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Development of safety profile evaluating pharmacokinetics, pharmacodynamics and toxicity of a combination of pioglitazone and olmesartan medoxomil in Wistar albino rats

Pinaki Sengupta, Utpal Nandi, Tapan Kumar Pal *

Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India

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ABSTRACT

Pioglitazone (PIO), an antidiabetic drug and olmesartan medoxomil (OLM), an antihypertensive drug were administered orally alone and in combination to Wistar albino rats for evaluation of pharmacokinetics, pharmacodynamics and repeated dose 28-day oral toxicity of individual drugs and their combination. Pharmacokinetic study was performed by orally administering PIO and OLM at single dose of 3 and 2 mg/kg, respectively alone and in combination analyzing the plasma samples using LC–MS/MS. Antidiabetic activity evaluation was done in type-2 diabetes mellitus induced animals at same dose level as in pharmacokinetic study daily for 30 days. PIO and/or OLM were administered orally to animals at daily doses of 50, 100 and 150 mg/kg for 28 days for toxicity study. There was no significant alteration in the pharmacokinetic parameters of either drug indicating absence of any pharmacokinetic interaction when co-administered. Positive pharmacodynamic interaction between PIO and OLM was established in this study. Two drugs in combination showed no evidence of potentiation of 28-day repeated dose toxicity in animals. Again, drugs, alone and in combination, caused only minor changes in clinical-laboratory tests and histopathological change was not found in the experiment performed. In conclusion, PIO and OLM combination can primarily be stated as safe in terms of present toxicity and pharmacokinetics findings.

1. Introduction

Hypertension is an important risk factor for cardiovascular mortality in diabetic patients. Patients with type-2 diabetes have a high prevalence of hypertension and show an elevated incidence of cardiovascular events and nephropathy (Zanchetti and Ruilope, 2002). Hypertension is twice as common in persons with diabetes as it is in others (Epstein and Sowers, 1992). Elevated blood pressure is known to contribute to diabetic microvascular and macrovascular complications (Bakris et al., 2000; UK Prospective Diabetes Study Group, 1998). Fortunately, reduction in blood pressure can decrease the risk of these complications. Angiotensin II receptor blockers shows promising treatment of hypertension in diabetic patients. In general, only 25% of patients with hypertension have adequate control of their blood pressure (The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure, 1997). Goals to decrease blood pressure are thus more difficult to achieve, in patients who also have diabetes.

Pioglitazone (PIO) [(±)-5-[*p*-[2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2,4-thiazolidinedione] (Lakshmi et al., 2009) is a peroxisome proliferator-activated receptor-γ agonist that enhances the action of insulin mainly by promoting glucose utilization in peripheral tissues and suppressing gluconeogenesis in the liver (Spiegelman, 1998; Miyazaki et al., 2001). It is a relatively new member of the thiazolidinedione class, improves hyperglycemia, reduces hyperinsulinemia, and ameliorates hypertriglyceridemia in a variety of insulin-resistant animal models of impaired glucose tolerance (Sengupta et al., 2009). Diabetes is strongly associated with kidney damage in addition to hypertension. First clinically detectable sign of renal disease is microalbuminuria which is a predictor for nephropathy as well as cardiovascular disease (Mogensen, 2002). Studies have shown that angiotensin II receptor antagonists have renoprotective effects in diabetes (Brenner et al., 2001; Lewis et al., 2001) and can slow the progression of microalbuminuria (Parving et al., 2001).

Abbreviations: LC–MS/MS, liquid chromatography–mass spectroscopy/mass spectroscopy; PIO, pioglitazone; OLM, olmesartan medoxomil; IS, Internal Standard; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; IAEC, Institutional Animal Ethics Committee; FBG, fasting blood glucose; RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; Rt, reticulocytes; WBC, white blood cell; TP, total protein; BUN, blood urea nitrogen; SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxaloacetate transaminase; ALP, alkaline phosphatase; AUC_{0-t}, mean area under the plasma concentration time curve from time zero to time t; $AUC_{0-\infty}$, area under the plasma concentration time curve from time zero to infinity; C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}; K_{el}, rate of elimination; $t_{1/2}$, plasma elimination half life.

* Corresponding author. Fax: +91 33 24146186.
E-mail address: proftkpal@gmail.com (T.K. Pal).

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Olmesartan medoxomil (OLM), an ester prodrug, which is rapidly converted in vivo to its active metabolite, olmesartan has a major role in treating the patients with type-2 diabetes with increased risk of cardiovascular disease (Brunner, 2002). Treatment of such patients by olmesartan medoxomil has been shown to reduce renal vascular resistance and oxidative stress, and increase renal perfusion (Fliser et al., 2005). Such effects may translate into beneficial long-term renoprotective effects. Olmesartan medoxomil [(5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-nethylethyl)-2-propyl-1-{4-[2-tetrazol-5-yl)-phenyl]phenyl}methylimidazol-5-carboxylate] is one of the newer angiotensin II receptor blockers dose dependently reduces blood pressure and provides nephroprotective effect in diabetes-induced nephropathy (Yanagisawa et al., 1996). It is the latest angiotensin II receptor blocker approved for use in the United State. The advantages of this drug include once-daily dosing, an absence of significant adverse reactions, a well-tolerated side-effect profile, and a cost-effective average wholesale price (Daryl et al., 2002).

