



Mutagenicity and teratogenicity studies of vitacoxib in rats and mice

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ABSTRACT

Vitacoxib is a new drug candidate for treatment of inflammation, pain and fever as selective cyclooxygenase-2 inhibitors. In the current study, the mice sperm abnormality, mammalian erythrocyte micronucleus and in vivo chromosome aberration, and teratogenicity in SD rats were evaluated. Vitacoxib did not cause an increase in the frequency of structural chromosome aberrations, nor did it produce an increase in the number of micro-nucleated polychromatic erythrocytes at dose of 1250–5000 mg/kg body weight (BW). There were no toxicological signs observed in teratogenicity test in female SD rats at dose of 200–5000 mg/kg BW. Based on these results of these studies, vitacoxib does not appear to be observed mutagenicity and teratogenicity.

1. Introduction

Vitacoxib [2-(4-chloro-5-*p*-tolyl-1H-imidazol-1-yl)-5-(methyl sulfonyl) pyridine (C₁₆H₁₄ClN₃O₂S)], as a novel non-steroid anti-inflammatory drug (NSAID) used in veterinary medicine, known as a specific cyclooxygenase-2(COX-2) inhibitor. The chemical structure is shown in Fig. 1.

These highly selective inhibitors of COX-2, have gained worldwide popularity. However, 4 out of 6 drugs belonging to coxibs (rofecoxib, valdecoxib, parecoxib and lumiracoxib) have already been withdrawn from the market due to serious adverse events [1]. Due to the high risk of myocardial infarction and blood hypertension related to rofecoxib use, the drug was withdrawn from the pharmaceutical market in 2004 by its manufacturer (Merck & Co., Inc.) [2]. In addition, valdecoxib and its prodrug, parecoxib, were also voluntarily withdrawn by manufacturer (Bextra, Pfizer Canada Inc.) and notified by the Food and drug Administration (FDA) in 2005 due to severe dermatological reactions [3]. Celecoxib and etricoxib are the only two members of this pharmacological group that continue to be marketed in many countries in the world. Besides, the oral tablet of firocoxib, deracoxib and robenacoxib have also been approved in clinical veterinary clinics in the European Union and FDA for use in dogs.

It reported that inhibition of prostaglandin synthesis may adversely affect the pregnancy and/or the embryo/fetal development, according to extensive experience of the use of NSAIDs (including

COX-2 inhibitors) [4]. Vitacoxib, a new anti-inflammatory drug, is developed by Beijing Orbiopharm Co., Ltd (PR China) for use in canine for the suppression of inflammation and pain. Preclinical studies show that vitacoxib has exhibited excellently clinical efficacy and safety in fast-acting COX inhibitor which is potently, selectively and high degree specific to COX-2 isozymes and has little effect on COX-1 isozymes in rodents [5]. The oral tablet of vitacoxib has been licensed for use in canine to treat pain and inflammation connected with osteoarthritis in China [6]. The predication of side-effects is a key issue in the Registration, Evaluation, Authorization and restriction of chemical (REACH) initiative on chemicals in the preclinical testing of drugs [7,8].

To date, there is few information on acute, sub-chronic, reproductive and developmental reports for vitacoxib according to relate to toxicology guidelines. Thus, it is necessary to evaluate the risk of vitacoxib. In the past five years, several pre-clinical toxicity studies were conducted in our laboratory. Previous study of vitacoxib reported that LD₅₀ was more than 5000 mg/kg in SD rats and ICR mice [9], and NOAEL (90 days and 180 days) was 20 mg/kg and 6 mg/kg bw in SD rats, respectively [9,10]. Vitacoxib did not induce dermal irritation in rabbits or skin sensitization toxicity in guinea pigs [11].

For development of vitacoxib, it is vital to characterize vitacoxib mutagenicity and teratogenicity. The goal of the current studies was to assess the safety of vitacoxib based on a battery of mutagenicity and teratogenicity studies, including the mice sperm abnormality test,

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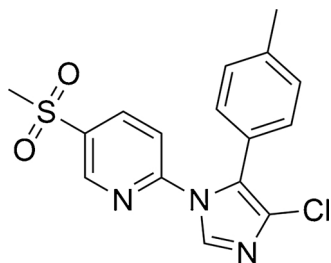


Fig. 1. Chemical structure of vitacoxib.

mammalian erythrocyte micronucleus, chromosome aberration and teratogenicity studies in rats and mice.

