system,

many clinical trials have failed to reduce the complications of these processes by concurrently administering a variety of antioxidants or vitamins [7–9].

The role of metformin as the first-line treatment for type-2 diabetes is widely accepted. Some of the benefits of using this drug in the early stages of the disease include the lack of weight gain, reduced risk of hypoglycemia, and insulin resistance [10]. In addition to the low risk of significant side-effects, the anti-atherosclerotic and cardioprotective effects of this drug, which reflect a set of independent glucose-lowering effects on the endothelium, have recently been confirmed in prospective and retrospective studies [11–13]. Wulfele M et al. claimed that metformin complements lifestyle management throughout the treatment of type-2 diabetes and forms a convenient pharmacological foundation for combinations therapy with other antidiabetic therapies, including insulin [14].

Our previous studies confirmed a reduction in rat CYP2D1 activity following type-1 and type-2 diabetes in animal

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models. Although metformin administration could balance enzyme activity in type-2 diabetes [2], And its effects in patients with type 2 diabetes are being studied [15], insulin therapy in type-1 diabetic rats could not modulate the enzyme activity to the observed levels in healthy control rats despite the good glycemic control [1].

All the reports above could confirm the positive effects of metformin on inflammatory processes and oxidative stress. Thus, this study was designed to investigate the benefits of metformin administration in modulating CYP activity in type-1 diabetes and further explain the related mechanism. Notably, the potential role of metformin administration, especially in type-1 diabetes, in combination with insulin therapy, is currently being investigated to evaluate any changes in the complications related to the inflammatory system. Meanwhile, to the best of our knowledge, this is the first study that is focused on the effects of metformin-insulin combination therapy in modulating liver enzyme activity in isolated perfused rat liver (IPRL) model [16].

It has been demonstrated that CYP2D1 is the rat orthologue of human CYP2D6 [17]. Considering the genetic and functional similarity of these enzymes, it seems the rat can be used as an appropriate animal model in early metabolic studies. The assessment of enzyme activity has also been confirmed in both human and animal studies concerning the changes in the metabolic rate of dextromethorphan as a reliable probe [18, 19]. Thus, this study mainly aimed to investigate the benefits of metformin administration in combination with insulin therapy to balance the CYP2D1 activity in type-1 diabetes in the IPRL model. Moreover, any changes in hepatic clearance in diabetic rats were assessed based on the inlet and outlet concentrations of dextromethorphan. Finally, and as a secondary indicator, blood glucose profiles were monitored in all groups.

Materials and methods

Materials and reagents Streptozocin injectable solution, was bought from thirteen Aban Pharmacy (Tehran, Iran). Nicotinamide was purchased from Merck (Darmstadt, Germany). The insulin NPH (Lansulin N®) was purchased from Exir Pharmaceutical Co. (Tehran, Iran). Metformin was purchased from thirteen Aban Pharmacy (Tehran, Iran). DM and its two metabolites, DXO (O-demethylated dextromethorphan) and HYM (N-demethylated dextromethorphan) as an Internal Standard (IS) were obtained from medicinal chemistry department, School of Pharmacy, Tehran University of Medical Science (Tehran, Iran) and their structures confirmed by H-NMR and CNMR in our laboratory. The HPLC (high performance liquid chromatography) grade acetonitrile and methanol were from Duksan Pure Chemicals Co.Ltd (Gyeonggi-do, Korea). Ultra-pure water was obtained from a Millipore Direct-Q™ system (Millipore

Corp., France) in our laboratory whenever it was necessary. All other analytical-grade chemicals were purchased from Merck (Darmstadt, Germany) unless otherwise stated.

