Effect of raw Radix *Rehmanniae* on the pharmacokinetics of pioglitazone in rats

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Abstract: Raw Radix *Rehmanniae* (RRR) is a frequently used traditional Chinese medicine in the treatment of diabetes mellitus according to the statistics on all of the anti-diabetic formulas recorded in New National Traditional Chinese Medicine. Pioglitazone and RRR may be co-administrated for presumably enhanced therapeutic effects because of the common indications. Therefore, the aim of the study was to evaluate the effect of RRR on the pharmacokinetics of pioglitazone in healthy rats and type 2 diabetic rats. The pharmacokinetic effect of RRR on pioglitazone was studied in healthy rats and type 2 diabetic rats. A validated UPLC-MS/MS method was used to analyze the concentration of pioglitazone in blood samples. The pharmacokinetic parameters were calculated using non-compartmental analyses by Winnonlin 5.0.1. In healthy group, the pre-treatment of RRR significantly (P<0.05) reduced the C_{max} but enhanced the V/F of pioglitazone; whereas in T2DM group, significant increase of C_{max} and decrease of V/F and $T_{1/2}$ were found after the rats were pre-treated with RRR. However, $AUC_{(0-t)}$ and CL/F remained unchanged in both healthy group and T2DM group. In conclusion, co-administration with RRR could alter the pharmacokinetic profiles of pioglitazone to statistically significant levels.

Keywords: Pioglitazone, raw Radix *Rehmanniae*, herb-drug interactions, pharmacokinetics, type 2 diabetes mellitus.

INTRODUCTION

Pioglitazone, an agent enhancing insulin sensitivity, has been used either as a monotherapy or in combination with other anti-diabetic drugs in the treatment of type 2 diabetic mellitus (T2DM) (Yuan *et al*., 2012). Although an association with the potential risk of bladder cancer was found, pioglitazone is still used clinically for its significant efficacy, especially after its first generic version of Actos tablets has been approved by the FDA recently (FDA, 2012). Chemical medicines are dominant in the treatment of T2DM, but herbal preparations are often used as an add-on therapy. Raw Radix *Rehmanniae* (RRR) is a frequently used traditional Chinese medicine in the treatment of diabetes mellitus. The statistics on New National Traditional Chinese Medicine (Song and Yang, 2002) shows that RRR is employed in 29 formulas among all the 52 anti-diabetic formulas recorded. Pioglitazone and RRR may be co-administrated for presumably enhanced therapeutic effects because of the common indications.

Pioglitazone is mainly metabolized by CYP2C8, 3A4, and 2C9 in human to several active and inactive metabolites (Gillies and Dunn, 2000), it was speculated that the combined usage may cause an increment in its plasma levels thus raise safety concerns. A previously published work has shown that quercetin, a CYP3A inhibitor, may increase the bioavailability of pioglitazone (Umathe *et al*., 2008). A recent study showed that RRR was also metabolized mainly by CYP2C9 and CYP3A4 (Or *et al*., 2012), rationally the presence of RRR may pose a potential competitive effect on the pharmacokinetics or pharmacodynamics of pioglitazone. Therefore, the aim of this study was to evaluate the effect of RRR on the pharmacokinetics of pioglitazone in both healthy and T2DM rats.

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MATERIALS AND METHODS

Materials

Streptozotocin (STZ) and nicotinamide were purchased from Sigma Chemical Company Inc. (St Louis, MO). RRR granules were produced by Guangdong Yifang Pharmaceutical Co., Ltd (Guangdong, China, batch No.1104067); 1 g granules were refined from 5 g RR crude drug. RRR granules was dissolved in distilled water and orally given to mice.