2. Materials and methods

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

2.1. Animal and animal housing

Female and male ICR mice and Sprague Dawley (SD) rats were obtained from Beijing Vital River Laboratories (Charles River Laboratories) (laboratory animal reproduction license #SCXK (Beijing) 2007-0001). All animals were examined for health condition to ascertain the suitability for study and the rats were acclimated to the laboratory environment for 7 days and the mice for 5 days. All animals were placed in a standard environmental condition, fed with rodent standard diets and water ad libitum. The animal facility was maintained at 22–24 °C, a relative humidity of 55% ± 10%, and a 12 h light/dark cycle at 160–290 lx throughout the testing period. Prior to initiation of dosing, all animals were fasted overnight, but free water access. These protocols for animal use were approved by the China Agricultural University Institutional Animal Care and Use Committee.

2.2. Study designs and methods

The study designs and methods were conducted according to OECD Genetic toxicology Guidance [12] and our published literature [13]. Some steps were modified. In Mice sperm abnormality test, mice in the experimental groups administrated vitacoxib via intragastric gavage (IG) at 1250 mg/kg (low-doses), 2500 mg/kg (medium-doses), and 5000 mg/kg (high-doses) bw for 5 consecutive days, respectively. Mice

Table 1
Sperm abnormality test for vitacoxib in mice.

Parameters	Group (mg/kg)				
	high-dose	medium-dose	low-dose	Negative	Positive
Number of mice	5	5	5	5	5
Number of sperm observed	5 × 1000	5 × 1000	5 × 1000	5 × 1000	5 × 1000
Number of sperm abnormality	96	110	103	104	369
Abnormal ratio (%)	1.96 ± 0.066	2.25 ± 0.68	2.10 ± 0.90	2.12 ± 0.72	7.99 ± 2.76
significance of difference	P > 0.05	P > 0.05	P > 0.05	–	P < 0.01
Abnormal sperms counted ratio (%)					
No hook	12.50 ± 1.00	13.64 ± 0.64	11.65 ± 0.60	11.54 ± 0.60	8.40 ± 1.32
Banana shape	14.58 ± 0.79	10.00 ± 0.51	9.71 ± 0.61	12.50 ± 0.67	6.23 ± 0.99
amorphous	41.67 ± 0.89	39.09 ± 1.09	36.89 ± 1.02	39.42 ± 1.00	53.39 ± 4.76
Large round head	12.50 ± 0.12	10.91 ± 0.60	10.68 ± 0.51	6.73 ± 0.49	11.38 ± 1.86
Kinks tail	7.29 ± 0.74	8.18 ± 0.60	2.91 ± 0.37	5.77 ± 0.47	4.07 ± 0.64
Two head	2.08 ± 1.00	2.73 ± 0.37	4.85 ± 0.44	1.92 ± 0.50	3.25 ± 0.75
Two tails	9.38 ± 0.01	15.45 ± 0.67	23.23 ± 0.89	22.12 ± 1.04	13.28 ± 1.76

Note: No significance of difference was observed that all treatment groups compared with negative control group.
Low dose = 1250 mg/kg bw; Medium dose = 2500 mg/kg bw; High dose = 5000 mg/kg bw.

in negative and positive control groups received IG 1% CMC-Na solvent and 40 mg/kg body weight of cyclophosphamide, respectively. In mammalian erythrocyte micronucleus assay, animals were treated with vitacoxib at 1250, 2500, 5000 mg/kg bw by IG (0.2 mL/10 g bw), twice within a 24 h interval. In vivo chromosome aberration test of bone marrow cells, Animals were treated with vitacoxib at 1250, 2500, 5000 mg/kg body weight by IG (0.2 mL/10 g BW), twice within a 24 h interval. Cyclophosphamide (40 mg/kg body weight) was used as the positive control while 1% of CMC-Na as the negative control. In teratogenicity study, the pregnant rats (12, 12, 12, and 12 for positive, 5000 mg/kg of vitacoxib, 1000 mg/kg of vitacoxib, 200 mg/kg of vitacoxib, and 300 mg/kg of aspirin, respectively) were administrated via IG. Using *t*-test for pair-wise comparisons to the control group was used to analyze the heterogeneous data and the significance of intergroup differences between the control and treatment groups. The significance level of $P < 0.05$ was used in all comparisons.

