Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 28 No. 2, 132-138, 2015
http://dx.doi.org/10.5806/AST.2015.28.2.132

Comparison of rosiglitazone metabolite profiles in rat plasma between intraperitoneal and oral administration and identifcation of a novel metabolite by liquid chromatography-triple time of flight mass spectrometry

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(Received August 18, 2014; Revised March 21, 2015; Accepted March 23, 2015)

액체크로마토그라피-삼중비행시간질량분석기를 사용한 rosiglitazone의 복강 및 경구투여 후 대사체 비교 분석

박민호[1] · 나숙희[2] · 이희주[2] · 신병희[3] · 안병준[3] · 신영근[1,★]

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(2014. 8. 18. 접수, 2015. 3. 21. 수정, 2015. 3. 23. 승인)

Abstract: Rosiglitazone metabolites in rat plasma were analyzed after intraperitoneal and oral administration to rats. Seven metabolites (M1-M7) were detected in rat plasma (IP and PO), and the structures were confirmed using liquid chromatography-triple time of flight (TOF) mass spectrometry; as a result, the most abundant metabolite was M5, a de-methylated rosiglitazone. Other minor in vivo metabolites were driven from mono-oxygenation and demethylation (M2), thiazolidinedione ring-opening (M1, M3), mono-oxygenation (M4, M7), and mono-oxygenation followed by sulfation (M6). Among them, M1 was found to be a 3-{*p*-[2-(*N*-methyl-*N*-2-pyridylamino)ethoxy]phenyl}-2-(methylsulfinyl)propionamide, which is a novel metabolite of rosiglitazone. There was no significant difference in the metabolic profiles resulting from the two administrations. The findings of this study provide the first comparison of circulating metabolite profiles of rosiglitazone in rat after IP and PO administration and a novel metabolite of rosiglitazone in rat plasma.

Key words: Rosiglitazone, Triple time of flight mass spectrometer, Metabolic profile

1. Introduction

Rosiglitazone is one of the thiazolidinedione class compounds which act as ligands for the gamma-subtype peroxisome proliferator-activated receptor and is involved in the glucose and lipid metabolism.[1,2]

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The first generation thiazolidinedione drug such as troglitazone was withdrawn from the market due to severe liver injuries.[3,4] Although the mechanism of liver injuries is not fully understood, it is expected that formation of reactive metabolites appears to be one of the possible mechanisms of liver toxicity.[5] Several researchers revealed the major metabolic pathway of troglitazone is CYP450 3A4-mediated oxidation of the chromane moiety to a quinone followed by reduction and sulfation.[6] Other minor pathways such as ring opening were also found from troglitazone. GSH-formation is the unique indication of reactive metabolites formation and several reports described the detection of GSH adducts of troglitazone in human hepatic microsomes.[6]

Other thiazolidinedione compounds such as rosiglitazone and pioglitazone were also developed with reduced liver toxicity.[7] However, pioglitazone is also subject to the similar metabolic pathways of troglitazone by CYP450 3A4 and 2C8 and generated ring-opening reactive metabolites in vitro.[7] Rosiglitazone is also predominantly metabolized by CYP450s to N-demethylated rosiglitazone and hydroxylated rosiglitazones in human.[8] GSH adducts of rosiglitazone were also reported from in vitro studies with human liver microsomes fortified with GSH and monitored by neutral loss scan of 129 using electrospray positive ionization mode.[7] Therefore, metabolic activation of thiazolidinedione by CYP450s appears to be the one of the possible formation mechanisms of reactive metabolites which could possibly contribute to the side effects including liver toxicity.

First-pass effect is a general term which refers to the metabolic reduction of a compound prior to reaching the general circulation. In orally-administered compounds, these metabolic processes generally take place in the intestine and the liver. Compounds administered via i.p. injection, via inhalation, and transdermally, *are also subject to* some degree of first-pass/pre-systemic metabolism and depending on the structures of compounds, the circulating metabolites after non-oral administration could be different from those after oral administration. Ali *et al.* demonstrated single dose pharmacokinetics, bioavailability and metabolism of glucosamine in rat after intraperitoneal and oral administration.[9]

Triple TOF mass spectrometer is a new state-of-the-art high resolution instrument with faster scan speed, decent sensitivity and linearity for both qualitative and quantitative analysis. Triple TOF mass spectrometer also offers mass defect filtering (MDF) function as well as information dependent acquisition (IDA) function to search novel metabolites in vivo and in vitro.

