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Evaluation of dermal irritation and skin sensitization due to vitacoxib

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ABSTRACT

The prediction of side-effects is a key issue in the REACH initiative on chemicals in the preclinical testing of drugs. The dermal irritation and skin sensitization toxicity potential of a new molecule, vitacoxib, were invested in rabbits and guinea pigs in compliance with the Organization for Economic Cooperation and Development guideline. To assess dermal irritation, rabbits were dermally attached to vitacoxib for 72 h or repeated application. The results showed that no adverse reactions such as erythema and edema were observed throughout the test. In skin sensitization test, guinea pigs were sensitized to vitaoxib, positive and negative article for 24 h. No sensitization reaction was shown in the vitacoxib and negative group whereas severe sensitization was observed in the positive group. Based on these findings, vitacoxib does not cause dermal irritation and skin sensitization toxicity, and seems to be safe for animal use.

1. Introduction

Vitacoxib [2-(4-chloro-5-*p*-tolyl-1H-imidazol-1-yl)-5-(methyl sulfonyl) pyridine (C16H14ClN3O2S), Fig. 1], as known as a newly developed compound drug in China, belongs to coxibs of NSAIDs which are selective inhibitors of cyclooxygenase-2. Preclinical studies show that vitacoxib has exhibited excellently clinical efficacy and safety in fast-acting COX inhibitor that is potently, selectively and highly specific to COX-2 and has little effect on COX-1 isozymes in rodents [1]. It has been approved in dogs on controlling pain and inflammation associated with osteoarthritis in China [2].

New substances require appropriate toxicology evaluation before human and animal consumption, especially those substance with daily uses [3]. 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 [4,5]. As animal skin is quite sensitive to most of the chemical thus all new formulations must be tried on skin for a specified period of time to check if any irritation or erythema will occur. Studies on dermal irritation and skin sensitization are essential components for minimum set of toxicity screening which provides a fundamental characterization of the potential hazards of vitacoxib. However, information studies on dermal irritation and skin sensitization, acute, sub-chronic, and reproductive and development studies according to the relative toxicology guidelines caused by vitacoxib is still lacking. It is necessary to evaluate the risk of vitacoxib. In the past five years, several pre-clinical toxicity studies were conducted in our laboratory. The toxicity experiments are soon be published. The results of the acute toxicity showed that acute toxicity of vitacoxib was more than 5 000 mg/kg in SD rats and ICR mice [6]. The sub-chronic toxicity of vitacoxib showed that NOAEL was considered to be 20 mg/kg in SD rats [6]. Recently, we have been putting effort in dermal irritation experiments in rabbits and shin sensitization experiments in guinea pigs using vitacoxib in compliance with OECD guidelines.

2. Materials and method

Vitacoxib (Lot#PH-OBP-2-RSI-A-0-1; purity 99.7%), prepared by Beijing Orbiepharm Co., Ltd. (Beijing, PR China). Healthy, adult New Zealand rabbits (weighting 2.5–3 kg, age 18 weeks, half of male) and healthy adult guinea pigs (weighting 260–320 g, age 5–8 weeks, half of male), obtained by Beijing Vital River Laboratories (Charles River Laboratories) (laboratory animal reproduction license #SCXK (Beijing) 2011-0006). They were placed in polypropylene cages, provided with

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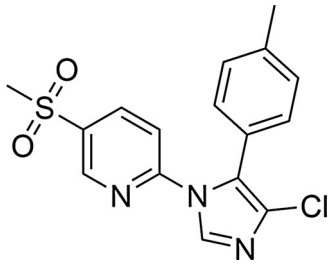

Fig. 1. Structure of the Vitacoxib.

Table 1
Dermal irritation study of vitacoxib at different time intervals in rabbits.

Materials	Erythema	Edema
Acute singled dermal irritation study		
1 h after removal of patches	0	0
24 h after removal of patches	0	0
48 h after removal of patches	0	0
72 h after removal of patches	0	0
Acute repeated dermal irritation study		
1 h after removal of patches	0	0
24 h after removal of patches	0	0
48 h after removal of patches	0	0
72 h after removal of patches	0	0

standard laboratory diet and water ad libitum. The animal facility was maintained at 22 °C–24 °C, a relative humidity of 55% ± 10%, and a 12 h light/dark cycle at 160–290 lx throughout the experiment. Animals were kept under acclimatization for eight days before application. This study was approved by the China Agricultural University Institutional Animal Care and Use Committee.