Ten male Sprague–Dawley (SD) rats (weighing about 250–300 g) were housed under constant conditions with a 12-h day/night cycle and granted free access to water and rodent chow and randomly divided into two equal groups:

1. Type-1 diabetic rats receiving both insulin and metformin.
2. Type-2 diabetic rats receiving insulin.

On the first day of study, a single intraperitoneal (IP) dose of Streptozotocin (STZ), 65 mg/kg, was injected into the overnight fasted rats to induce type-1 diabetes. To induce type-2 diabetes, 110 mg/kg of Nicotinamide (NAM) was firstly administered, and after 15 min, 65 mg/kg of STZ was injected under the same conditions. To confirm the induction of both types of diabetes, fasting blood glucose (FBG) levels were checked. FBG levels greater than 400 mg/dL and 200 mg/dL after seven days of starting the study, were considered as an index of type-1 and type-2 diabetes induction in rats, respectively.

The type-1 diabetic rats were treated with simultaneous subcutaneous injection of 8 IU of isophane insulin and oral administration of metformin, 200 mg/kg once a day for 14 days. Type-2 diabetic rats were treated with daily subcutaneous administration of 6 IU insulin.

After three weeks of diabetes induction, the IPRL model was used to evaluate the hepatic CYP2D1 activity and hepatic clearance in designed animal groups. The results of the following groups were considered from the previous study [1] in our lab:

1. Control group of healthy rats.
2. Type-1 diabetic rats not receiving any treatment.
3. Type-1 diabetic rats receiving insulin.
4. Type-2 diabetic rats not receiving any treatment.
5. Type-2 diabetic rats receiving metformin.

This protocol was approved by the Institutional Review Board of Pharmaceutical Research Centre, Tehran University of Medical Sciences. The ethical approval code number was [IR.TUMS.TIPS.REC.1398.014].

Laboratory techniques

Isolated liver perfusion study The rats were anesthetized with injection of 4:1 mixture of ketamine 10% and xylazine 2% intraperitoneally. In order to perfusing of isolated liver, the portal vein and inferior vena cava were cannulated in all rats. The bile duct and superior vena cava was blocked. The perfusion medium, Krebs–Henseleit buffer containing DM (300 µM) as probe, was freshly prepared. The inferior vena cava was used as the outlet duct for sampling and the perfusate

samples (1 milliliter) were collected immediately after wash and then every 5 min, for the first 30 minutes and every 10 min, for the second 30 minutes and stored at -70°C until analysis after centrifugation. Liver transaminases activities (AST and ALT) were also continuously monitored by a spectrophotometric method used as a measure of liver viability as it has been described by Kebis, A. et al. [20].

Apparatus and chromatographic condition The HPLC fluorescence method was performed according to the same method and conditions was previously described by Lin et al., [21] with a slight modification: The chromatographic apparatus consisted of a low-pressure gradient HPLC pump coupled with a fluorescence detector (320 excitation- 285 emission nm), a 100 μlit loop, and a Rheodyne model 7725i injector, all from Knauer (Berlin, Germany). A ChromolithTM Performance RP-18e 100 mm $\times$ 4.6 column coupled with ChromolithTM guard cartridge RP- 18e 5 mm $\times$ 4.6 mm were applied for chromatographic separation (Merck, Darmstadt, Germany). A mixture of 20% methanol, 30% acetonitrile and 50% KH_2PO_4 buffer (10 mM, 0.02% of TEA, and pH 3.5) was employed as mobile phase and was passed through the system with a constant flow rate of 1 ml/min. Data acquisition and analyses were achieved by ChromGate chromatography software (Knauer, Berlin, Germany). Using this method, the retention time of dextrophan and dextromethorphan were measured to be 5.5 and 10 min, respectively.

Pharmacokinetic parameters

The metabolic rates were calculated using metabolite concentration divided by parent drug concentration at each sampling time point.

To determine the steady-state concentration of both metabolite and the parent drug, the mean concentration of each compound at three last sampling time points were considered.

Hepatic clearance of dextromethorphan was calculated using the following equation:

$$CL_{(\text{ml}/\text{min})} = E \times Q$$

Q is the perfusion flow rate, which was 8.3 ml/min.