Instrumentation and analytical conditions

Quantitative analysis of pioglitazone in rat plasma was carried out using a UPLC-MS/MS method developed and validated in our laboratory (Yuan *et al*., 2012). Briefly, after a liquid-liquid extraction process with ethyl acetate as the extracting solvent, plasma samples were subjected to UPLC-MS/MS analysis to generate the concentration of pioglitazone with rosiglitazone serving as the IS. The mobile phase consisted of 30% acetonitrile and 70% aqueous buffer (5 mM ammonium acetate and 0.1% formic acid). An isocratic elution lasting 2.5 min was obtained at a flow rate of 0.3 mL/min. The detection system was a tandem quadrupole mass spectrometer operating in the positive ion mode. The precursors to

product ions for analytes in multiple-reaction monitoring (MRM) are as follows: pioglitazone, 357.1→133.8; rosiglitazone, 358.2→134.8. Pioglitazone possessed good linearity within the concentration range 4.4-4400 ng/mL.

The development of type 2 diabetic model in rats and pharmacokinetic experiments

Healthy male Wistar rats (180-200 g body weight) were supplied by Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China). All experiments were conformed to the Chinese regulations on protection of experimental animals and approved by the Animal Ethical Committee of Sun Yat-sen University. Type 2 diabetic model was developed in overnight fasted rats by an intraperitoneal injection of 110 mg/kg nicotinamide, followed by another intraperitoneal injection of 65 mg/kg STZ within 15 mins. STZ was dissolved in 0.1M citrate buffer (pH 4.4) and nicotinamide was dissolved in saline. The glucose levels in plasma were determined at 72 h and on Day 7 after injection. Rats with fasting plasma glucose higher than 200 mg/dl were designated as Type 2 diabetic model rats and used for further study (Masiello *et al*., 1998).

A total of 24 experimental rats were divided into four groups: Healthy-control group (saline treated healthy rats), Healthy-RRR group (healthy rats administered orally with RRR 7.8 g/kg daily for 14 days), T2DM-control group (saline treated diabetic model rats), T2DM-RRR group (diabetic model rats administered orally with RRR 7.8 g/kg daily for 14 days) (Chinese Pharmacopoeia Commission 2010).

At the end of the 14 days, the experiment rats were fasted overnight prior to pharmacokinetic study and were orally given a single dose of 1.5 mg/kg pioglitazone (equalling to the dose of 15 mg per day human takes) dispersed in 5 g/l CMC-Na solution. Blood samples were collected at 0.083, 0.5, 1, 1.5, 2, 2.5, 3.5, 5, 8, 12, 24 and 36 hour post-dose. 25 µl plasma was separated immediately from each collection and stored at -20ºC until analysis. The pharmacokinetic parameters were estimated using non-compartmental analyses by Winnonlin 5.0.1. Statistical analysis was conducted using the t-test and $P<0.05$ was considered to be statistically significant.

RESULTS

Concentration-time curves of pioglitazone following oral dosing of 1.5 mg/kg to healthy rats and T2DM rats in the presence or absence of RRR are shown in fig. 1. The pharmacokinetic parameters for pioglitazone are summarized in table 1.

In healthy groups, the pre-treatment of RRR significantly ($P<0.05$) reduced the C_{max} but enhanced the V/F of pioglitazone, whereas in T2DM groups, significant increase of C_{max} and decrease of V/F and $T_{1/2}$ were found after the rats were pre-treated with RRR. However, $AUC_{(0-t)}$ and CL/F remained unchanged in both healthy group and T2DM group.

DISCUSSION

The present study showed that the combined use of RRR significantly decreased plasma level of pioglitazone in healthy rats despited a steady AUC which indicated a decrease of pioglitazone absorption and metabolism. However, the pharmacokinetic change in T2DM group was quite a reverse. Significant increase of C_{max} and decrease of V/F was found after diabetic rats were pretreated with RRR, yet AUC once again remain steady compared to T2DM-control group. Additionally, the $T_{1/2}$ in T2DM-RRR group was also reduced significantly which reflected a better elimination. The results indicate the coadministration of RRR increases the rate and the extent of absorption but not the bioavailability of pioglitazone in rats; however, it did seem to alter the pharmacokinetics of pioglitazone to clinically significant levels because such variations would lead to toxicity concentrations or subtherapeutic concentrations and it will be difficult to maintain the drug plasma concentration within the therapeutic window.