2.1. Acute dermal irritation

The acute dermal irritation study was performed in accordance with the OECD Guidelines 404 "Acute dermal irritation/corrosion" [7]. A positive control group received 0.8% w/v aqueous solution of formaldehyde as a standard irritant; a control group received placebo patch and a treated group received vitacoxib-loaded transdermal patch. Vitacoxib was mixed in a minimum amount of olive oil to create paste preparation for dermal application (2 mg/kg). Around 5 cm × 5 cm of rabbit's trunk was unclipped for experimental use. The test article was then applied under a 2.5 cm × 2.5 cm gauze patch to one intact site per rabbit and wrapped with an occlusive dressing. The animals were fitted with Elizabethan collars during the application. The test article was attached to skin for 4 h after which the wrappings and patches were removed. The remaining test articles were removed from the test site by gently washing with soaked in lukewarm water at the end of the exposure period, prior to scoring for dermal reactions. No dermal reactions were observed at 3 min, 1 h and 4 h after patch removal. The test was repeated with two additional rabbits to confirm the initial findings, since the rabbits in the initial test did not exhibit any dermal reaction. Meanwhile, three repeated dermal application studies were conducted. Applications were made for 7 consecutive days.

The test sites were scored for erythema and edema at 1 h, 24 h, 48 and 72 h post exposure with vitacoxib for rabbits in single dermal study and last administrated in repeated dermal study. Dermal responses were determined in accordance with OECD guideline [7]. Erythema and edema were scored on a scale of 0–4, with 0 showing no effect and 4 representing severe symptoms. For each animal, dermal response scores at 1 h, 24 h, 48 and 72 h after removal of the patches were summed and then divided by three to obtain a mean irritation score per time point. The results were compared to those of the control animals which received distilled water. The mean scores were summed and averaged to obtain the primary irritation index.

2.2. Skin sensitization experiment

The skin sensitization test was conducted in accordance with the OECD guideline [8] and modified per Banerjee method [9]. A day before the first induction, forty healthy guinea pigs were assigned to three groups: a positive control group (n = 10) that received 0.1% w/v 1-chloro-2,4-dinitrobenzene (CDNB) in 10% propylene glycol as a standard skin sensitizing agent, a placebo group (n = 10), and a transdermal patch-treated group (n = 20). Around 4 cm × 6 cm of left flank of each guinea pig was unclipped for experimental use. Transdermal patch was applied to the shaved area of each animal during the induction phase. On day 0, the first day of the first stage of induction, the agent was evenly spread on a lint attached to a patch of test tape. The patch was then applied to the shaved area, covered with an impermeable, adhesive plaster and secured in place by wrapping the trunk with