3. Results

3.1. Mice sperm abnormality test

The proportion of sperm abnormal morphology and rate of mal-formation type were showed in Table 1. No significant differences were noted in sperm abnormality of the vitacoxib treatment groups at all dose levels (vitacoxib, 1250 mg/kg–5000 mg/kg) compared to the negative control group. All treatment groups and the negative control group were significantly lower than in the positive control group, indicating that vitacoxib at the doses above did not result in abnormal sperm morphology.

3.2. Mammalian erythrocyte micronucleus

The results were summarized in Table 2. The positive control value was significantly greater than that of the negative control, indicating that the current study was capable of showing micronuclear toxicity. There was no significant increase in micronucleus of the vitacoxib treatment groups at all dose levels, compared with the negative control group at the endpoint under evaluation ($P > 0.05$).

3.3. In vivo chromosome aberration test of mammalian bone marrow cells

The results were summarized in Table 3. No significant differences were found between the negative control group and the vitacoxib treatment groups (vitacoxib, 1250 mg/kg–5000 mg/kg). Compared with the negative control group at the endpoint under evaluation, similarly, the chromosome aberration of vitacoxib treatment groups at all

Table 2
Effects of vitacoxib on mouse bone marrow micronucleus and PCE/RBC ratio.

Sex	Dose (mg/kg)	PCE/RBC	PCE micronucleus (%)	P
Female	high-dose	0.89 ± 0.04	1.43 ± 1.97	P > 0.05
	medium-dose	0.86 ± 0.06	1.72 ± 2.13	P > 0.05
	low-dose	0.91 ± 0.09	1.80 ± 2.01	P > 0.05
	Negative	0.80 ± 0.07	1.62 ± 2.22	–
	Positive	0.45 ± 0.04	14.70 ± 5.52	P < 0.01
Male	high-dose	0.90 ± 0.06	1.01 ± 1.97	P > 0.05
	medium-dose	0.83 ± 0.07	1.99 ± 2.64	P > 0.05
	low-dose	0.80 ± 0.06	1.40 ± 2.15	P > 0.05
	Negative	0.82 ± 0.08	1.45 ± 2.23	–
	Positive	0.47 ± 0.06	12.92 ± 7.04	P < 0.01

Note: No significance of difference was observed that all treatment groups compared with negative control group. RBC: red blood cells; PCE: poly-chromatic erythrocytes.

Low dose = 1250 mg/kg bw; Medium dose = 2500 mg/kg bw; High dose = 5000 mg/kg bw.

dose levels did not show a significant increase ($P > 0.05$), suggesting that vitacoxib did not result in chromosome aberration.

3.4. Teratogenicity study

No signs of illness, gastrointestinal intolerance or abnormal behavior were observed between the control and treated groups. There were no females aborted, delivered prematurely, or died throughout the experiment. Table 4 shows the data of body weight gain, water intake and food consumption in gestation, respectively. Compared to the control group, the daily body weight gain of pregnant rats was significantly decreased ($P < 0.05$). However, no significant differences were noted in high-dose (5000 mg/kg BW), medium-dose (1000 mg/kg BW) groups and low-dose group (200 mg/kg BW), compared to the control group in food consumption and water intake of pregnant rats, respectively. No toxicity signs were observed in pregnant rats in any groups. The results of fetal rat malformations examination and reproductive toxicity, and the type and incidence of visceral and skeletal alteration are showed in Table 5.

3.4.1. Maternal reproductive performance

No significant differences were noted with respect to gravid and empty uterus weights, placenta weight and ovary weight in vitacoxib treated groups and control groups. The mean numbers of corpora lutea and implantation sites, and the number of live fetuses were similar between any of the vitacoxib treated groups and control group.

