Effects of Vinclozolin on Nrf2 Testicular Expression in Sexually Mature Boars

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• Blood samples were collected on Day 0 prior to the beginning of treatment and on Days 3 and 10 for analyses of oxidative stress within the testes of sexually mature boars. Vinclozolin (VCZ), a dicarboximide fungicide, has been associated with ovidendrigenic effects in animal models. Preliminary data suggest that adult boars are more susceptible to oxidative effects of VCZ and its metabolites. Further development and refinement of a porcine reproductive model might be a more accurate predictor of the reproductive risks associated with human EDC exposures. Vinclozolin (VCZ) will affect the testicular expression as well as reproductive effects of VCZ and its metabolites.

**OVERALL RESEARCH OBJECTIVE**

• Further development and refinement of a porcine model for human risk assessment involving exposures to endocrine disrupting chemicals (EDCs).

**In Vivo Experimental Approach**

Thirty-two post-pubertal boars were dosed orally with 0, 0.25, 5 and 100 mg of VCZ/kg BW/day (four replicates of n = 2 in each treatment group; total of eight animals per replicate) for 15 days (Days 0-14).

• Blood samples were collected on Day 0 prior to the beginning of treatment and on Days 3 and 10 for analyses of testicular oxidative stress and total estrogen concentrations.

• Semen samples were collected prior to Day 0 and on Days 3, 7, 10, and 14 for computer-assisted sperm analysis (CASA) and were stored in cryopreservation medium.

• Enzymes were used to prepare tissue sections for histological analysis.

• Sertoli cells, pachytene spermatocytes, round spermatids of the boar in the 100 mg VCZ/kg BW/day were stained with muscle and kidney sections stained as positive controls.

• After rinsing with deionized water, the sections were treated with Romulin Red (RAE810) chromogen to detect the antigen, and counterstained with hematoxylin.

• Sections were incubated with 3% H2O2 buffer block to reduce non-specific background staining and were pretreated with BLOX (BG1000, pH 9).

• After rinsing with deionized water, the slides were loaded into the Intellipath FIX for automated IHC staining.

• A Sniper (BS966H) incubation was performed to further reduce non-specific background staining.

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**Nrf2 Background Information**

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor which regulates endogenous antioxidant responses. Under normal conditions, Nrf2 is localized in the cytoplasm. Under conditions of oxidative stress, Nrf2 translocates to the nucleus to induce the transcription of genes regulating cellular responses to oxidative stress. Nrf2 localization and expression in rodent testes change in response to oxidative stress. Oxidative stress is one mechanism by which EDCs damage testes, and VCZ and its metabolites can induce oxidative damage.

**RESEARCH HYPOTHESES FOR TESTICULAR EXPRESSION OF Nrf2**

• Subacute oral exposure of sexually mature boars to VCZ will affect the testicular expression as well as localization of Nrf2, in a dosage-dependent fashion.

**Specific Aims**

• Evaluate the effects of VCZ dosage on oxidative stress within the testes of sexually mature boars.

• Characterize VCZ-related changes in Nrf2 expression using immunohistochemical (IHC) staining.

**Endocrine Disruptor Background Information**

Many chemicals can interfere with normal endocrine function (i.e., endocrine disrupting chemicals or EDCs) and cause adverse reproductive effects, especially in experimental rodent models. Swine are more similar to humans than rodents, so the bovine model might be a more accurate predictor of the reproductive risks associated with human EDC exposures. Vinclozolin (VCZ), a dicarboximide fungicide, has been associated with ovidendrigenic effects in animal models. Preliminary data suggest that adult boars are more susceptible to the adverse reproductive effects of VCZ and its metabolites.

**Mechanism of Action of Nrf2**

Figure 3. Without oxidative stress, Nrf2 is kept in the cytoplasm by Kelch-like-ECH-associated protein 1 (KEAP1) and degraded by ubiquitination. In the presence of reactive oxygen species (ROS), more Nrf2 protein is produced, and Nrf2 translocates into the nucleus. Nuclear Nrf2 forms a heterodimer with a small Maf protein and binds to the Antioxidant Response Element (ARE) in the upstream promoter region of many genes involved in cellular responses to oxidative stress.

**Effects of VCZ and Metabolites on Interstitial Cell Density, Hormone Concentrations, and Sperm Parameters**

Effect of VCZ on testicular interstitial cell density in this study are shown in A (** and *** denote P < 0.05 and P < 0.001 with respect to pairwise comparisons between treatment groups), and are reflective of Interstitial Cell hyperplasia and associated changes in the mean serum concentrations of immunoreactive testosterone and total estrogen. Boars treated with either 5 or 100 mg VCZ/kg BW/day had increased mean percentages of abnormal sperm on Day 14 (29 ± 16.8% and 54.5 ± 9.9%, respectively), as compared to pre-exposure and Day 7 sperm, as reflected by ubiquitination of abnormal sperm (B, C, and D).

**Preliminary Nrf2 IHC Experimental Results**

The samples were incubated with 3% H2O2 buffer block to reduce non-specific background staining and were pretreated with BLOX (BG1000, pH 9).

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**SUMMARY OF PRELIMINARY FINDINGS**

• Porcine testicular Nrf2 expression and localization appear to be dependent on the dosage of Vinclozolin.

• Nrf2 appears to be a useful biomarker for porcine testicular responses to EDC-induced oxidative stress.