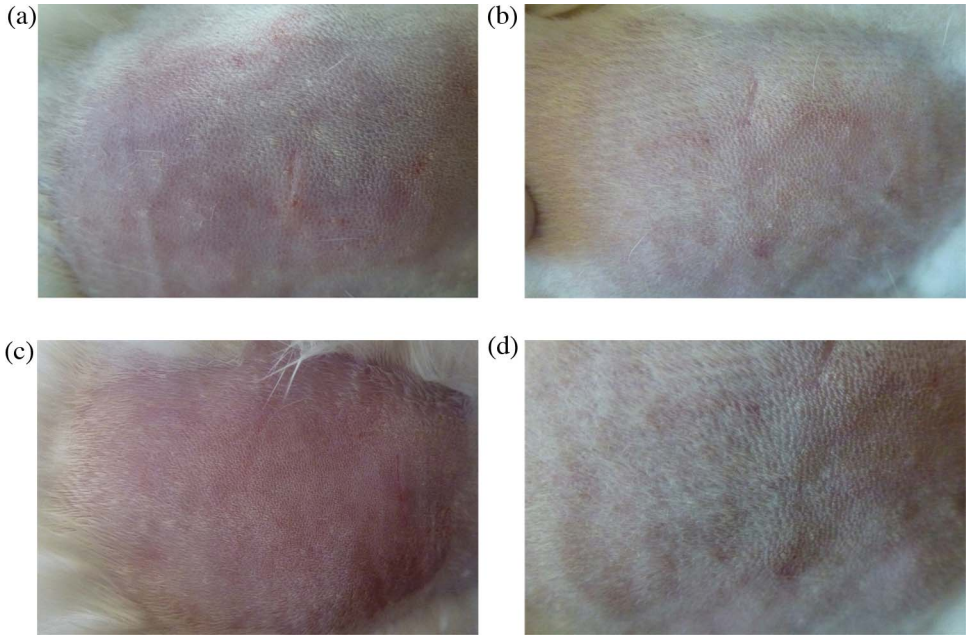

Fig. 2. (A) Dermal in singled group before administration. (B) Dermal in singled group at 72 h after administration. (C) Dermal in repeated group before administration. (D) Dermal in repeated group at 72 h after last administration.

Table 2
Group average weight grained, respectively.

Groups	Vitacoxib treated		Positive groups		Negative groups	
	♀(n = 10)	♂(n = 10)	♀(n = 5)	♂(n = 5)	♀(n = 5)	♂(n = 5)
0–7 day	3.63 ± 0.79	5.17 ± 1.15	3.14 ± 0.65	4.14 ± 1.36	2.95 ± 0.30	4.00 ± 0.86
8–14 day	3.26 ± 0.81	2.86 ± 1.66	3.62 ± 1.19	4.19 ± 0.68	4.19 ± 2.03	3.29 ± 2.10
15–31 day	3.71 ± 2.15	3.48 ± 1.41	3.69 ± 1.74	3.73 ± 1.35	3.18 ± 1.22	3.53 ± 0.87
Total average weight grained (g/d/guinea pig)	3.59 ± 1.31	3.72 ± 0.26	3.55 ± 1.19	3.92 ± 0.86	3.35 ± 0.31	3.58 ± 1.03

Note: Data were analyzed with SPSS 20.0 followed by T's test, all values are expresses as mean ± S.D. of each group.

Table 3
Skin sensitization study of vitacoxib in guinea pigs.

Number of sensitization animals		Erythema					Edema				Positive ratio
		0	1	2	3	4	0	1	2	3	
24 h	Positive groups	0	0	3	7	0	0	0	5	5	100
	Negative groups	10	0	0	0	0	10	0	0	0	0
	Vitacoxib treated	20	0	0	0	0	20	0	0	0	0
48 h	Positive groups	0	0	2	7	1	0	0	4	6	100
	Negative groups	10	0	0	0	0	10	0	0	0	0
	Vitacoxib treated	20	0	0	0	0	20	0	0	0	0
72 h	Positive groups	0	0	2	6	2	0	0	3	7	100
	Negative groups	10	0	0	0	0	10	0	0	0	0
	Vitacoxib treated	20	0	0	0	0	20	0	0	0	0

an elastic bandage for a 6-h closed application. Additional hair-removal and induction were carried out once weekly (on the day 6–7 and day 13–14). At 14 days after the third induction (day 28), the test was conducted. Treated sites in both induction and challenge phases were observed and scored 24, 48 and 72 h after patch removal. All reactions were evaluated using stand scoring code [10]. Body weights of all animals were measured before the study initiation; the animals were also observed for signs of toxicity, systemic effects and misbehaviors.

3. Results

3.1. Dermal irritation

The results of acute dermal irritation experiments (singled and repeated exposure) are summarized in Table 1. No clinical signs or changes in body weight were observed in any groups treated with vitacoxib. No dermal responses erythema/eschar or edema, were found in rabbits (Fig. 2).

3.2. Skin sensitization

The results of sensitization are shown in Tables 2 and 3. No clinical signs or changes in body weight were observed in any group. There were no statistically significant mean weight differences in body weights between the control and the treated groups from the first day of patch application through the end of the experiment (Table 2). The skin sensitization experiments were validated using the positive control group (CNDB), where positive dermal sensitization responses were observed (Fig. 3A). No sensitization was noted among guinea pigs that were challenged with transdermal patch or the placebo patch (Fig. 3B and C). Erythema and edema were not observed after the challenge in this experiment.

