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Safety assessment of vitacoxib: 180-day chronic oral toxicity studies

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ABSTRACT

Vitacoxib, a selective COX-2 inhibitor, is approved for the relief of pain and inflammation associated with orthopedic surgery and osteoarthritis in dogs. In the current study, a chronic toxicity research was performed to evaluate the safety of vitacoxib in male and female rats for long-term. Vitacoxib was dosed orally to groups of rats for 180 days at 1.2, 6, 30 mg/kg bw/day by gavage. The chronic study oral administration of vitacoxib did not show observational or toxicological effects on the body or organ weights, food consumption, hematology and biochemistry at dose 6 mg/kg bw. However, vitacoxib (30 mg/kg) showed minor alterations to histopathology of liver, kidney and stomach related to treatment. These results provide further indication that vitacoxib is safe and well-tolerated in rats after 180 days of daily oral administration at 6 mg/kg bw and the NOAEL for both sexes was 6 mg/kg bw for 180 consecutive days.

1. Introduction

Vitacoxib, a novel imidazole derivate, is a highly selective cyclooxygenase-2(COX-2) inhibitor that shows potent anti-inflammatory and analgesic activities. Its chemical name is 2-(4-chloro-5-p-tolyl-1H-imidazol-1-yl)-5-(methyl sulfonyl) pyridine ($C_{16}H_{14}ClN_3O_2S$, Fig. 1) and molecular weight is 347.82 g mol^{-1}. The substance represents good oral activity when tested in experimental models of acute and chronic inflammation and pain, comparable to celecoxib (Wang et al., 2017a-d; Li, 2015). The oral tablet of vitacoxib is labeled for use for osteoarthritis and inflammation in dogs (MOA, 2016).

Nonsteroidal anti-inflammatory drugs (NSAIDs) block pathologic processes via inhibition of cyclooxygenase enzymes. There are two cyclooxygenases, designated COX-1 and COX-2 (Needleman and Isakson, 1997). COX-1 is a housekeeping enzyme and COX-2 is inducible and is predominantly expressed in association with inflammation (Masferrer et al., 1994). COX-2-specific inhibitors have anti-inflammatory activity without the associated gastrointestinal (GI) adverse effects of classical NSAIDs (Paulson et al., 2001). There is a trend for the replacement of NSAIDs with selective COX-2 inhibitors in treatment protocols to reduce the gastrointestinal and/or platelet disorders triggered by COX-1 inhibition (Kim and Giorgi, 2013). However, all NSAIDs inhibit both COX-isoforms, suppressing the synthesis of homeostatic and proinflammatory prostaglandins and consequently have a narrow therapeutic index with primary adverse effects being gastric irritation, hepatic and renal damage (Steagall et al., 2007).

For over a decade, several COX-2 selective drugs have been marketed in the veterinary usage including deracoxib (2002), firocoxib (2007), mavacoxib (2008), robenacoxib (2009) and the latest cimicoxib (2011). However, 4 out of 6 drugs belonging to coxibs (rofecoxib, valdercoxib, parecoxib and lumiracoxib) have already been withdrawn from the market due to serious adverse events in human (Bessone et al., 2016). Several studies conducted to investigate COX-2 selective inhibitor (coxib) drugs in human and veterinary medicine since the severe adverse effects are often associated with chronic NSAID use. Moreover, long-term use of coxibs can be associated with an increased risk of cardiovascular (CV) adverse events (AEs) (Shi and Klotz, 2008). Thus, there is a concern about the adverse effects of NSAIDs, particular because their most common use is for long-term administration (Luna et al., 2007).

To date, information from acute, sub-chronic, and reproductive and developmental studies caused by vitacoxib is still lacking. A series of analytic and toxicological studies were conducted in our laboratory in the past five years. Our research showed that acute toxicity of vitacoxib was more than 5000 mg/kg in Sprague Dawley (SD) rats and Institute of Cancer Research (ICR) mice (Wang et al., 2017a-d). The sub-chronic

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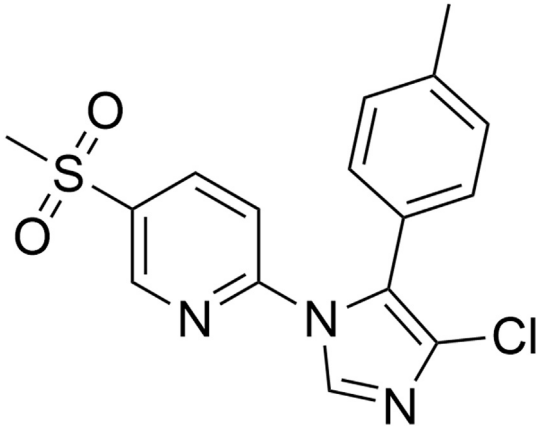

Fig. 1. The structure of the vitacoxib.

toxicity of vitacoxib showed that no observed adverse effect level (NOAEL) was considered to be 20 mg/kg in SD rats (Wang et al., 2017a-d). All the results including mutagenicity and teratogenicity test were negative (Wang et al., 2017a-d). Vitacoxib did not induce dermal irritation in rabbits or skin sensitization toxicity in guinea pigs (Wang et al., 2017a-d). It is evident that vitacoxib is well tolerated in animals.