3.4.2. Fetal examination

Average number of live fetuses, number of live fetus's ratio (%), fetus death rate (%) and number of embryo resorption (%) indexes were

Table 3
Summary of chromosomal aberration frequencies in the bone marrow of the male and female mice dosed with vitacoxib.

Sex	Dose(mg/kg)	Number of mice	Number of cells at metaphase	Number of cells with chromosome aberration	Chromosome aberration (%)	P
Female	high-dose	5	5 × 100	5	1.0 ± 0.45	P > 0.05
	medium-dose	5	5 × 100	5	1.0 ± 0.45	P > 0.05
	low-dose	5	5 × 100	4	0.8 ± 0.40	P > 0.05
	Negative	5	5 × 100	6	1.2 ± 0.48	–
	Positive	5	5 × 100	203	40.6 ± 2.90	P < 0.01
Male	high-dose	5	5 × 100	5	0.8 ± 0.42	P > 0.05
	medium-dose	5	5 × 100	4	0.8 ± 0.40	P > 0.05
	low-dose	5	5 × 100	5	1.0 ± 0.45	P > 0.05
	Negative	5	5 × 100	4	0.8 ± 0.42	–
	Positive	5	5 × 100	196	39.2 ± 2.60	P < 0.01

Note: No significance of difference was observed that all treatment groups compared with negative control group.

Low dose = 1250 mg/kg bw; Medium dose = 2500 mg/kg bw; High dose = 5000 mg/kg bw.

statistically significantly different ($P < 0.05$) in positive (300 mg/kg BW of aspirin) groups compared to the vitacoxib treatment group and control groups. But there were no statistically significance differences ($P > 0.05$) in vitacoxib groups compared to the control group. These results showed that there was no embryo toxicity in the low-dose (200 mg/kg BW), medium-dose (1000 mg/kg BW) and high-dose (5000 mg/kg BW).

No significant differences were noted with respect to type and incidence of visceral and external malformations in all groups. No statistically significant differences were noted with respect to fetal malformations and maternal alterations in control, medium-dose groups and high-dose groups. Therefore, no external malformations, skeletal alterations or visceral alterations were observed in the low-dose and medium-dose groups.

4. Discussion

There is no literature reported regarding on the mutagenicity and teratogenic evaluation of vitacoxib, the current studies are important for this substance.

The mammalian erythrocyte micronucleus assay, mice sperm abnormality test and in vivo chromosome aberration test of mammalian bone marrow cells were chosen to evaluate genotoxicity of vitacoxib. It reported that celecoxib is no genotoxic under the proposed clinical conditions [14] and mavacoxib is not genotoxicity [4]. In the present study, vitacoxib at the doses (1250 mg/kg–5000 mg/kg) did not cause abnormal chromosome aberration. Mavacoxib was not found clastogenic in rat bone marrow at dose of 400 mg/kg for 2 days [4]. The clastogenic effect of both parecoxib and robenacoxib were not found in vivo rat bone marrow micronucleus assay [15,16]. Cimicoxib did not induce mutations or chromosome aberrations in vitro and appeared to be negative in vivo micronucleus test [17]. In this study, vitacoxib did not cause mouse bone marrow micronucleus or sperm malformation, even at high dosage levels. Thus, these results supplied strong evidence that indicated the low risk of genotoxicity under the proposed oral exposure.

It reported that inhibition of prostaglandin synthesis may adversely affect the pregnancy and/or the embryo/fetal development, according to extensive experience of the use of NSAIDs (including COX-2 inhibitors) [4]. The teratogenic studies were conducted to further assess the potential effects of vitacoxib on reproduction and development of rats. Open literature sources indicated that maternal toxicity of firocoxib was characterized by a transient decrease with respect to feed consumption and reduction in body weight very early in gestation (days 6–12) and the maternal NOEL was considered to 3 mg/kg/day [18]. In this research, exposure of vitacoxib to SD rats at concentrations of 200, 1000 or 5000 mg/kg bw during the 3-wk pre-gestation period and from day 1 though day 19 of gestation, causing neither fetal toxicity nor malformation. Maternal toxicity was evident in the positive group.

