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Pharmacokinetics, pharmacodynamics and toxicity of a combination of metoprolol succinate and telmisartan in Wistar albino rats: Safety profiling

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ABSTRACT

Metoprolol succinate (MET), a cardioselective β blocker and telmisartan (TEL), an angiotensin receptor blocker were administered orally, both individually and in combination to Wistar albino rats for evaluation of their pharmacokinetics, pharmacodynamics and repeated dose oral toxicity (28 days). Pharmacokinetic study was performed by analyzing drug concentration in plasma by a developed and validated LC–MS/MS method following oral administration of MET and TEL at 2.5 mg/kg and 2.0 mg/kg dose, respectively, both individually and in combination. Antihypertensive activity of MET and TEL in above dose and manner was evaluated on artificially induced hypertension on laboratory animals. In repeated dose oral toxicity study, MET (60, 120 and 240 mg/kg/day) and/or TEL (12, 24 and 48 mg/kg/day) were administered to animals for 28 days followed by a recovery period of 14 days. Pharmacokinetic data revealed the probable absence of any pharmacokinetic interaction when co-administered. Improved blood pressure lowering effect was observed by combination therapy. Moreover, toxic effects obtained at high dose level of each treatment groups were transient and reversible and no evidence of additive toxic effects were observed due to concomitant administration. So, this combination can primarily be stated as safe which will be confirmed after clinical interaction studies in humans.

1. Introduction

Cardiovascular disease (CVD) is a growing concern globally as it is responsible for one in every three deaths (Panda et al., 2006). As per the report of WHO (2007), an estimated 17 million people die of CVD particularly heart attack and stroke, every year. Various studies have shown that strict control of blood pressure (BP) is required to produce the maximum reduction in clinical cardiovascular end points, especially in patients with comorbidities like diabetes mellitus, chronic renal failure, heart failure, thyroid disorders, etc. (Lewington et al., 2002; Hansson et al., 1998). Achieving recommended goal of BP (<140/90 mmHg in all hypertensives and <130/80 mmHg in hypertensives with diabetes mellitus) is difficult in majority of patients with hypertension (Ong et al., 2007). The Framingham Heart Study (Cook et al., 1995) indicated that a 2-mmHg reduction in average diastolic blood pressure could result in a 14% decrease in the risk of stroke and transient ischemic attacks and a 6% reduction in the risk of coronary artery disease. Though single drug treatment may be effective in some, more than 50% will require more than one drug for appropriate control of their BP (Kalra et al., 2010). So, proper control of blood pressure at its recommended goal to treat hypertension can be achieved by using more than one drug either as multiple individual drugs or a fixed dose combination therapy (Stanton and Reid, 2002). In addition, The Seventh Report of the Joint National Committee (2003) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) and European Society of Hypertension (ESH) guidelines (2003) recommend that therapy with more than one antihypertensive agent be considered in patients with systolic blood pressure (SBP) more than 20 mmHg or diastolic blood pres-

Abbreviations: LC–MS/MS, Liquid Chromatography–Mass Spectroscopy/Mass Spectroscopy; MET, metoprolol succinate; TEL, telmisartan; IS, Internal Standard; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; IAEC, Institutional Animal Ethics Committee; BP, systolic blood pressure; 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.

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sure (DBP) more than 10 mmHg above the recommended goal and among patients at high cardiovascular risk, as determined by elevated BP level, and the presence of other risk factors. They also pointed out that when used in combination, antihypertensive drugs additively lower blood pressure and can often do so employing low doses of the individual agents, thereby minimizing dose-related adverse effects (Chobanian et al., 2003; Ofili, 2006).

Choice of anti-hypertensive drug in hypertensive patients is influenced by many factors including the possibility or risk of pharmacokinetic and pharmacodynamic drug–drug interactions. To understand such potential, it is necessary to evaluate their drug–drug interaction as well as toxicity in experimental animals following concomitant administration of both drugs. Data from such studies in laboratory animals help in deciding whether the new combination is suitable for further study in human volunteers.

Based on pharmacokinetic and pharmacodynamic properties, metoprolol, a well known cardioselective β blocker and telmisartan, a new angiotensin II receptor blocker were selected to study concomitantly as well as individually in animal model to achieve the goal of formulating these as a fixed dose combination (FDC) to treat patients with ischemic heart disease especially in essential hypertension and heart failure through once daily dosing. This reduces drug intake frequency leading to improved patient compliance as well as reduction of the cost of therapy.

