European Journal of Pharmaceutical Sciences 68 (2015) 18–26

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Inhibitory effect of atenolol on urinary excretion of metformin via down-regulating multidrug and toxin extrusion protein 1 (rMate1) expression in the kidney of rats

Yan-rong Ma [a,b], Jing Huang [a,b], Yun-yun Shao [a,b], Kang Ma [a,b], Guo-qiang Zhang [a], Yan Zhou [a], Zhi Rao [a], Hong-yan Qin [a], Xin-an Wu [a,*]

[a] Department of Pharmacy, the First Hospital of Lanzhou University, Lanzhou 730000, China
[b] School of Pharmacy, Lanzhou University, Lanzhou 730000, China

ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form 10 October 2014
Accepted 1 December 2014
Available online 5 December 2014

Keywords:
Metformin
Atenolol
rMate1
rOct2
Urinary excretion

ABSTRACT

Renal tubular secretion is an important pathway for the elimination of many clinically used drugs. Metformin, a commonly prescribed first-line antidiabetic drug, is secreted primarily by the renal tubule. Many patients with type 2 diabetes mellitus (T2DM) receiving metformin may together be given selective β1 blockers (e.g., atenolol). Therefore, it is of great use to evaluate the effect of atenolol on metformin urinary excretion for exploring drug interactions and predicting the adverse effect of drugs. The aim of this study was to investigate the effect of atenolol on the pharmacokinetic of metformin and plasma lactate (LCA) level in rats, for high LCA is a serious adverse reaction of metformin after long-term metformin treatment. In this study, rats were treated with metformin alone or in combination with atenolol. Plasma, urine and tissue concentration of metformin was determined by HPLC method, while Western blotting and immunohistochemical analysis were used to evaluate the renal expression of rat organic cation transporter 2 (rOct2) and multidrug and toxin extrusion protein 1 (rMate1). The results showed that, after 7 days drug treatment, the $AUC_{0\to t}$ of metformin in atenolol and metformin co-administration group was significantly increased by 19.5% compared to that in metformin group, while the 24 h cumulative urinary excretion of metformin was significantly decreased by 57.3%. In addition, atenolol treatment significantly decreased the renal expression of rMate1, but had no effect on rOct2 expression, renal blood perfusion and glomerular filtration. Moreover, plasma LCA level in atenolol and metformin co-administration group was significantly increased by 83.3% compared to that in metformin group after 60 days drug treatment. These results indicated that atenolol can inhibit urinary excretion of metformin via decreasing renal rMate1 expression, and long-term atenolol and metformin co-administration may induce potential lactic acidosis. Our results, for the first time, provided an important experimental evidence that rMate1 is the target of transporter-mediated drug interactions concerning metformin and atenolol.

1. Introduction

Metformin, a biguanide derivative, is one of the first-line drugs for the treatment of type 2 diabetes mellitus (T2DM), and it is often used alone or in combination with other drugs to control blood glucose level (Quaile et al., 2010). Although the mechanism that is responsible for blood glucose improvement is not well understood, metformin, to some extent, decrease hepatic glucose production and improve insulin sensitivity by increasing the uptake and utilization of peripheral glucose (Li et al., 2011; Ou et al., 2006). The main adverse effects of metformin involve gastrointestinal intolerance and lethal lactic acidosis (Scheen, 2011; Scheen and Paquot, 2013). In vivo, metformin is not metabolized by hepatic cytochrome P450 (Tornio et al., 2012) and excreted into urine via active renal tubular secretion (Scheen, 1996).

The epidemiological studies have shown that the prevalence of hypertension in T2DM is higher than that in the general population. It was reported that around 40% of patients with T2DM suffered from hypertensive at the age of 45, and the proportion was further increased to 60% by the age of 75 (Hu et al., 2005; UK Prospective Diabetes Study Group, 1998). Therefore, it is very likely that a large number of patients who received metformin may together be given anti-hypertensive agents, such as atenolol, in

* Corresponding author. Tel./fax: +86 931 8616392.
E-mail address: xinanwu6511@163.com (X.-a. Wu).

http://dx.doi.org/10.1016/j.ejps.2014.12.002
0928-0987/

clinical practice (Zaman Huri and Fun Wee, 2013). Atenolol is one of the most widely used blockers of β1 receptor, and it mainly acts on the heart by competing the sites of β1 receptor on the cardiac muscle (Wadworth et al., 1991). Atenolol could slow down the strength of heart contractions, reduce oxygen consumption and reduce the volume of blood flow, and it is often used for the treatment of hypertension (Damiani, 2011).

Kidney plays a vital role in the urinary excretion of drugs and their metabolites via glomerular filtration and/or tubular secretion. Rat organic cation transporter 2 (rOct2/*slc22a2*) is highly expressed on the basolateral membrane of proximal tubules, and mediates uptake of organic cations from the blood to the proximal tubular cells (Koepsell, 2004; Koepsell and Endou, 2004). It is notable that, at the brush-border membranes of the proximal tubules, rat multidrug and toxin extrusion protein 1 (rMate1/*slc47a1*) was found to mediate the extrusion of organic cations from cells into the tubular lumen by using transmembrane H^+ gradient as driving force, which is considered to be responsible for the final step of urinary excretion of cationic drugs (Yonezawa and Inui, 2011). Nowadays, it is well accepted that rMate1 cooperates with the rOct2 for the renal secretion of cationic drugs in the proximal tubular epithelium (Omote et al., 2006; Otsuka et al., 2005; Terada and Inui, 2008).