Table 4
Effects of vitacoxib on the daily body weight gain, food consumption and water intake during gestational day.

Parameter day (s) of pregnancy	Dose level (mg/kg/day)				
	High-dose	Medium-dose	Low-dose	Control	Positive
Number of rats	12	12	12	12	12
Daily body weight gain M ± SD(g/rat/day)					
Day 0–6	5.44 ± 0.54	5.33 ± 0.51	5.10 ± 0.76	5.00 ± 0.86	4.67 ± 0.52
Day 7–12	7.61 ± 0.76	7.46 ± 1.24	7.86 ± 1.06	7.50 ± 1.58	6.25 ± 0.79*
Day 13–20	5.98 ± 1.33	7.50 ± 1.70	7.43 ± 1.12	7.07 ± 1.58	6.49 ± 1.99
Day 0–20	6.32 ± 0.65	6.80 ± 0.96	6.83 ± 0.66	6.55 ± 0.53	5.84 ± 0.57*
Net gain	2.33 ± 0.33	2.52 ± 0.62	2.31 ± 0.44	2.49 ± 0.43	2.21 ± 0.42
Food consumption M ± SD(g/rat/day)					
Day 0–6	19.88 ± 1.11	20.89 ± 1.18	20.66 ± 1.24	19.83 ± 1.38	21.51 ± 1.42
Day 7–12	28.60 ± 1.77	29.95 ± 1.75	29.14 ± 1.71	28.59 ± 1.44	26.68 ± 1.09
Day 13–20	36.82 ± 1.36	36.66 ± 1.45	36.11 ± 2.13	36.39 ± 1.48	36.52 ± 1.50
Water intake M ± SD(g/rat/day)					
Day 0–6	43.71 ± 1.90	45.28 ± 2.59	45.17 ± 2.02	44.69 ± 1.81	45.10 ± 2.63
Day 7–12	50.74 ± 3.15	50.82 ± 4.25	52.36 ± 2.89	50.63 ± 2.63	51.13 ± 2.58
Day 13–20	67.16 ± 7.14	65.67 ± 4.80	63.18 ± 8.97	66.96 ± 6.95	66.71 ± 6.66

Note: No significance of difference was observed that all treatment groups compared with negative control group.

N, total of pregnant rats.

Low dose = 200 mg/kg bw; Medium dose = 1000 mg/kg bw; High dose = 5000 mg/kg bw; Positive = 300 mg/kg bw (aspirin).

* Denote significance differences (P < 0.05).

Maternal body weight daily gains and food consumption for the vitacoxib treated groups were no statistically significantly compared with the negative control group throughout the dosing and/or post-dosing

periods. Foetal body weight and length remained were unaffected by vitacoxib treatment. Fetal mortality of firocoxib, alterations to growth, and structural alterations were observed in the foetuses of dams

Table 5
Effects of vitacoxib on the reproductive toxicity and fetal rat malformations examination.