The hepatic uptake (E) of dextromethorphan was calculated as follows:

$$E = 1 - F$$

The bioavailability (F) was considered as follows:

$$F = C_{\text{outlet}}/C_{\text{inlet}}$$

C_{inlet} and C_{outlet} are mean dextromethorphan concentrations in the last three sampling time points at the liver input and output, respectively.

Statistical analysis

All data, in this study, were expressed as mean $\pm$ SEM. An unpaired t-test was employed in this study using SPSS 16.0 for the determination of the differences between the means of groups ($p < 0.05$).

Results

The effects of type-1 diabetes on CYP2D1 activity and hepatic clearance parameters

The results of our previous study [1] showed that type-1 diabetes could remarkably reduce the activity of CYP2D1. Thus, the mean metabolic rates of dextromethorphan changed from 0.012 ± 0.004 in the control group to 0.006 ± 0.001 in the untreated type-1 diabetic rats ($p < 0.05$) (Fig. 1a). As it has been presumed, the FBG of diabetic rats (471 ± 32 mg/dl) significantly ($p < 0.001$) increased compared to the control group (75 ± 4 mg/dl) (Fig. 3a).

Although the metabolic rate increased after treatment, the improvement in enzyme activity was not enough to reach the observed levels in the control group despite the good glycemic control in insulin-receiving type-1 diabetic rats (0.008 ± 0.001 in treated rats vs. 0.012 ± 0.004 in the control group) ($p < 0.05$).

Diabetes had a similar effect on the hepatic clearance of dextromethorphan, where this parameter decreased significantly after the induction of type-1 diabetes (6.3 ± 0.1 ml/min vs. 5.2 ± 0.2 ml/min) ($p < 0.001$). Although hepatic clearance increased after insulin administration ($CL = 5.4 \pm 0.1$ ml/min), the enhancement was not enough to reach the observed levels in the control group ($p < 0.001$) (Fig. 2a).

The effects of insulin plus metformin administration on type-1 diabetic rats

CYP2D1 activity

A significant increase ($p < 0.01$) in CYP2D1 activity following 14 days of simultaneous insulin and metformin administration was observed in the treated group compared to the untreated type-1 diabetes, where the metabolic rate increased from 0.006 ± 0.001 in the untreated diabetic rats to 0.0112 ± 0.0008 in the group receiving treatment (Fig. 1a). The average FBG in this group significantly decreased to 93 ± 3 mg/dl compared to untreated type-1 diabetic rats (471 ± 32 mg/dl) ($p < 0.001$).

Fig. 1 Perfusate mean metabolic ratio profile (3 endpoints) in insulin received type 1 diabetic group [1] and insulin plus metformin received type 1 diabetic group compared to both control healthy group and untreated type 1 diabetic rats, following the passage of the perfusion buffer containing 300 μ M dextromethorphan through the portal vein (mean $\pm$ SD) (a). Perfusate mean metabolic ratio profile (3 endpoints) in metformin received type 2 diabetic group and insulin [1] received type 2 diabetic group compared to both control healthy group and untreated type 2 diabetic rats, following the passage of the perfusion buffer containing 300 μ M dextromethorphan through the portal vein (mean $\pm$ SD) (b). Each group contains 5 rats. Each experiment was repeated independently three times in triplicate tests and data are shown as mean $\pm$ SD. * $P \leq 0.05$; ** $P \leq 0.01$

Hepatic clearance

The hepatic clearance increased significantly in this group (CL = 6.2 ± 0.1 ml/min) compared to the untreated diabetic rats (CL = 5.2 ± 0.2 ml/min) ($p < 0.01$) (Fig. 2a), where the clearance was comparable to the level observed in the healthy control rats ($p < 0.05$).