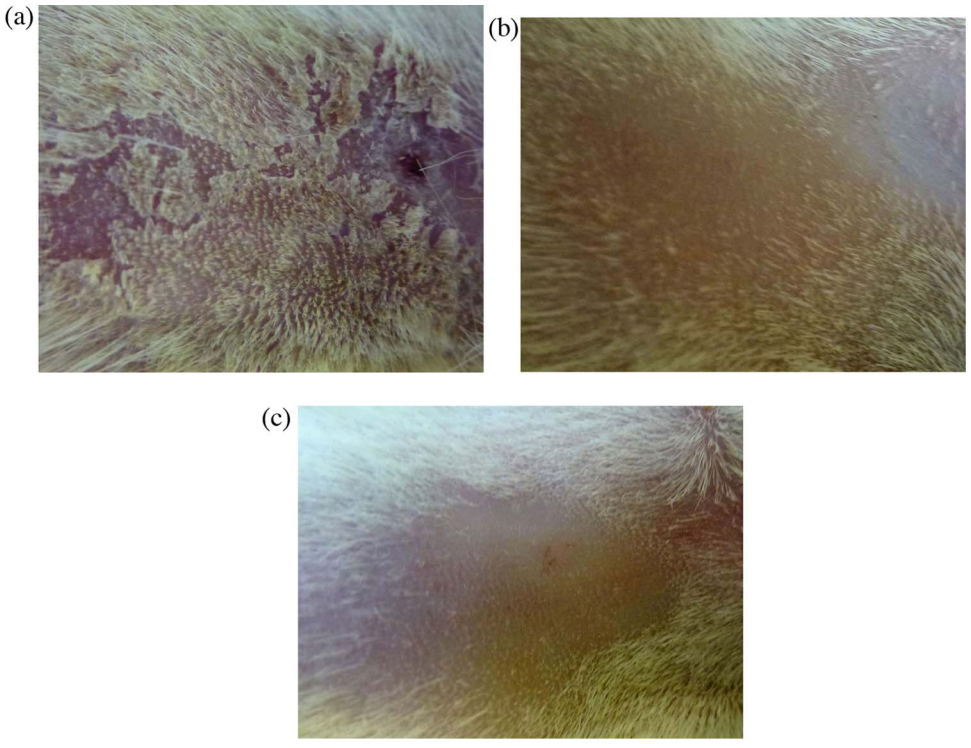

Fig. 3. (A) Positive group. (B) Treated group. (C) Negative group.

4. Discussion

Assessing risk is a function of hazard and exposure data. For new substances introduced into the marketplace, it is the recommended stepwise testing approach for developing scientifically sound data on the irritation of the substance[11,12]. This study was performed to evaluate if vitacoxib can cause dermal irritation and skin sensitization.

Erythema is redness of the skin or mucous membranes, caused by hyperemia of superficial capillaries [13]. Edema means swelling caused by fluid in body's tissue. The dermal irritation study in rabbits showed no dermal responses, including erythema and edema. The results of skin sensitization exhibited that vitacoxib do not cause skin sensitization among guinea pigs except for the positive control group. None of these animals showed any clinical signs and any overt signs of toxicity in dermal irritation study (singled and repeated administration) from the first day until the end of the experiment. No treatment-related misbehavior was noted in vitacoxib treated and negative groups, any gender of guinea pigs in the following 14 days before the patch administration was removed.

Loss of body weight is an important marker of gross toxicity which drastic toxicity or interference with absorption of nutrients will be reflected in body weight reduction [9]. There were no statistically significant mean weight differences in body weights between the control and the treated groups from the first day of patch application through the end of the experiment. Thus, it can be inferred that the vitacoxib has no tendency to produce drastic tissue destruction nor does it seem to interfere with absorption of the nutrients.

Based on the analysis of all the available parameters studied, it can be inferred that vitacoxib was tolerated in experimental rabbits and there were no dermal irritation and skin sensitization in animals. Further investigation in the areas of acute, mutagenicity, teratogenicity and 180-day toxicity effects of vitacoxib are soon to be published, to confirm the safety before using in clinical therapy.

Conflict of interest

The authors declare there are no conflicts of interest.

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