Table 1: Pharmacokinetic parameters following oral administration of pioglitazone in the four experimental groups

Parameter	Healthy		T2DM	
	Healthy-control	Healthy-RRR	T2DM-control	T2DM-RRR
$T_{1/2}$ (hr)	3.2±0.2	3.7±0.7	2.7±0.4	1.9±0.1[b]
T_{max} (hr)	1.8±0.4	2.0±0.5	1.6±0.6	1.2±0.3
C_{max} (ng/ml)	3575.1±217.2	2894.2±325.1[a]	1934.3±391.0.	2742.9±362.9[b]
Cl/F (ml/min)	15.9±1.9	14.4±1.4	31.3±7.1	31.2±6.7
V/F (ml)	66.3±4.3	78.1±11.0[a]	116.6±13.7	82.6±19.0[b]
AUC (ng/ml·hr)	22032.1±2322.2	21539.8±2439.8	10476.4.0±2415.0	10658.1±2253.7

Data are expressed as mean ± SD (n=6).
[a]$p<0.05$, significant difference compared with Healthy-control. [b]$p<0.05$, significant difference compared with T2DM-control.

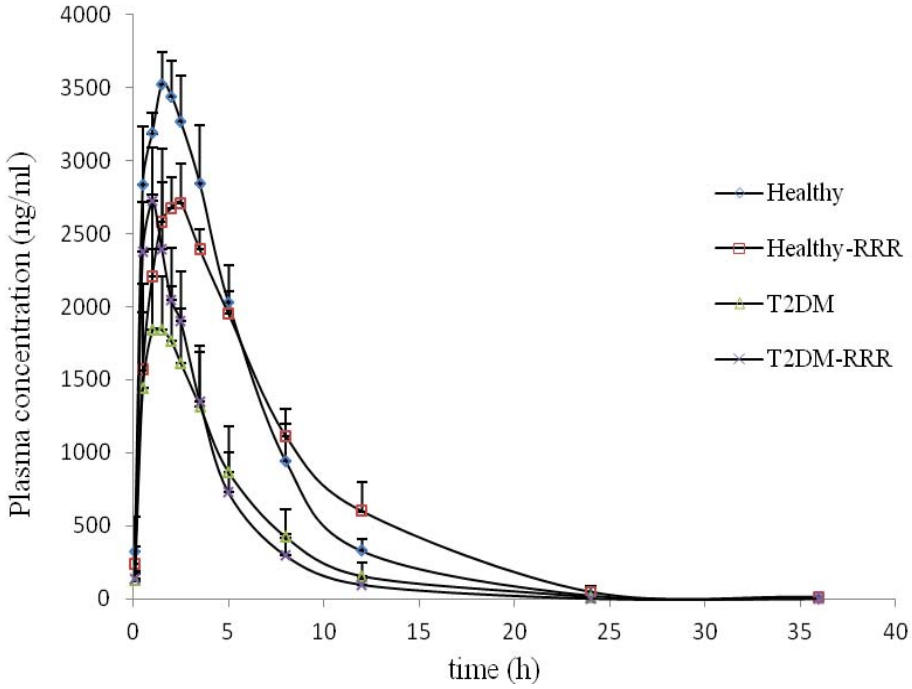

Fig. 1: Plasma concentration-time curves of pioglitazone following oral dosing of 1.5 mg/kg to healthy rats and T2DM rats in the presence or absence of RRR

It has been known that diseases may affect the pharmacokinetic profiles of a drug through different ways, the different results in healthy group and T2DM group may have several causes. On one hand, it was a reflection of the complicate effects of traditional Chinese medicine which contains various components; on the other hand, neither pioglitazone nor RRR is metabolised by a single enzyme, the complex mechanism of the results may also involve conversion of the common metabolic pathway.

In conclusion, the study showed that RRR could alter the pharmacokinetic profiles of pioglitazone to statistically significant levels. However, further confirmation of the finding is still required.

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