The effects of type-2 diabetes on CYP2D1 activity and hepatic clearance parameters

In our previous study [1], the FBG levels in untreated rats confirmed the induction of type-2 diabetes (269 ± 17 mg/dl in induced type-2 diabetes vs. 75 ± 4 mg/dl in the healthy control group) (Fig. 3b). The results demonstrated that the activity of CYP2D1 reduced after the induction of type-2 diabetes in a way that the mean metabolic rates changed from 0.012 ± 0.004 in the healthy rats to 0.008 ± 0.003 in the untreated type-2 diabetic rats; however, this reduction was not statistically significant ($p > 0.05$) (Fig. 1b). In contrast to the results of insulin therapy in type-1 diabetes, good

glycemic control through metformin administration significantly increased the enzyme activity such that the metabolic rate increased from 0.008 ± 0.003 in the untreated type-2 diabetic rats to 0.013 ± 0.003 in the group receiving treatment (Fig. 1b).

The hepatic clearance decreased in type-2 diabetic rats compared to the control group (5 ± 0.6 ml/min vs. 6.3 ± 0.01 ml/min) ($p < 0.01$). It also increased in the metformin-receiving group compared to untreated type-2 diabetic rats (CL = 6.1 ± 0.4 ml/min). This increase was enough to reach the observed levels in the control group ($p > 0.05$) (Fig. 2b).

The effects of Insulin administration on type-2 diabetic rats

CYP2D1 activity

A significant increase ($p < 0.01$) in enzyme activity following 14 days of insulin therapy was observed in the treated group compared to the untreated diabetic rats, where the metabolic rate increased from 0.008 ± 0.003 to 0.0149 ± 0.0012

Fig. 2 Clearance profile (based on 3 endpoints of dextromethorphan concentrations) in insulin-received type 1 diabetic group [1] and insulin plus metformin-received type 1 diabetic group compared to both control healthy group and untreated type 1 diabetic rats, following the passage of the perfusion buffer containing 300 μ M dextromethorphan through the portal vein (mean $\pm$ SD) (a). Clearance profile (based on 3 endpoints of dextromethorphan concentrations) metformin-received type 2 diabetic group [1] and insulin-received type 2 diabetic group compared to both control healthy group and untreated type 2 diabetic rats, following the passage of the perfusion buffer containing 300 μ M dextromethorphan through the portal vein (mean $\pm$ SD) (b). Each group contains 5 rats. Data are shown as mean $\pm$ SD. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

(Fig. 1b). The FBG level in the insulin-receiving group significantly decreased compared to the untreated diabetic rats (106 ± 5 mg/dl vs. 269 ± 17 mg/dl) ($p < 0.05$) (Fig. 3b).

Hepatic clearance

The hepatic clearance increased in insulin-receiving type-2 diabetic rats compared to the untreated group (6.03 ± 0.06 ml/min vs. 5 ± 0.6 ml/min), and this increase was enough to reach the observed levels in the control group ($p > 0.05$) (Fig. 2b).

Discussion

In the modern medicine, most of patients take various medications on a daily basis to treat one or more diseases [22]. Increased number of drug-drug interactions has been the

certain result of these drug combinations [23]. Many diseases particularly those involving inflammatory processes cause one or more physiological and biochemical alterations in absorption, distribution, metabolism, and elimination (ADME) of medicines [24]. So, there are several studies that evaluate the effects of chronic inflammatory disease like diabetes mellitus on the biotransformation capacity of diabetic animals and patients with diabetes mellitus [2, 15, 25].

The metabolism process of drug mainly happens in the liver followed by the important organs of intestine and kidneys as well as some additional organs with less significant roles (e.g., blood, skin, and brain) [26, 27]. According to the biotransformation type undertaken, drug-metabolizing enzymes are categorized as two groups of phase 1 and phase 2 enzymes [28]. Cytochrome P450 (CYP) enzyme family is considered among the most important typical phase 1 oxidative enzymes. As the predominant catalysts, the CYP family

Fig. 3 Fasting Blood Glucose (mg/dl). FBG of untreated type 1 diabetic group, insulin [1] and insulin plus metformin receiving groups in comparison with control healthy rats (a). FBG of untreated type 2 diabetic group, insulin and metformin [1] receiving groups in comparison with control healthy rats (b). Each experiment was repeated independently three times in triplicate tests and data are shown as mean $\pm$ SD. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

of enzymes serve/act in biotransformation pathway or elimination process for the most cases of marketed drugs [29].

