VALIDATION OF A FUNCTIONAL OBSERVATIONAL BATTERY IN THE MINIPIG FOR REGULATORY NEUROTOXICITY ASSESSMENTS

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INTRODUCTION
Background: A functional observational battery (FOB) is recommended as the first tier neurotoxicity screening in the preclinical safety pharmacology testing guidelines. The minipig has increasingly been used in regulatory toxicology studies; however, no current FOB protocol is available for the neurotoxicity testing in this species.

Purpose: To validate a minipig functional observational battery protocol.

Methods: A crossover study with Sinclair minipigs was performed to capture effects of amphetamine, ketamine, and diazepam. The minipigs were observed in their home cage, were video-recorded for 10 minutes in a confined open field and went through a neurological exam.

Results: Both ketamine and diazepam treated minipigs showed reduced interest in environmental changes and increased exploration interest, and had muscle dysfunction and gait impairment. On the other hand, unique and even opposite effects were also observed between ketamine and diazepam, which might reflect their unique mechanisms of action in treated minipigs. Effects of ketamine and diazepam were consistent with their roles in suppressing central nervous system (CNS) functions. Amphetamine is a CNS stimulant, which promotes dopamine-associated brain activities. Amphetamine treated minipigs were consistently shown to be hypoactive and to display increased interest to environmental changes and reduced exploring activities. Amphetamine also increased locomotion and induced biphasic behavior effects in the treated animals. The shared effects of ketamine and diazepam might result from their interference with dopamine functions in CNS, in contrast to those of amphetamine.

Conclusion: The Sinclair minipig is suitable for functional observational battery (FOB) evaluation of chemicals in preclinical safety pharmacology testing, and a reproducible quantitative approach is described.

Keywords: Neurotoxicity Testing, Functional Observational Battery, Sinclair Minipig, Amphetamine, Ketamine, Diazepam

EXPERIMENTAL METHODS
Animals:
- Minipig (4 male)
- Age: 7 - 10 months

Procedure:
- Accommodate for 7 days
- Weekly body weight
- Intramuscular dosing (vehicle, amphetamine, ketamine, & diazepam (test article))
- Each animal had no more than one test article treatment in each week.

Functional Observational Battery:
- Specification of FOB parameters: 1) normal behavior/response, 2) above-normal behavior/response, 3) absence of normal behavioral responses
- One-time observation of animals in their home cages
- Open field observation: video-recorded for 10 minutes in an 8.8 x 8 feet enclosure
- Neurological examination: examined in a slin or on the ground by a clinical veterinarian

SUMMARY
- Variability (moderate to high) was observed only in 12 continuous and count variables.
- Variability attributed to individual animals was higher than those from the repeated daily observations for most of the 12 parameters.
- Both ketamine and diazepam were shown to reduce the interest in environmental changes and to increase exploration interest of treated minipigs, as well as to cause muscle dysfunction and gait impairment.
- Ketamine was shown to impair pupillary reflex and to induce nystagmus and to affect proprioceptive positioning whereas diazepam to impair blink reflex in multiple animals.
- Consciousness and alertness were compromised in diazepam treated but not in ketamine treated animals.
- Ketamine increased gland secretion and heart and respiratory rates, while diazepam decreased respiration.
- Unlike ketamine which reduced desire of motility, diazepam increased ambulation and bouts of locomotion in treated animals.
- Amphetamine increased the interest of treated minipigs to environmental changes, and reduced exploring activities.
- Amphetamine increased the desire of motility and induced biphasic effect at low and high doses in a number of behavior parameters.

DISCUSSION & CONCLUSION(S)
- Amphetamine is a CNS stimulant, and ketamine and diazepam are CNS suppressors.
- A FOB protocol has been validated in the minipig.

REFERENCES