Choice of anti-hypertensive drug in diabetic hypertensive patients is influenced by many factors including the possibility or risk of pharmacokinetic and pharmacodynamic drug–drug interactions. To understand such potential, pharmacokinetic drug interaction and toxicity study should be conducted following concomitant administration of both drugs. Again, in order to establish the safety and efficacy of a new combination of two existing drugs, it is necessary to evaluate their drug–drug interaction as well as toxicity in experimental animals. Data from such studies in laboratory animals helps in deciding whether the new combination is suitable for further study in human volunteer.

Though there is reported pharmacokinetic, pharmacodynamic and toxicity/safety study of either pioglitazone (Yukiyoshi et al., 2003; Berria et al., 2007; Spiegelman, 1998; Miyazaki et al., 2001) or olmesartan (Ogata et al., 2004; Yamagishi et al., 2005) separately or with other drugs, this is first time to report the pharmacokinetics, pharmacodynamics and toxicity of these two drugs in combination in any type of biological system. Current study was conducted to determine their single dose oral pharmacokinetics, antidiabetic potential when co-administered and to assess whether the toxicity of pioglitazone in combination with olmesartan medoxomil was additive, synergistic, or abrogated in rats following repeated dose 28-day oral toxicity study in Wistar albino rats.

2. Materials and methods

2.1. Chemicals

Olmesartan medoxomil (purity >98%) (Fig. 1A), pioglitazone (purity >99%) (Fig. 1B) and rosiglitazone (Internal Standard; IS) (>98%) were obtained from Burgeon Pharmaceuticals (Chennai, India). Streptozotocin and nicotinamide was bought from Sigma Aldrich (Germany) and Ranbaxy Chemicals Ltd. (Mumbai), respectively. Formic acid, ethyl acetate, isopropyl alcohol (analytical-reagent grade) and methanol (HPLC-grade) were purchased from Merck Pvt. Ltd. (Mumbai, India). HPLC grade water (resistivity of 18.2 MΩ cm) generated from Milli Q water purification system (Elix, Milli Q A10 Academic, Molsheim, France) was used throughout the analysis. All biochemical kits obtained from Merck Pvt. Ltd. (Mumbai, India). Anticoagulant (EDTA-2K) and other reagents used were of analytical grade (Merck Pvt. Ltd. Mumbai, India).

2.2. Animals husbandry and maintenance

Wistar albino rats of each sex were obtained from the animal house of Indian Institute of Chemical Biology (IICB), Kolkata, India at 6 weeks of age. The animals were grouped and housed in wire cages with not more than six animals per cage, under good laboratory conditions (temperature 25 ± 2 °C; 50 ± 20% relative humidity) with dark and light cycle (12/12) for minimum of 7 days before the beginning of experiment to adjust the new environment and to overcome stress possibly incurred during transit. Only healthy animals were assigned for the study. During this period they had free access to standard dry pellet diet (Hindustan Liver, Kolkata, India) and water *ad libitum*.

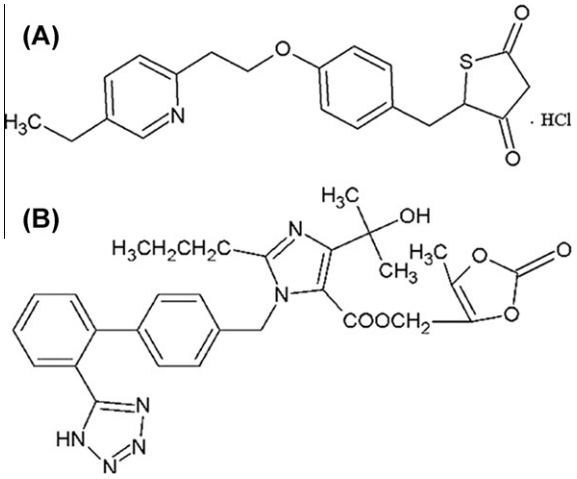

Fig. 1. Structural representation of (A) pioglitazone hydrochloride and (B) olmesartan medoxomil.

The study was approved by Institutional Animal Ethics Committee of Jadavpur University (CPCSEA, Reg. No. 367), Kolkata, India. Animals were maintained in accordance with the "Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)" guide lines.

2.3. Pharmacokinetic study

2.3.1. Experimental design

Adult male healthy Wistar albino rats, weighing 118–130 g were used as experimental animals for the pharmacokinetic study to evaluate if there was any pharmacokinetic interaction between PIO and OLM in combination. A single dose study comprising of 24 healthy male Wistar albino rats divided into four groups ($n = 6$) was performed. Treatments include oral administration of normal saline (control group), 3 mg/kg PIO alone, 2 mg/kg OLM alone and 2 mg/kg OLM in combination with 3 mg/kg PIO. Dose of the drugs in Wistar albino rat was calculated based on the body surface area following this formula (Shannon et al., 2007):

$$\text{Human Theraputic Dose (mg/kg)} = \text{Animal Dose (mg/kg)} \times \frac{\text{Animal Km}}{\text{Human Km}}$$

$$\text{Km of Rat} = 6; \text{Km of Human} = 37]$$

(K_m : Surface Area to Weight Ratios)

The values for human dose used to calculate the animal dose for olmesartan medoxomil and pioglitazone were 20 and 30 mg/kg, respectively.

Blood samples were collected from each rat by retro-orbital puncture at a predetermined time interval of pre-dose, 0.5, 1.5, 2, 3, 5, 12, 24, 36 and 48 h into the tubes containing EDTA-2K. Plasma was separated by centrifuging the blood samples at 5000 rpm and stored frozen at −20 °C until analysis.

2.3.2. Instrumentation and chromatographic condition

Liquid chromatography system used consists of solvent delivery (LC 10ADVP), controller (LC10ADVP) and column oven (CTO10ASVP)