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Subchronic toxicity and toxicokinetics of LZB, a new proton pump inhibitor, after 13-week repeated oral administration in dogs

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Abstract

The subchronic toxicity and toxicokinetics of a novel proton pump inhibitor, pymeprazole (LZB), were investigated in beagle dogs by daily oral administration for 13 consecutive weeks. Three test groups received doses of 30, 100 and 300 mg/kg/day of LZB. Rabeprazole of 60 mg/kg/day was used as positive control. The 13-week repeated oral doses of LZB resulted in objective signs of mild gastrointestinal disturbance for high-dose group animals. One individual dog of high-dose group was found to be lethargy and astasia at the last month of administration; for hematology, mild anemia was observed at high-dose females; for clinical chemistry, higher cholest, trigly and gastrin were observed at high-dose females, higher ASAT, ALAT, cholesterol, triglyceride and gastrin at high-dose males were also observed; for histopathology, the primary effects of LZB were related to gastric mucosa of high-dose group seen by H and E or Grimelius stain. Impairment of surface epithelium was observed by SEM. The treat-related effects basically were reversible for a 4-week drug-free period. As for positive control group, 13-week oral administration of rabeprazole resulted in more severe toxicity than high-dose group of LZB although much lower dose was employed. The accumulation of LZB after 13-week oral administration was not notable at the toxic dose of 300 mg/kg/day. The toxic dose was considered to be 100 mg/kg/day and the no-observed-adverse-effect level (NOAEL) to be 30 mg/kg/day, which is much higher than other PPIs. The toxicological target could be stomach, liver, hematological system and nervous system.

Keywords: LZB; Subchronic toxicity; Toxicokinetics; 13-week oral administration; Beagle dogs

1. Introduction

Proton pump inhibitors (PPIs) are now the mainstay of treatment of peptic ulcers, reflux esophagitis and the *Zollinger–Ellison* syndrome, and have overtaken histamine-2-receptor antagonists due to their superior effectiveness and faster onset of action. Current PPIs are mainly substituted pyridyl methyl sulfinyl benzimidazole or imidazopyridine prodrug that selectively inhibits the gastric acid secretion. After absorption into the circulation, PPIs readily permeate the basolateral parietal cell membrane. When the parietal cell is activated and H^+/K^+-ATPase is exposed in the canaliculi, PPIs are activated by the acid environment. The activated form of the PPIs, sulfenamides, has a reactive sulfhydryl group that bonds with the cysteine residues of H^+/K^+-ATPase. The covalent bond permanently deactivate the proton pump-associated ATPase. Acid secretion can only occur when new pumps are synthesized (Jai et al., 2006).

From the result of toxicity study in animals (Ekman et al., 1985; Abe et al., 1990; ACIPHEX™, 1999; Okamoto et al., 1998a,b), the main toxicity of PPIs were vomit, some clinical parameters changes and stomach lesions.

The most common clinical adverse effects associated with PPIs are gastrointestinal disturbances, upper respiratory infection, dizziness, asthenia, back pain, reversible hypergastrinemia, enterochromaffin-like (ECL) cell proliferation, hindrance of nutrition absorption, atrophic gastri-

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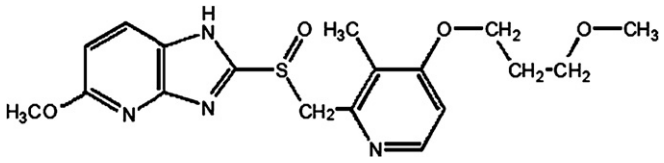

Fig. 1. Chemical structure of LZB.

tis, interstitial inflammation of liver, and so on (Valuck and Ruscin, 2004; Marchetti et al., 2003; Bardhan et al., 2005; Gerald et al., 1986; Byrne and Murray, 1999; Feret et al., 1999; Garnett, 1998; Freston, 1997; Fitton and Wiseman, 1996). Deaths have been reported with proton pump inhibitor use (Watson et al., 2005).

LZB (recently developed by Research laboratory of Haoseng Pharmaceutical Manufacturing Company, Ltd., Shanghai, China) is a novel reversible proton pump inhibitor. The structure was shown in Fig. 1. It has demonstrated low acute toxicity for dogs and rats and is absorbed quickly following oral administration for rats (unpublished data). LZB is acid labile and white to off-white powder with a purity of 99.5%, which is made to enteric capsule, and is now being considered for evaluation in phase I clinical trial. The purpose of this study is to determine the subchronic toxicity and toxicokinetics of LZB during 13-week oral administration at doses of 0 (to serve as a control), 30, 100 and 300 mg/kg/day. The toxic effects of LZB and rabeprazole (serve as positive control drug) were also compared.