Our previous study [1] showed a significant reduction in CYP2D1 activity following the induction of type-1 diabetes compared to the control group ($p < 0.05$) based on the mean metabolic rates of the last three sampling points of liver perfusion. As expected, FBG level decreased significantly ($p < 0.001$) after insulin therapy; however, this increase was not sufficient to reach the observed levels in healthy control rats ($p < 0.05$) despite an indication of significant modulation of enzyme activity in rats compared to the untreated group [1].

Like the changes observed in enzyme activity, the induction of type-1 diabetes significantly reduced the hepatic clearance of dextromethorphan compared to the control group ($p < 0.001$). On the contrary, insulin therapy failed to modulate it [1]. There are two possible explanations for the inefficiency of insulin therapy in balancing enzymatic function and hepatic clearance despite good glycemic control. First, human insulin used may stimulate the immune system, thereby increasing rather than reducing inflammatory cytokines. To test the

hypothesis, a group was added to the present study in which a lower dose of insulin was administered to type-2 diabetic rats. The results showed that both metformin [1] and insulin could effectively modulate the enzyme activity. Therefore, human insulin appears to have no significant effect on the results of the study. Notably, in both groups of type-2 diabetic rats treated with metformin [1] and insulin, FBG levels were effectively controlled, possibly contributing to selecting the right dose in this study.

In other words, it has been demonstrated that glycemic control plays an essential role in reducing the complications of diabetes by reducing vascular complications. The results of this study also confirmed the importance of glycemic control in modulating the activity of liver enzymes.

It should be noted that metformin administration in type-2 diabetic rats [1] showed a more significant effect on the hepatic clearance of dextromethorphan than insulin therapy. Thus, there was no significant difference with the control group after metformin administration. The benefits of this compound in this area should be further considered in future studies.

Despite good glycemic control after insulin therapy in type-1 diabetic rats, the modulation of these two pharmacokinetic parameters to the normal value was not observed [1]. This observation may be explained by varying degrees of inflammation caused by these two types of diabetes. Good glycemic control alone seems to fail to return the enzyme activity due to the advanced inflammatory conditions as well as higher vascular complications in type-1 diabetes compared to type-2 diabetes mellitus. To evaluate this explanation, both enzyme activity and hepatic clearance factors were measured after adding an adjuvant anti-inflammatory drug, such as metformin, to type-1 diabetes regimens. It should be noted that the addition of other anti-inflammatory drugs, such as NSAIDs or corticosteroids, is not recommended because of the increased risk of adverse effects. However, the positive effect of metformin administration in type-1 diabetic patients is currently being investigated and has been proven in some cases such as lower BMI and reduced insulin requirements, TC and LDL-c according to a literature review [30–32].

Surprisingly, combination therapy with insulin and metformin in type-1 diabetic rats modulated both the CYP2D1 activity and hepatic clearance to the observed levels in the healthy control group ($p > 0.05$). The results indicated the possible positive effects of metformin on liver function in type-1 diabetic patients, necessitating further studies in this field. In other words, changes in enzyme activity are likely due to both glycemic and inflammatory conditions. Therefore, continuous monitoring of enzyme activity in these patients is necessary to improve personalized drug therapy.

Conclusions

In a nutshell, the results of this study revealed the possibility of a reduction in cytochrome 2D1 activity and hepatic clearance due to type-1 diabetes mellitus. Combination therapy with insulin and metformin can modulate the activity of CYP2D1 to the observed levels in the control group (healthy rats), making it more predictable to treat diabetes mellitus and levels of other drugs this enzyme is involved in their biotransformation. Overall, the results of this study indicated the need for further studies on the possible changes in the activity of liver enzymes induced by diabetes, as well as the benefits of using metformin in this field.

Acknowledgements This work was fully supported by a grant from *National Institute for Medical Research Development of Iran (NIMAD)* (grant no. 957596). The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

Compliance with ethical standards

Conflicts of interest The authors report no conflicts of interest.

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