Preclinical Toxicology of AN2728, A Novel Borinic Acid Ester with Anti-Inflammatory Activity

Emily Y. Ip, Carolyn Bellinger-Kawahara, Irwin Heyman, Kirk R. Maples

Anacor Pharmaceuticals, Inc., 1060 East Meadow Circle, Palo Alto, CA 94303, USA

ABSTRACT

AN2728 is a new anti-inflammatory agent in development for the topical treatment of psoriasis. Summarized here are the preclinical toxicity studies with AN2728.

METHODS AND RESULTS

Rat 28-day subcutaneous toxicity studies

0, 10, 30, 100, and 300 mg/kg/day. Twenty male Sprague-Dawley rats were treated subcutaneously with AN2728 (in 100% propylene glycol) at 0, 10, 30, 100, or 300 mg/kg/day for 28 days. Rats were observed and scored daily at 1 h after dosing for clinical parameters or changes in behavior and appearance. On day 29, approximately 24 h after the last dose, the rats were humanely euthanized for blood and tissue collection. Blood samples were collected for clinical chemistries. Necropsies were performed, selected organs were removed and weighed, and the tissues were fixed, paraffin-embedded, sectioned, mounted, and stained with hematoxylin and eosin. Slides were evaluated by a board-certified veterinary pathologist.

Results. The subcutaneous administration of AN2728 at dosages of 10, 30, 100 or 300 mg/kg/day for 28 consecutive days resulted in drug-related histopathologic changes in the liver and spleen. Injection site changes were present in all treatment groups including vehicle control. A NOAEL dosage was not established for this study.

0, 0.3, 1, 3, and 10 mg/kg/day. Twenty male Sprague-Dawley rats were treated subcutaneously with AN2728 (in 100% propylene glycol) at 0, 0.3, 1, 3, or 10 mg/kg/day for 28 days. Rats were observed and scored daily at 1 h after dosing for clinical parameters or changes in behavior and appearance. On day 29, approximately 24 h after the last dose, the rats were humanely euthanized for blood and tissue collection. Blood samples were collected for clinical chemistries. Necropsies were performed, selected organs were removed and weighed, and the tissues were fixed, paraffin-embedded, sectioned, mounted, and stained with hematoxylin and eosin. Slides were evaluated by a board-certified veterinary pathologist.

Results. The only observed effects that can be attributed to AN2728 were the decrease in lymphocyte number and increase in neutrophil and monocyte numbers. These alterations were only significant at the 3 and 10 mg/kg/day doses. The no-observed-effect level (NOEL) for this study was determined to be 1 mg/kg/day. The no-observed-adverse-effect level (NOAEL) for this study was determined to be 10 mg/kg/day.

Minipig 14-day dermal toxicity study

Four male Göttingen minipigs were treated with daily occluded dermal application of 7 test materials. Each minipig was treated on 7 test sites with Ointment Vehicle, 0.25% AN2728 Ointment, 1% AN2728 Ointment, 5% AN2728 Ointment, Cream Vehicle, 1% AN2728 Cream, and 5% AN2728 Cream. Each test site was approximately 4 x 4 cm (approximately 0.25% total body surface area). The dose level was 5 µL/cm^2 for all test sites. Parameters included mortality, clinical signs, body weight, food consumption, clinical pathology, and gross necropsy. Blood samples for toxicokinetic analysis were collected on Day 14.

Results. There were no test article-related effects on hematology, coagulation, clinical chemistry, gross necropsy observations, absolute organ weight or organ-to-body weight ratios. AN2728 was not detected in plasma at any time point following 14 consecutive days of dosing when sampled on Day 14 (< 0.125 µg/mL). Repeated dermal dose administration of AN2728 at various concentrations and vehicle formulations was well tolerated systemically. Concentrations of 0.25%, 1%, and 5% AN2728 in Ointment Vehicle produced only minor localized irritation whereas concentrations of 1% and 5% AN2728 in Cream Vehicle produced more incidences of pronounced but transient localized irritation including eschar formation in some but not all animals. In conclusion, although both vehicle formulations were well tolerated, the 5% AN2728 in Ointment Vehicle produced a lower incidence of localized irritation at the site of application.