Though there is reported pharmacokinetic, pharmacodynamic and toxicity/safety study of metoprolol succinate (Mostafavi and Foster, 2000; Deore et al., 2005; Shimasaki et al., 1999; Vermeulen et al., 1993; Höcht et al., 2004) and telmisartan (Hao et al., 2007; Wienen et al., 2000) separately as well as 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, antihypertensive potential of the FDC and to assess whether the toxicity of metoprolol succinate (MET) in combination with telmisartan (TEL) was additive, synergistic, or abrogated following repeated dose 28 day oral toxicity study in Wistar albino rats.

2. Materials and methods

2.1. Chemicals

Metoprolol succinate (purity >99%) (Fig. 1), telmisartan (purity >99%) (Fig. 1) and atorvastatin calcium (Internal Standard, IS) (>99%) were obtained from Stadmed Pvt. Ltd. (Kolkata, India), Akums Drugs & Pharmaceuticals Ltd. (Delhi, India) and Micro Labs Limited (Bangalore, India), respectively. N^G-nitro-L-arginine methyl ester L-NAME was bought from Sigma–Aldrich (Germany). Formic acid, ethyl acetate (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, anticoagulant (EDTA-2K) and other reagents of analytical grade were obtained from Merck Pvt. Ltd. (Mumbai, India).

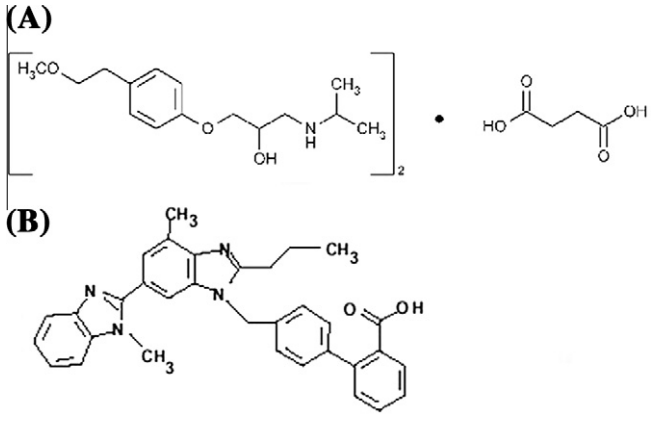

Fig. 1. Structural representation of (A) metoprolol succinate and (B) telmisartan.

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 6–8 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; relative humidity 50 ± 20%) with dark and light cycle (12/12) for a minimum of 7 days before the start of experiment to adjust to 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*.

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 150–180 g were used as experimental animals for the pharmacokinetic study to evaluate if there was any pharmacokinetic interaction between MET and TEL in combination. A single dose study comprising of 24 healthy male Wistar albino rats divided into four groups ($n = 12$) was performed. Treatments include oral administration of normal saline (control group), 2.5 mg/kg MET alone, 2.0 mg/kg TEL alone and 2.5 mg/kg MET in combination with 2.0 mg/kg TEL. Dose of the drugs in Wistar albino rat was calculated based on the body surface area by this formula (Shannon et al., 2007):

$$\text{animal dose (mg/kg)} = \frac{\text{human equivalent dose (mg/kg)} \times \text{human } K_m}{\text{animal } K_m}$$

K_m is the surface area to weight ratios.

The values for human daily dose used to calculate the animal dose for metoprolol succinate and telmisartan were 23.75 mg (equivalent to 25 mg metoprolol tartrate) and 20 mg, respectively.

Blood samples were collected from each rat by retro-orbital puncture at a predetermined time interval of pre-dose, 10 min, 20 min, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 24.0 and 48.0 h into the tubes containing EDTA-2K. Plasma was separated by centrifuging the blood samples at 5000 rpm and finally stored by freezing at −20 °C until analysis.

2.3.2. Instrumentation and chromatographic condition

Liquid chromatographic system consisting of solvent delivery (LC 10ADVP), controller (LC10ADVP) and column oven (CTO10ASVP) from Shimadzu (Kyoto, Japan) were used. Aliquots (30 µl) of the processed samples were injected using SIL HTC autosampler from Shimadzu (Kyoto, Japan) on a Gemini C_{18} column (50 mm × 4.6 mm, 5 µm particle size; Phenomenex, USA) column maintained at ambient temperature. Isocratic mobile phase con-