We explore the metabolism of rosiglitazone in rat plasma by two different routes, intraperitoneal and oral administration and compare the metabolites in plasma collected by two different routes. We also report a novel metabolite of rosiglitazone which appears to be generated by CYP450 mediated thiazolidinedione ring-opening mechanism of rosiglitazone. The results of this study provide the first comparison of circulating metabolite profiles of rosiglitazone in rat after IP and PO administration and a novel metabolite of rosiglitazone in rat plasma.

2. Experimental

2.1. Materials

Rosiglitazone (*Fig.* 1) was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (ACN) and HPLC grade water were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (minimum of 95%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical grade or better and were used prior to their respective expiration dates.

2.2. Animal study and sample preparation

SD rats (~250 g) were purchased from Samtako (Chungbuk, Korea). Animals were quarantined for a

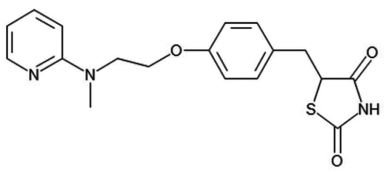

Fig. 1. Structure of rosiglitazone.

minimum of 7 days prior to treatment and maintained on a 12-h light/dark cycle. The animals were fasted overnight prior to administration of the dose. Water was provided *ad libitum* to the animals. All studies were approved by the institutional animal care and use committee, Chungnam National University.

A group of six SD rats was housed individually in stainless steel cages. Among them, a single dose rosiglitazone was orally administered to three rats at a target dose level of 10 mg/kg. The dose was formulated in phosphate buffer solution (pH 2.3) at a target concentration of 2.0 mg/mL. Another single dose of rosiglitazone was intraperitoneally administered to the remaining three rats at the same target dose level. The dose was formulated in phosphate buffer solution (pH 2.3) at a target concentration of 2.0 mg/mL.

Plasma was pooled using Hamilton's method across all animals per group at the collected time points (i.e., 15 min, 30 min, 1 h, 1.5 h, 2 h and 3 h).[10] Pooled plasma (110 μL) and acetonitrile (220 μL) were combined, vortex-mixed (5 min), and followed by centrifugation at 10,000 rpm for 5 min at 4 °C. One hundred microliter of supernatant was collected and mixed with 200 μL of water before LC-MS analysis.

2.3. Liquid chromatography-mass spectrometry

The liquid chromatography-mass spectrometry system consisted of two Shimadzu LC-20AD pumps, a Shimadzu CBM-20A HPLC pump controller (Shimadzu Corporation, Columbia, MD, USA), a CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC, USA) and an AB Sciex 5600 tripleTOF® mass spectrometer (AB Sciex, Foster City, CA, USA). The analytical column used for this assay was a Phenomenex C18 column, 2.1×50 mm (2.6 μm). The mobile phase consisted of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was 0.3 mL/min. The HPLC gradient was held at 5% B for 0.5 min, increased to 50% B at 25.5 min, to 95% B at 25.6 min, and then to 95% B at 27 min, decreased to 5% B at 27.1 min and equilibrated for 7.9 min. The injection volume was 10 μL. Analyst TF® Version 1.6 (AB Sciex) operated with Windows® (Microsoft) was used for instrument control, data acquisition and data analysis.

The AB Sciex 5600 TripleTOF® mass spectrometer was operated in the positive ion mode using a Duospray™ ion source. High purity nitrogen gas was used for the nebulizer/Duospray™ and curtain gases. The source temperature was set at 400 °C with a curtain gas flow of 30 L/min. The ion spray voltage was set at 5500 V, declustering potential was 93 V and the collision energy was 35 V for rosiglitazone and its metabolites. Full TOF scan (range from *m/z* 100~1000) followed by information dependent acquisition (IDA) using mass defect filtering with triggering criteria of 100 mDa was used. Additional IDA scans using low (CE=10 V) and high (CE=45 V) collision energy to search for GSH adducts were also conducted. MetabolitePilot® was used to process the data of rosiglitazone and its metabolites.

