Kinetics of the Absorption, Distribution, Metabolism, and Excretion of Lobeglitazone, a Novel Activator of Peroxisome Proliferator-Activated Receptor Gamma in Rats

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ABSTRACT: This study was performed to determine biopharmaceutical properties of lobeglitazone (LB), a novel thiazolidinedione-based activator of peroxisome proliferator-activated receptor gamma, in rats. In parallel artificial membrane permeability assay and Madin–Darby canine kidney (MDCK) cell permeability assays of LB, the activator was found to interact with multidrug-resistance protein 1 (MDR1) and OATP1B1. The concentration resulting in 50% inhibition value for LB in MDR1 expressing MDCK cells was approximately 12.5 ± 3.61 μM. LB had adequate stability (i.e., 56% remaining at 0.5 h) in rat liver microsomes. A cytochrome P450 (CYP) inhibitory potency study indicated that LB is primarily interacted with CYP1A2, 2C9, and 2C19. In rats, LB appeared to be readily absorbed after an oral administration (an absolute bioavailability of ~95%). Following intravenous administration, LB exhibited linear pharmacokinetics in the dose range of 0.5–2 mg/kg. The primary distribution site was the liver but it was also distributed to heart, lungs, and fat tissue. The excretion of LB to the urine, bile, feces, and intestine was insignificant (i.e., <10% of the dose) in rats. These observations suggest that, despite the fact that it interacts with some drug transporters and metabolizing enzymes, the pharmacokinetics of LB were linear with a high oral bioavailability. J Pharm Sci 104:3049–3059, 2015
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INTRODUCTION

Lobeglitazone (LB), an activator of thiazolidinedione-peroxisome proliferator-activated receptor gamma (TZD-PPAR-γ), is a new drug that is under development for use in the treatment of diabetes in Korea. The compound is reported to have at least a 1.11- and 16.6-fold higher affinity for PPAR-γ in comparison to pioglitazone and rosiglitazone (i.e., clinically used TZD-PPAR activators), respectively. As a result, it is expected that LB would have a lower effective dose and reduce cardiovascular side effects (i.e., common for TZD-PPAR activators), provided that the kinetic properties of LB are comparable to the other PPARs activators. Unfortunately, however, a comprehensive pharmacokinetic study of TZD-PPAR activators, including LB, has not been reported in the literature.

In the case of the biopharmaceutical properties of TZD-PPAR activators, the metabolic characteristics are relatively well understood. For example, although pioglitazone and rosiglitazone are primarily eliminated via hepatic metabolism, the major cytochrome P450 (CYP) isozyme for their elimination is different (i.e., CYP2C8/3A4 for pioglitazone; CYP2C8/2C9 for rosiglitazone). As the activators, including pioglitazone and rosiglitazone, contain a common TZD moiety but with different side chains, this structural difference appears to be the main determinant of the heterogeneity in metabolic outcome. TZD-PPAR activators are relatively hydrophobic [i.e., partition coefficient (log *P*) of 2.95, 3.17, and 4.16 for rosiglitazone, pioglitazone, and troglitazone, respectively; the values were determined using the ALOGPS software] and their extent of absorption is relatively high (95%, 83%, and 50% for rosiglitazone, pioglitazone, and troglitazone, respectively).[1–3] The involvement of drug transporters in their pharmacokinetics has not been systemically studied in the case of TZD-PPAR activators.

The objective of this study was to characterize the biopharmaceutical properties of LB. Although such properties have been shown to be important in understanding the pharmacokinetics, the information may also be closely linked to clinically relevant issues (e.g., drug–drug interaction) in the later stages of the development of a new drug. We were particularly interested in the *in vitro* properties of the metabolism and transport of LB, and their relationship to pharmacokinetics, as such information may have a direct consequence in the elucidation/prediction of drug interactions. Our findings indicate that, despite its interaction with transporters and metabolizing

Abbreviations used: LB, lobeglitazone; PAMPA, parallel artificial membrane permeability assay; MDCK, Madin–Darby canine kidney; MDR1, multidrug-resistance protein 1; OATP, organic anion transporting polypeptide; OAT, organic anion transporter; OCT, organic cation transporter; BCRP, breast cancer-resistance protein; IC_{50}, concentration resulting in 50% inhibition; CYP, cytochrome P450; TZD-PPAR, thiazolidinedione-peroxisome proliferator-activated receptor; log *P*, partition coefficient; HPLC, high-performance liquid chromatography; DMSO, dimethyl sulfoxide; TEER, transepithelial electrical resistance; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid; LC–MS/MS, liquid chromatography–tandem mass spectrometry; CO_2, carbon dioxide; NADPH, nicotinamide adenine dinucleotide phosphate oxidase; DMEM, Dulbecco's modified Eagle's medium; RED, rapid equilibrium dialysis.

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enzymes, LB exhibited linear kinetics with a nearly complete bioavailability.

MATERIALS AND METHODS

Chemicals and Reagents

Lobeglitazone (98.5% purity), LB sulfate (99.1% purity), and rosiglitazone [99.0% purity, an internal standard (IS) of LB assay] were provided by Chong Kun Dang Pharmaceuticals (Seoul, Korea). Acetonitrile [high-performance liquid chromatography (HPLC) grade] and formic acid were obtained from J. T. Baker (Phillipsburg, New Jersey) and from Fluka (Cambridge, Massachusetts), respectively. Other chemicals, including testosterone and 7-hydroxy coumarin, were purchased from Sigma–Aldrich (St. Louis, Missouri). Pooled rat liver microsomes and uridine diphosphate (UDP) reaction mix solution were purchased from Corning Gentest (Woburn, Massachusetts). Solvents were of HPLC grade (Fisher Scientific, Pittsburgh, Pennsylvania), and other chemicals were of the highest grade available.