Transporter-mediated drug interactions has aroused much attention in recent years, previous studies showed that the effect of transporter-mediated drug interactions involves inhibitory effect, inductive effect, or even both (Kaliszczak et al., 2013; Konig et al., 2013; Limtrakul et al., 2005; Muller and Fromm, 2011). We all known that transporters can be inhibited in a competitive manner, namely, when two substrates compete the same binding site in which only one substrate can be bound, competitive effect will be occurred (Mandery et al., 2012; Perez et al., 2009). It has been reported that metformin is a substrate of OCT2 and MATE and mainly renally cleared (Graham et al., 2011; Staud et al., 2013; Takane et al., 2008). β1-blockers such as metoprolol and bisoprolol are found to be the potential substrate or inhibitor of renal OCT2 (Bachmakov et al., 2009; Belzer et al., 2013; Zolk et al., 2009). However, it is not clear whether transporter-mediated drug interactions may occur when metformin and atenolol are both eliminated through kidney, and whether there is a competitive effect on the renal excretion of metformin and/or atenolol. To our knowledge, there is no study to provide information about the interaction of metformin and atenolol concerning renal transporter. In view of the important role of metformin in blood glucose level and the critical effect of atenolol on blood pressure, the potential effect of transporter-mediated drug interactions is of great importance for clinical drug safety.

In this present study, we investigated the effect of atenolol on urinary excretion, plasma concentration and tissue distribution of metformin in rats. To further investigate the transporter-mediated drug interactions, the renal expression of rOct2 and rMate1 was evaluated by Western blot and immunohistochemical technique after drugs administration.

2. Methods and materials

2.1. Materials

Metformin (97% purity) and atenolol (99% purity) were both obtained from Sigma–Aldrich (St Louis, MO, USA). Norethindrone and diazepam (internal standard) were purchased from the Nation Institute for the Control of pharmaceutical and Biological Products (Beijing, China). Methanol was of high performance liquid chromatography (HPLC)-grade (Fisher Scientific, NJ, USA). All other reagents and solvents were of analytical grade and were commercially available.

2.2. Animals

Male Wistar rats aged 8 weeks with body weigh about 180–220 g, were obtained from the Experimental Animal Center of Lanzhou University (Lanzhou, China). Rats were housed in plastic cages and maintained at 25 °C under 12–12 h alternating light–dark cycle with free access to food and water. All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Study design

The first series of experiments aimed to investigate the effect of atenolol on pharmacokinetic profile of metformin. Rats were divided into two groups ($n = 8$). Rats in co-administration group were orally treated with atenolol (20 mg/kg) plus metformin (100 mg/kg) once a day for successive 7 days, while rats in metformin group were treated with metformin (100 mg/kg) for 7 days. After the final drug administration, serial blood samples (0.25 mL) were collected through the right side of the femoral artery with heparinized syringes at 0, 10, 20, 40, 60, 90, 120, 180, 240, 360, 480, and 720 min post metformin, respectively. For the intravenous pharmacokinetic study, rats in co-administration group were orally administered atenolol (20 mg/kg) plus metformin (100 mg/kg) once a day for 6 days, while rats in metformin group were treated with metformin (100 mg/kg). On day 7, rats in co-administration group were orally given atenolol (20 mg/kg) plus metformin (25 mg/kg) intravenous administration, and rats in metformin group were given metformin (25 mg/kg) by intravenous injection. Blood samples were collected at 2, 5, 10, 20, 30, 60, 90, 120, 240, and 480 min post metformin, respectively. Plasma concentration of metformin was determined by HPLC, and the pharmacokinetic parameters were calculated using DAS2.0 program.

The second series of experiment aimed to test the effect of atenolol on renal blood flow and glomerular filtration rate. Rats were divided into two groups ($n = 8$), the groups setting and drug treatment was as same as that of orally pharmacokinetic study. After 7 days drug treatment, blood samples were collected from orbital at 2 h post metformin for the determination of creatinine concentration by LC–MS/MS (Agilent 6460, Agilent Technology Inc., CA, USA). In addition, renal blood flow and glomerular filtration rate were determined using Single-Photon Emission Computed Tomography (SPECT, Infinia VC Hawkeye 4 SPECT–CT, GE Medical Systems, WI, USA)

The third series of experiment aimed to investigate the effect of long-term drug administration on biochemical parameters as well as plasma concentration, tissue distribution, and urinary excretion of metformin. The groups setting and drug administration were as same as that of orally pharmacokinetic study, but drugs were administered for 60 days. On day 1, 3, 7, 15, 30, and 60 post drug treatment, and blood samples were collected from orbital at 2 h post metformin, urine samples were collected during the interval of 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, and 12–24 h post metformin. For tissue distribution study and biochemical analysis, rats that had drugs for 60 days were used. All the rats were given metformin, and then sacrificed at 2 h post metformin. Blood were collected for the determination of LCA, glucose (GLU), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. After blood sample collection, the residual blood in the body was removed by saline cardiac perfusion, and then tissues, i.e., heart, liver, brain, lung, kidney and small intestine, were dissected, weighed and homogenized in saline for metformin determination.