3. Results and Discussion

3.1. Metabolic profiles

In order to directly compare in vivo metabolites

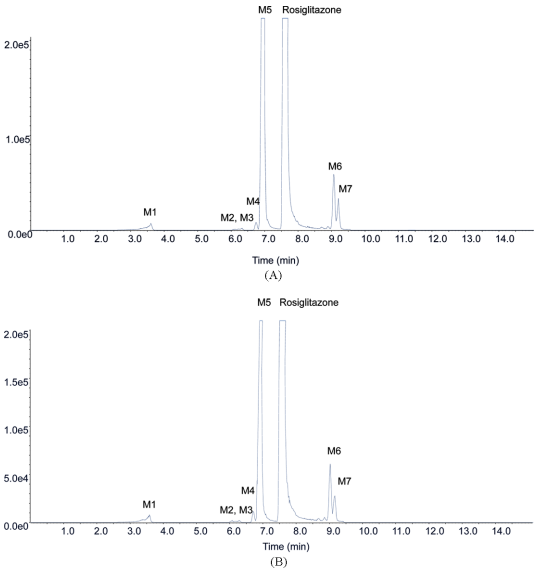

Fig. 2. Representative TOF MS scan chromatograms of rosiglitazone metabolites in PO plasma (A) and IP plasma (B).

difference of rosiglitazone in rat plasma between IP and PO administration, pooled plasma samples per each group were extracted using protein precipitation method. Representative total ion scan chromatograms of oral (PO) plasma and intraperitoneal (IP) plasma are shown in *Fig*. 2. The relative percentages of metabolite peak response were obtained by integration of the respective peaks (data not shown).

Approximately seven metabolites were detected in the circulation from rat plasma. The majority of circulating entities was attributed to unchanged parent rosiglitazone (70%) followed by M5 (25%). The remaining metabolites each represented less than 5% of total peak response. Apparently metabolite profiling in rat plasma between IP and PO administration were quite similar.

3.2. Metabolite identification and characterization

The structures of metabolites were mainly elucidated by comparing the high resolution collision-induced dissociation (CID) product ion spectra of metabolites with that of rosiglitazone.

3.2.1. Rosiglitazone

Rosiglitazone had a retention time of 7.6 min on HPLC and a protonated molecular ion at m/z 358 (accurate mass: 358.1234; error: 3.9 ppm). Its product ion spectrum showed diagnostic fragment ions at m/z 107.0631, 119.0599 and 135.0906 (*Fig*. 3). The

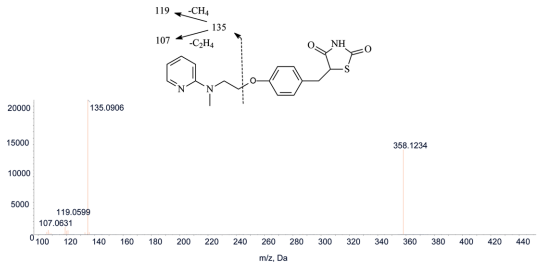

Fig. 3. Product ion mass spectrum of rosiglitazone.

ions at m/z 135.0906 was formed via cleavage of the 5-(4-hydroxybenzyl)-2,4-thiazolidinedione moiety from the ion at m/z 358 and two other ions at m/z 107.0734 and 119.0599 were formed via neutral loss of ethylene or methane from the ion at m/z 135.0906, respectively, and were confirmed by accurate mass measurement. The diagnostic fragment ions are listed in *Table* 1.