From the literature, the long-term effect of vitcoxib in animals has, however, not been well studied in veterinary medicine. The purpose of the present study was undertaken to fully characterize the potential long-term systemic toxicity of vitacoxib administered by the oral route for precise risk assessment, following Organization for Economic Cooperation and Development (OECD) test guideline 452 chronic toxicity studies with an exposure period of 180 days (OECD, 2009). As an evaluation of preclinical safety, this study will provide guidance for the design of further preclinical toxicity studies and clinical trials of vitacoxib.

2. Materials and methods

Vitacoxib (Lot#PH-OBP-2-RSI-A-0-1; purity 99.7%), prepared by Beijing Orbiepharm Co., Ltd. (Beijing, PR China). Cyclophosphamide and carboxyl methyl cellulose sodium (CMC-Na), purchased from Tianjin Chemical Reagent Company (Tianjin, China).

2.1. Animals and animal housing

Female and male Sprague Dawley rats were purchased from Beijing Vital River Laboratories (Charles River Laboratories) (laboratory animal reproduction license #SCXK (Beijing) 2012-0001). The animal housing conditions were in compliance with our previous study (Wang et al., 2017a-d). This study was approved by the China Agricultural University Institutional Animal Care and Use Committee.

2.2. Study designs

The chronic research project was designed according to OECD Guideline 452 chronic toxicity studies (OECD, 2009). Two hundred SD rats were randomly divided into four groups, each group containing 25 females and 25 males, respectively. Animals were assigned to three treatment groups and a control (1% of CMC-Na) group. Based on the OECD 452 dosage guidelines and sub-chronic studies, the rats in treatment groups were fed with diets incorporated with 1.2, 6, 30 mg/kg of vitacoxib, respectively, for 180 days. The testing sample solutions were gavaged at 2 mL/100 g body weight/day. The control group was given the diet without any drugs.

2.3. Clinical observations, body weight, food and water consumption

During the study, all animals were observed twice daily for their conditions and behaviors before and after dosing for changes in posture, skin, fur, eyes, mucous membranes, bowel movement, morbidity and mortality. Body weight were measured and recorded before experimental diet administration, weekly during the treatment and at necropsy. Food consumption was recorded weekly and prior to necropsy, and water intake was monitored daily.

2.4. Hematology and blood chemistry

Blood was obtained from the tail vein for the examination of hematology and serum biochemistry, 10 rats (5 for each sex) per group, at the study (days 45, 90, 135 and 180) for examination of hematology and clinical chemistry after animals had been fasted overnight, respectively.

The hematology tests were conducted by HEMASTAR II (YSBAERT) using the whole blood supplemented with ethylene diamine tetra-acetic acid (EDTA) as the anticoagulant. White blood cells count (WBC), hemoglobin (HGB), platelet count (PLT), red blood cell count (RBC), and leukocyte differential counts were measured. Plasma alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), total protein, aspartate aminotransferase (AST), glucose, triglyceride, creatinine (CREA), cholesterol, lipoprotein (CHOL), and blood urea nitrogen (BUN) were determined using ELLIPSE (YSBAERT).

2.5. Necropsy and histopathology

Necropsy was conducted in each group consisting of both 5 females and 5 males in 45, 90, 135 and 180 days. After the blood samples had been collected, the rats were sacrificed by cervical dislocation. The heart, liver, lung, kidneys, spleen, stomach, duodenum, and testes/ovaries were excised and weighed. Meanwhile, the absolute organ weight and the relative organ-to-body weight ratio were calculated for all organs.

All excised organs were preserved in 10% formalin (pH 7.4) followed by dehydration and then enclosed in paraffin. Random tissue sections were then stained with hematoxylin and eosin (H&E) and observed with an optical microscope (DM750, Leica, Germany).

2.6. Statistical analysis

All numerical data are expressed as mean ± stand deviation (SD). The differences were performed using one-way analysis of variance (ANOVA) in SPSS 20.0 program. Heterogeneous data and the significance of intergroup differences was tested with *t*-test for pairwise comparisons with control. P-values of $^{<}$ 0.05 were considered statistically significant.

3. Results

3.1. Clinical observation

No death, abnormal behavior or gastrointestinal disturbances were observed at the end of the treatment. Generally, consumption of vitacoxib was well tolerated. There were no significant differences ($P > 0.05$) in the food intake, water consumption and daily weight gain between the treated groups and controls (Fig. 2).

3.2. Hematological examination

On hematological examination, the values were within the normal historical range for the laboratory (data shown in supplemental materials). No significant differences were found between treated groups and controls ($P > 0.05$).