Parameter	Dose level (mg/kg/day)				
	High-dose	Medium-dose	Low-dose	Control	Positive
Number of females pregnant	12	12	12	12	12
Number of implantations	160	152	159	160	149
Average number of implantations	13.50 ± 2.20	12.67 ± 2.35	13.25 ± 1.48	13.33 ± 2.23	13.92 ± 3.37
Number of live fetuses (♀/♂)	158(76/82)	149(79/70)	156(72/84)	156(81/75)	123(64/59)
Average number of live fetuses	13.33 ± 2.15	12.42 ± 2.15	13.17 ± 1.70	13.00 ± 1.17	9.08 ± 2.07*
Number of live fetus's ratio (%)	98.75(158/160)	98.03(149/152)	99.37(158/159)	97.50(156/160)	82.55(123/149)*
Fetus death rate (%)	0(0/160)	0(0/152)	0(0/159)	0(0/160)	1.34(2/149)*
Number of embryo resorption (%)	1.25(2/160)	1.97(3/152)	0.63(1/159)	2.50(4/160)	18.45(24/149)*
Number of corpora lutea	13.75 ± 2.38	14.08 ± 2.15	14.83 ± 1.11	14.67 ± 2.23	14.75 ± 2.49
Ovary weight (g)	0.13 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.03	0.12 ± 0.03
Uterus weight (g)	5.82 ± 0.75	5.52 ± 0.73	5.43 ± 0.73	5.27 ± 0.55	5.52 ± 0.95
Placental weight (g)	0.47 ± 0.05	0.49 ± 0.03	0.49 ± 0.03	0.50 ± 0.04	0.49 ± 0.03
Fetal weight (g)	3.63 ± 0.08	3.80 ± 0.15	3.76 ± 0.12	3.75 ± 0.17	3.76 ± 0.13
Fetal body length (cm)	3.61 ± 0.05	3.65 ± 0.07	3.68 ± 0.05	3.62 ± 0.11	3.67 ± 0.03
Fetal tail length (cm)	1.25 ± 0.02	1.28 ± 0.03	1.29 ± 0.03	1.26 ± 0.04	1.29 ± 0.02
External malformations					
Number of fetuses examined	158	149	158	156	123
Fetus malformations (%)	0	0	0	0	0
Maternal malformations (%)	0	0	0	0	0
Skeletal malformation					
Number of fetuses examined	79	75	79	78	62
Fetus malformations (%)	11.25 (9/80)	9.33 (7/75)	12.66(10/79)	10.26(8/78)	41.94(26/62)*
Maternal malformations (%)	41.67 (5/12)	33.33 (4/12)	50.00 (6/12)	41.67 (5/12)	100.00(12/12)*
Visceral alterations					
Number of fetuses examined	79	74	79	78	61
Fetus malformations (%)	0	0	0	0	0
Maternal malformations (%)	0	0	0	0	0

Note: No significance of difference was observed that all treatment groups compared with negative control group.

Number of embryo resorption (%) = Number of embryo resorption/ Number of implantations.

Fetus death rate (%) = Number of fetus death/ Number of implantations.

Number of live fetus's ratio (%) = Number of live fetuses/Number of implantations.

Fetus malformations (%) = Number of Fetus malformations/Number of fetuses examined.

Maternal malformations (%) = number of pregnant rats with abnormal fetus/Number of pregnant examined.

Low dose = 200 mg/kg bw; Medium dose = 1000 mg/kg bw; High dose = 5000 mg/kg bw; Positive = aspirin treated.

* Denote significance differences (P < 0.05).

administered 1000 mg/kg/day [18]. The results of rat and rabbit teratology studies indicated that Celecoxib acts as a teratogen in both species without any treatment-related clinical sign or change in body weight of dams [14]. It reported that robenacoxib is not approved for use in breeding animals of both target animal species, including pregnant and lactating animals [16]. It is interesting that the fertility and reproductive performance did not found any vitacoxib harmful effects in the present study. Thus, it can be concluded that pregnant rats and fetus were exposed to the test article and its potential metabolites without any toxicity. In addition, the rabbit is much more sensitive to showing reproductive effects. Future reproductive studies in rabbits are necessary to conduct.

In summary, the results of the mutagenicity and teratogenic test studies described here provide a comprehensive toxicity profile of vitacoxib. No obvious mutagenicity and teratogenic test toxicity was revealed, and the results indicated that vitacoxib is relatively safe for animals. Based on these results of the present oral exposure studies, we concluded that vitacoxib is no genotoxicity or teratogenic toxic. These studies will supply information pertinent to the establishment of doses which could be administered to these species via the diet in chronic studies to establish safety for use as a drug. As a part of preclinical safety, the findings of these studies will also furnish guidance for the design of further preclinical toxicity research and clinical studies of vitacoxib. However, the health risk should be carefully evaluated when the use of vitacoxib is aiming to achieve the therapeutic anti-inflammatory effect. Therefore, more detailed toxicological studies are necessary to assess the efficacy and safety of vitacoxib as a promising NSAIDs drug.

Conflict of interest

The authors declare that there are no conflicts of interest.

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