3.2.2. Metabolite M1

Metabolite M1 was observed in rat IP and PO plasma. It had a retention time of 3.6 min on HPLC and a protonated molecular ion at m/z 362 (accurate mass: 362.1540; calculated elemental composition: $C_{18}H_{23}N_3O_3S_1$; error: 1.9 ppm) which is 4 daltons (Da) higher than rosiglitazone. Its product ion spectrum showed diagnostic fragment ions at m/z 107.0611, 119.0604, 135.0904, 255.1474 and 298.1536 (*Table* 1). Similar to those of parent drug, the product ions at m/z 107.0611, 119.0604 and 135.0904 suggested that the N-methyl-(pyridinylamino)ethoxy moiety was

Table 1. Characterization of rosiglitazone metabolites in rat plasma after a single oral or intraperitoneal administration of rosiglitazone (10 mg/kg)

Metabolite	Retention time (min)	[M+H]+	Elemental composition	Error (ppm)	Product ions
Rosiglitazone	7.6	358.1234	C18H19N3O3S1	3.9	107.0631, 119.0599, 135.0906
M1	3.6	362.1540	C18H23N3O3S1	1.9	107.0611, 119.0604, 135.0904, 255.1474, 298.1536
M2	6.1	360.1021	C17H17N3O4S1	2.3	121.0726, 255.1106, 300.1305
M3	6.3	376.1330	C18H21N3O4S1	1.2	107.0579, 119.0572, 135.0886, 287.1164, 333.1220
M4	6.7	374.1176	C18H19N3O4S1	1.8	107.0590, 119.0573, 135.0886, 269.1249, 314.1458
M5	6.9	344.1072	C17H17N3O3S1	2.5	121.0755
M6	9.1	454.0748	C18H19N3O7S2	2.5	135.0506, 151.0858, 374.1153
M7	9.3	374.1183	C18H19N3O4S1	3.6	135.0508, 151.0832

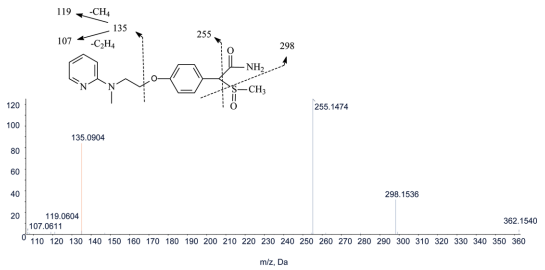

Fig. 4. Product ion mass spectrum of M1.

unchanged and the modification was due to modification of the thiazolidinedione moiety of parent drug. Unlike rosiglitazone, two unique product ions at m/z 255.1474 and 298.1536 indicated ring-opening of thiazolidinedione moiety (*Fig*. 4). In order to elucidate the metabolic pathway of this unique metabolite, several thiazolidinedione compounds were examined and we confirmed that one of the metabolites of Rivoglitazone was also generated by the same metabolic pathway.[11]

On the basis of these data, M1 was proposed to be 3-{*p*-[2-(*N*-methyl-*N*-2-pyridylamino)ethoxy]phenyl}-2-(methylsulfinyl)propionamide, which is a novel metabolite of rosiglitazone.

3.2.3. Metabolite M2

Metabolite M2 was observed in rat IP and PO plasma. It had a retention time of 6.1 min on HPLC and a protonated molecular ion at m/z 360 (accurate mass: 360.1021, calculated elemental composition: $C_{17}H_{17}N_3O_4S_1$; error: 2.3 ppm), 2 Da higher than the parent compound. Its product ion spectrum showed diagnostic fragment ions at m/z 121.0726, 255.1106 and 300.1305 (*Table* 1). The ions at m/z 121.0726 suggested that the N-demethylation was occurred on the N-methyl-(pyridinylamino)ethoxy but overall mass increase by 2 daltons indicated mono-oxygenation on the other side of the structure. On the basis of these data, M2 was identified as a mono-oxygenation and demethylation metabolite.