FIGURE 1. AN2728 (5-(4-cyanophenoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole)

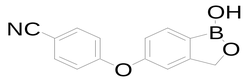

TABLE 1. Summary of AN2728 toxicity studies

Study	Species	AN2728 Doses Tested	Results
28-Day Systemic Safety	Rat	10, 30, 100, & 300 mg/kg/day (subcutaneous)	Histopathological liver effects at all doses
28-Day Systemic Safety	Rat	0.3, 1, 3, & 10 mg/kg/day (subcutaneous)	NOAEL = 10 mg/kg/day
14-Day Topical Safety	Minipig	0.25%, 1%, 5% Ointment 1%, 5% Cream (topical)	No systemic exposure & no significant dermal irritation
Functional Observational Battery & Maximum Tolerated Dose	Rat	100, 300, 500, & 1000 mg/kg (oral)	NOAEL = 500 mg/kg
HERG Channel	*in vitro*	1 µM	No significant HERG inhibition
Bacterial Reverse Mutation	*in vitro*	15 - 5000 µg/plate	No mutagenic activity +/- S9
Skin Sensitization	Mouse	1%, 5%, and 10% Solution (topical)	No skin sensitizing activity

CONCLUSION

AN2728, a novel compound in development for the topical treatment of psoriasis, exhibits a good safety margin in terms of systemic exposure after dermal application compared to systemic toxicity.

METHODS AND RESULTS (continued)

Safety pharmacology studies

Functional observational battery and maximum tolerated dose. Fifteen male Sprague-Dawley rats were dosed orally with 0, 100, 300, 500, or 1000 mg/kg AN2728. Behavioral, neurological (sensorimotor)/neuromuscular, autonomic observations, and body temperatures were recorded at 1 h post-dosing.

Results. At 1 h post-dose, a mild effect on gait was noted in 2 of 3 rats in the 300 mg/kg group, and in 1 of 3 rats in the 500 mg/kg group. At this same time, a moderate effect on gait was noted in 3 of 3 rats in the 1000 mg/kg group. No gait effect was seen in any of the rats in the 100 mg/kg group; all 3 rats appeared normal at this time. At 24 and 48 h, symptoms had disappeared in all of the AN2728 groups, with the exception of one rat in the 1000 mg/kg group which had a mild gait effect at 48 h, but not at 24 h. The NOEL and NOAEL of AN2728 were 100 and 500 mg/kg, respectively, when given by the oral route to male Sprague-Dawley rats. The maximum tolerated dose of AN2728 is greater than 1000 mg/kg.

Receptor binding, CYP450, and HERG channel. AN2728 was tested at 10 µM against a panel of 50 transmembrane and soluble receptors, ion channels, and monoamine transporters and against 5 cytochrome P450 isoforms. AN2728 was tested at 1 µM against the HERG channel.

Results. AN2728 did not significantly inhibit any of the receptors, ion channels, or transporters tested. AN2728 significantly inhibited CYP2C9 at 55%. Based on its HERG channel tail current inhibition, No significant HERG inhibition by AN2728 was identified.

Bacterial reverse mutation study

AN2728 was tested against four *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and one *Escherichia coli* strain (WP2 uvrA) using the plate incorporation method in the presence and absence of S9 activation. In the initial toxicity-mutation assay, toxicity was observed beginning at 500, 1500, or 5000 µg per plate. In the confirmatory mutagenicity assay, AN2728 was plated in triplicate at dose levels of 15, 50, 150, 500, 1500, and 5000 µg per plate.

Results. AN2728 demonstrated no mutagenic activity in the presence or absence of S9 activation.

Sensitization study

Twenty-five female CBA/J mice were used in the local lymph node assay. Five groups of 5 mice each were treated on the dorsal surface of both ears once per day for 3 days with 1%, 5% or 10% (w/v) of AN2728 or with the vehicle (acetone/ethanol 50:50, v/v) or the positive control (35% hexylcinnamaldehyde [HCA], v/v). The dose volume was 25 µL/ear. On Day 6, the mice were injected iv with 20 µCi of ^{3}H-thymidine in sterile saline. Five hours later, the mice were euthanized and the draining auricular lymph nodes were removed. The lymph node cells were precipitated with 5% trichloroacetic acid (TCA) and the pellets counted in a ß-scintillation counter to determine incorporation of the ^{3}H-thymidine. The positive control, HCA, resulted in a stimulation index (SI) of 3.46 indicating a positive response. This response was also statistically significant when compared to the vehicle control group ($p = 0.0001$). Exposure to AN2728 at 1%, 5% or 10% (w/v) resulted in stimulation indices of 0.87, 0.88, and 0.95, respectively. No statistically significant differences were observed when the mean log DPM of the test article treatment groups was compared to the vehicle treated group.

Results. Based on the criteria of this study, treatment with AN2728 at 1%, 5% or 10% (w/v) did not result in a stimulation index of 3 or greater and hence was not considered to have skin sensitizing activity.

Poster will be available at www.anacor.com after February 6th, 2007