2. Materials and methods

2.1. Test substance

LZB and rabeprazole enteric capsule were supplied by Research Laboratory of Haoseng Pharmaceutical Manufacturing Company, Ltd. Other chemicals were of reagent grade or high-performance liquid chromatographic (HPLC) grade and therefore were used without further purification. LZB and rabeprazole were stored refrigerated at 2–8 °C, protected from light and wet.

The appropriate amount of LZB enteric capsules required for daily administration was calculated and was adjusted every two weeks based on the most recently recorded body weight. The control animals received empty enteric capsules of the same size and number as those given to the mid-dose group animals. Dogs were dosed daily (6 days per week), at approximately 8:00 AM.

2.2. Animal

Twenty male (weighing 6.0–7.5 kg) and 20 female (weighing 5.5–7.0 kg) purebred beagle dogs of 5.5–6.5 months of age were purchased from ZhongKe experimental animal Co. Ltd. (Suzhou, China). After quarantine and acclimation periods of 30 days, oral studies were performed (when male dogs weighed 7.0–8.5 kg and female dogs weighed 6.0–8.0 kg). Each animal was identified by an ear tattoo as well as kennel number and housed in individual kennel. Room temperature and humidity were monitored continually, with target ranges for room temperature of 20 ± 3 °C, relative humidity 30–70% and a photocycle of 12 h. Diet (500 g of dry feed) (Japan Oriental Yeast Company, Tokyo, Japan) was provided to the animals at 10:00 each morning and removed at 15:00. The food and water used in the study was assayed for chemical as well as for microbiological contaminants and the levels were found to be under acceptable limits. Water was supplied ad libitum.

Beagle dogs (4/sex/group) with LZB administered orally at dose levels of 0 (control), 30, 100, 300 mg/kg/day and rabeprazole at 60 mg/kg/day (positive control). Half animals were sacrificed after 13-week of treatment, the remained half (2/sex/group) were kept for a 4-week recovery period and then killed.

2.3. Selection of doses

In the previous acute safety study for LZB in rat and dogs, all animals survived the 2-week observation period following a single oral administration of 5000 mg/kg and 2000 mg/kg in rats and dogs, respectively. No abnormal signs, clinical test parameters, or necropsy findings were observed except that vomit was observed at the dog of 2000 mg/kg at the first day after administration. It can be concluded that LZB has a low order of acute toxicity and that the oral MTD (maximum tolerated dose) >2000 mg/kg for dogs.

Otherwise, in a 21-day repeated oral dose pretest (data not shown), slight vomiting were observed in dogs at doses of 300 mg/kg of LZB in both sexes, while severe anorexia, vomiting and diarrhea were observed in positive controls at dose of 100 mg/kg of rabeprazole in both sexes.

According to the results of acute toxicity study and pretests, a dose of 300 mg/kg/day of LZB (900 times as rabeprazole's clinical dose, rabeprazole enteric tablet, 20 mg/day) was selected for the highest dose in this study. Dose of 100 and 30 mg/kg/day (300 and 90 times as rabeprazole's clinic dose) were selected as middle and low dose, using a common ratio of 3. Dose of 60 mg/kg/day (180 times as rabeprazole's clinic dose, two times as low-dose of LZB) of rabeprazole was selected as positive control.

2.4. Experimental design

The purpose of this study was to determine the subchronic toxicity, and the potential for recovery from any adverse effects, associated with repeated exposure to LZB when administered orally to beagle dogs 6 days per week for a period of 13-week, following an additional 4 weeks to determine the reversibility, persistence, or delayed occurrence of toxic effects.

This nonclinical laboratory study was carried out in compliance with the Testing Guidelines for Safety Evaluation of Drugs (Notification [H] GPT2-1 issued by China Food and Drug Administration on March 2005) and the Organization for Economic Cooperation and Development under the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAA LAC), and animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

2.5. Clinical observations

Observations for mortality and viability were recorded from the beginning of the quarantine period. Each animal was examined at least twice daily for any change in behavior, reaction to treatment or ill. Observations included, but were not limited to, changes in skin and fur; eyes and mucous membranes; respiratory, circulatory, autonomic, and central nervous systems; somatic motor system; and behavior patterns.

Ophthalmoscopic examinations were performed on each animal every two weeks from the quarantine period to the end of recovery for abnormalities of the eyes, at least 20 min after the instillation of 0.5% tropicamide solution, using a binocular indirect ophthalmoscope. The observation area included the cornea, conjunctiva, sclera, iris, lens and fundus.

2.6. Body weights, weight gain, and food consumption

Food consumption and body weight were measured every two weeks. Dose administration was based on the most recent individual body weights.