3.2.4. Metabolite M3

Metabolite M3 was observed in rat IP and PO plasma. It had a retention time of 6.3 min on HPLC and a protonated molecular ion at m/z 376 (accurate mass: 376.1330, calculated elemental composition: $C_{18}H_{21}N_3O_4S_1$; error: 1.2 ppm), 18 Da higher than the parent compound. Its product ion spectrum showed diagnostic fragment ions at m/z 107.0579, 119.0572, 135.0886, 287.1164 and 333.1220 (*Table* I). Similar to those of parent drug, the ions at m/z 107.0579, 119.0572, 135.0886 suggested that the N-methyl-(pyridinylamino)ethoxy moiety was unchanged and the modification was due to hydrolysis of the

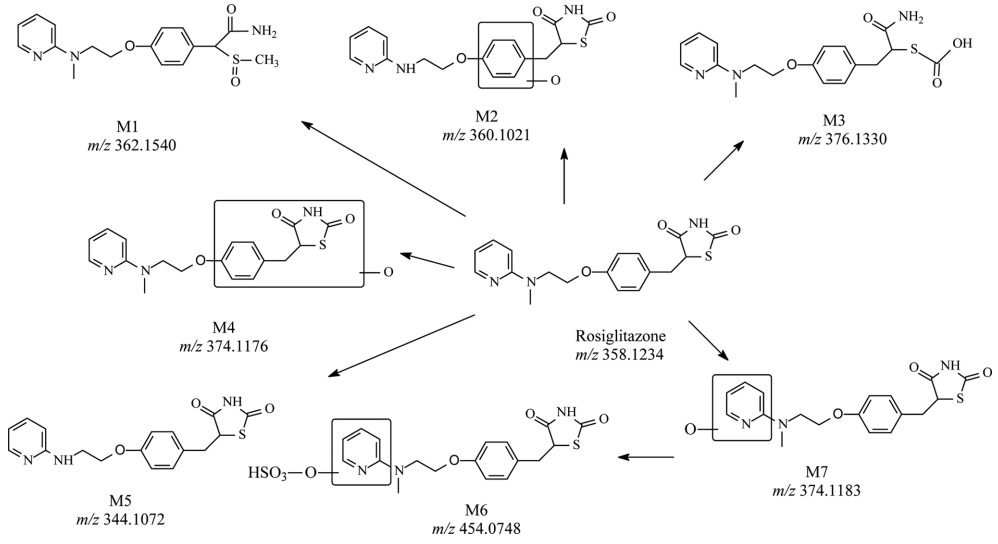

Fig. 5. Proposed metabolic pathways of rosiglitazone in rat plasma.

thiazolidinedione moiety of parent drug. On the basis of these data, M3 was identified as a thiazolidinedione-ring hydrolysis metabolite which appears to be an intermediate metabolite of M1.

3.2.5. Metabolite M4, M6 and M7

Metabolite M4 and M7 were observed in rat IP and PO plasma. These had retention times at 6.7 min and 9.3 min on HPLC and the protonated molecular ions at m/z 374 (accurate mass: 374.1176 (M4) and 374.1183 (M7), calculated elemental composition: $C_{18}H_{19}N_3O_4S_1$; error: 1.8 and 3.6 ppm), 16 Da higher than the parent compound. The product ion spectrum of M4 showed diagnostic fragment ions at m/z 107.0590, 119.0573, 135.0886, 269.1249 and 314.1458, whereas that of M7 showed 135.0508 and 151.0832 (*Table* 1). The ion at m/z 135.0886 of M4 clearly suggested that the N-methyl-(pyridinylamino)ethoxy moiety was unchanged and the modification was due to mono-oxygenation of the benzene ring or thiazolidinedione moiety of the parent drug. However, the ion at m/z 151.0832 of M7 indicated the site of mono-oxygenation of M7 was on the pyridinylamino-ethoxy moiety. Metabolite M6 was observed in rat IP and PO plasma with a retention time of 9.1 min on HPLC and a protonated molecular ion at m/z 454 (accurate mass: 454.0748, calculated elemental composition: $C_{18}H_{19}N_3O_7S_2$; error ppm: 2.5 ppm), 96 Da higher than the parent compound. Its product ion spectrum showed diagnostic fragment ions at m/z 135.0506, 151.0858, and 374.1153 (*Table* 1). On the basis of these data, M6 was identified as mono-oxygenation and sulfation metabolite.