In Vitro Absorption, Distribution, and Elimination Studies

Parallel Artificial Membrane Permeability Assay

To estimate the intestinal permeability of LB via diffusional transport, parallel artificial membrane permeability assay (PAMPA) was carried out following the standard procedure.[4,5] Briefly, a dodecane solution containing phosphatidyl choline (20 mg/mL) was added to the membrane (multiscreen PAMPA assay plate, #MAIP-N4550; millipore, Bedford, Massachusetts) of the insert. Phosphate-buffered saline (PBS) containing LB (200 μg/mL) in 15% dimethyl sulfoxide (DMSO) and 15% polyethylene glycol (PEG) 400, or LB sulfate (100 μg/mL) in 10% DMSO, was added to the well before artificial membrane material had evaporated (<10 min). The reaction was initiated by inserting the donor plate into the acceptor plate, and allowing the process to proceed at room temperature. When necessary, the permeability of verapamil (i.e., the high-permeability marker for this assay) was measured in parallel. The acceptor buffer was collected at 16 h after the initiation and the concentration was measured using an HPLC–UV spectrophotometer (Waters e2695, 2489, Waters, Milford, Massachusetts). The mobile phase involved isocratic condition of 0.1% formic acid–acetonitrile (70:30, v/v) at a flow rate of 1 mL/min at 25°C. After 50 μL of samples were injected onto a reverse-phase HPLC column (Kinetex XB-C18, 150 mm × 4.6 mm, 5 μm; Phenomenex, Torrance, California), the eluent from the column was monitored at a wavelength of 290 nm. The apparent permeability of each compound was calculated using the standard equation.[4]

Permeability Study in Madin–Darby Canine Kidney II Cells

Madin–Darby canine kidney II-wild type (MDCKII-WT) and MDCKII–multidrug-resistance protein 1 (MDR1) cell lines were generously provided by Dr. Borst (The Netherlands Cancer Institute, Amsterdam, The Netherlands). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Welgene Inc., Daegu, Korea) containing 10% fetal bovine serum (FBS; Welgene Inc.), 1% nonessential amino acid solution, 100 units/mL penicillin, and 0.1 mg/mL streptomycin under a humidified atmosphere of air containing 5% carbon dioxide (CO_2) at 37°C. Collagen-coated 12 mm Transwell (Costar, Corning, New York) was incubated with medium at 37°C for 1 h to improve cell attachment. Cells were seeded at a density of 2.5 × 10^5 cells/well and the medium was replaced at 2-day intervals. Bidirectional transport experiments were performed on 5 days after seeding. The confluence of the cell monolayer and the integrity of tight junction were confirmed by microscope and measurement of transepithelial electrical resistance (TEER; 130–180 Ω), respectively. Apical or basolateral chambers were washed twice and preincubated with the transport buffer [25 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose (pH7.4)] at 37°C for 30 min.

For the inhibition study, the basolateral to apical transport of LB was measured in the presence and the absence of verapamil (i.e., inhibitor). In this assay, cells were preincubated with the transport buffer containing verapamil (500 μM) for 15 min. The transport buffer containing only LB (i.e., 5 μM) or LB with the inhibitor was added to the donor chamber (500 μL for apical chamber and 1.5 mL for basolateral chamber) and the drug-free transport buffer was placed in the receiver chamber and the cells were then incubated at 37°C. The final concentration of the organic solvent (e.g., DMSO) in the transport buffer was below 1% in all experiments. Aliquots were collected from the receiver chamber at 30, 60, 90, and 120 min, and the solution was replenished with the same volume of fresh transport buffer. Samples were stored at −80°C until the analysis. LB was quantified using liquid chromatography–tandem mass spectrometry (LC–MS/MS).[6] The apparent permeability coefficient (P_{app}, cm/s) was calculated by using the equation below:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A} \times \frac{1}{C_0}$$

where dQ/dt is the transport rate, A is the surface area of the membrane, and C_0 is the initial concentration of LB in the donor side. When necessary, the net efflux ratio was also determined using the equation below using the apparent permeability from the basolateral to apical direction ($P_{app,\ B\ to\ A}$) and the apparent permeability from the apical to basolateral direction ($P_{app,\ A\ to\ B}$):

$$\text{Efflux ratio} = \frac{P_{app,B\ to\ A}}{P_{app,A\ to\ B}}$$

Interaction of LB with Carrier-Mediated Transports in MDCK Cells Expressing Organic Anion Transporting Polypeptide 1B1, Organic Anion Transporting Polypeptide 1B3, Organic Anion Transporter 1, Organic Anion Transporter 3, Organic Cation Transporter 2, and Breast Cancer-Resistance Protein

To determine the interaction of LB with major transporters, organic anion transporting polypeptide 1B1 (OATP1B1), OATP1B3, organic anion transporter 1 (OAT1), OAT3, and organic cation transporter 2 (OCT2) were cloned and functionally expressed in MDCK cells containing Flip-In system (Invitrogen, Carlsbad, California) (Supplementary Information). In addition, MDCKII–breast cancer-resistance protein (BCRP) cells, generously provided by Dr. Borst in the Netherland Cancer Institute, were also used. Cells were typically cultured in DMEM (Hyclone, Thermo Scientific, Rockford, Illinois) containing 10% FBS, 1% nonessential amino acids, 100 units/mL penicillin, 0.1 mg/mL streptomycin, 40 μg/mL gentamicin, and