3.2.6. Metabolite M5

Metabolite M5 was observed in rat IP and PO plasma. It had a retention time of 6.9 min on HPLC and a protonated molecular ion at m/z 344 (accurate mass: 344.1072; calculated elemental composition: $C_{17}H_{17}N_3O_3S_1$; error: 2.5 ppm), 14 Da lower than the parent compound. Its product ion spectrum showed diagnostic fragment ions at m/z 121.0755 and no peak was observed at m/z 135 (*Table* 1). Similar to those of parent drug, the ions at m/z 121.0755 suggested that the modification was due to loss of the N-methyl moiety of parent drug. On the basis of these data, M5 was identified as N-demethylated rosiglitazone.

4. Conclusions

In summary, the results of this study provided the first comparison of circulating metabolite profiles of rosiglitazone in rat after IP and PO administration. On the basis of metabolite profiling and the structures of metabolites, major metabolic pathways of rosiglitazone in rat appear to involve de-methylation (M5). Other minor in vivo metabolites were generated by mono-oxygenation and de-methylation (M2), thiazolidinedione ring-opening (M1, M3), mono-oxygenation (M4, M7), mono-oxygenation followed by sulfation (M6). Among them, M1 was proposed to be a 3-{*p*-[2-(*N*-methyl-*N*-2-pyridylamino)ethoxy]phenyl}-2-(methyl-sulfinyl)propionamide, which is a novel metabolite of rosiglitazone.

The metabolic profiles between two different administrations were similar and no significant difference was observed. The results of this study provide the first comparison of circulating metabolite profiles of rosiglitazone in rat after IP and PO administration and a novel metabolite of rosiglitazone in rat plasma. Metabolic pathway of M1 metabolite from rosiglitazone is still under investigation but we anticipate that rosiglitazone would also follow the similar thiazolidinedione ring-opened methyl sulfoxide amide pathway of rivoglitazone.[11] The thiazolidinedione ring opened hydrolysis metabolite would also support the hypothesis of M1 metabolic pathway and to our best knowledge, this is the first report as a rosiglitazone metabolite.

Acknowledgements

This work was supported by research fund of Chungnam National University.

References

1. J. M. Lehmann, L. B. Moore, T. A. Smitholiver, W. O.

Wilkison, T. M. Willson and S. A. Kliewer, *J. Biol. Chem.,* **270**(22), 12953-12956 (1995).
2. A. R. Saltiel and J. M. Olefsky, *Diabetes,* **45**(12), 1661-1669 (1996).
3. J. Kohlroser, J. Mathai, J. Reichheld, B. F. Banner and H. L. Bonkovsky, *Am. J. Gastroenterol,* **95**(1), 272-276 (2000).
4. K. V. N. Menon, P. Angulo and K. D. Lindor, *Am. J. Gastroenterol,* **96**(5), 1631-1634 (2001).
5. R. Alvarez-Sanchez, F. Montavon, T. Hartung and A. Pahler, *Chem. Res. Toxicol.,* **19**(8), 1106-1116 (2006).
6. S. L. Pearson, M. A. Cawthorne, J. C. Clapham, S. J. Dunmore, S. D. Holmes, G. B. T. Moore, S. A. Smith and M. Tadayyon, *Bioche. Biophys. Res. Commun.,* **229**(3), 752-757 (1996).
7. R. Alvarez-Sanchez, F. Montavon, T. Hartung and A. Pahler, *Chem. Res. Toxicol.,* **19**(8), 1106-1116 (2006).
8. P. J. Cox, D. A. Ryan, F. J. Hollis, A. M. Harris, A. K. Miller, M. Vousden and H. Cowley, *Drug Metab. Disposit.,* **28**(7), 772-780 (2000).
9. A. Aghazadeh-Habashi, A. Ibrahim, J. Carran, T. Anastassiades and F. Jamali, *J. Pharm. Sci.,* **9**(3), 359-364 (2006).
10. C. E. C. A. Hop, Z. Wang, Q. Chen and G. Kwei, *J. Pharm. Sci.,* **87**(7), 901-903 (1998).
11. M. Uchiyama, H. Iwabuchi, F. Tsuruta, K. Abe, M. Takahashi, H. Koda, M. Oguchi, O. Okazaki and T. Izumi, *Drug Metab. Disposi.,* **39**(4), 653-666 (2011).