TOXICOLOGY STUDIES CONDUCTED IN BEAGLE DOGS AT IITRI

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ABSTRACTS PREPARED BY IITRI

[1] Silvestrol, NSC783538

[2] Rocaglamide, NSC326408

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ABSTRACT

In this study undertaken to determine the toxicity profile and plasma elimination kinetics of Silvestrol (NSC-783538) in beagle dogs, NSC-783538 was administered to male and female dogs (one per sex per group) at dose levels of 0 (vehicle), 0.03, 0.015 and 0.0075 mg/kg/day in a vehicle of 20% 2-hydroxypropyl beta-cyclodextrin (HPBCD) in sterile water. NSC-783538 or vehicle was administered by intravenous bolus injection once daily for 5 days followed by 2 days of no treatment, then another cycle of once daily administration for 5 days followed by 2 days of no treatment (observation period). Dosing occurred on a sequential weekly basis. The dose level for Group 3 was determined based on observations from Group 2; likewise, the dose level for Group 4 was determined based on observations from Groups 2 and 3. Experimental endpoints consisted of moribundity/mortality and clinical observations; body temperature; body weight; clinical pathology parameters (hematology, clinical chemistry, blood gas and coagulation); pulse oximetry; respiratory parameters; plasma drug levels; and necropsy and histopathological evaluations. Plasma drug results were provided to the Sponsor for determination of pharmacokinetics parameters.

Intravenous bolus injection of NSC-783538 once daily for 5 days followed by 2 days of no treatment, then another cycle of once daily administration for 5 days followed by 2 days of no treatment (observation period) to one dog/sex/group at dose levels of 0.03, 0.015 and 0.0075 mg/kg, resulted in mortality of the male dog at the 0.03 mg/kg dose level. This dog exhibited a loss of body weight prior to death. Treatment-related effects on the respiratory system as evidenced by clinical observations of dyspnea, rapid respiration and cyanosis were also observed in this dog prior to its death. These clinical observations were associated with decreases in pO_2 and $\%O_2$ blood levels, along with respiratory changes consisting of increased respiratory rate, decreased tidal volume and increased minute volume. At necropsy, the lungs of this dog were mottled, which correlated microscopically with neutrophilic infiltration in the septum of the lung. The neutrophilic infiltration was associated with an increase in total leukocyte count (WBC) and absolute and relative neutrophil counts. Fibrinogen levels were also increased in this dog. The female dog at the 0.03 mg/kg dose level survived the dosing; however, treatment-related effects seen in this dog consisted of body weight loss, a decrease in $\%O_2$, mottled lungs at necropsy and neutrophilic infiltration in the septum of the lungs.

ABSTRACT

Treatment-related respiratory system effects seen at the 0.015 mg/kg dose level consisted of labored (irregular/shallow) breathing in the male dog and rapid/irregular respiration in the female dog. Microscopic changes were seen in the lung of the male dog only and consisted of neutrophilic infiltration in the septum, which correlated with an increase in WBC and absolute and relative neutrophil counts.

A treatment-related decrease in reticulocyte count was seen in the male dog at the 0.03 and 0.015 mg/kg dose levels. However, the decrease was not associated with any evidence of bone marrow toxicity.

The only treatment-related effect seen at the 0.0075 mg/kg dose level was minimal septal neutrophilic infiltration in the lung of the male dog.

Kinetics for the drug were linear over the dose range studied. AUC (area under curve; plasma concentration vs. time) and C_{max} (maximum observed concentration) increased in a roughly dose proportional manner. As would be expected for a drug with a terminal half-life of approximately 2-3 hours, the drug did not accumulate when dosed as in this study, as evidenced by the similarity between Day 1 and Day 12 AUC and C_{max} values.

In conclusion, intravenous bolus injection of Silvestrol (NSC-783538) for two 5-day dosing cycles resulted in mortality at the 0.03 mg/kg level and toxic effects on the respiratory system at 0.03, 0.015 and 0.0075 mg/kg. Given that a male animal was found dead and that inflammatory infiltration was noted microscopically, male dogs may be more sensitive to the toxic effects of Silvestrol than females.

DOSE RANGE-FINDING AND PHARMACOKINETICS STUDY OF SILVESTROL (NSC-783538) IN BEAGLE DOGS

COMPREHENSIVE SUMMARY

Study Title: Dose Range-Finding and Pharmacokinetics Study of Silvestrol (NSC-783538) in Beagle Dogs

Test Article: NSC-783538

Vehicle: 20% HPBCD (2-hydroxypropyl-beta-cyclodextrin) in sterile water

Dose Groups:

Group Number	Treatment	Dose Level (mg/kg/day)
1	Vehicle	0
2	NSC-783538	0.03
3	NSC-783538	0.015
4	NSC-783538	0.0075

Dose Route and Administration: Intravenous bolus injection once daily for 5 days followed by 2 days of no treatment, then another cycle of once daily administration for 5 days followed by 2 days of no treatment (observation period).

Dosing occurred on a sequential weekly basis. The dose level for Group 3 was determined based on observations from Group 2; likewise, the dose level for Group 4 was determined based on observations from Groups 2 and 3.

Species: Canine (purebred beagle)

Number and Sex: 4 males and 4 females (1 dog per sex per group)

Age at Dosing: Approximately 5 to 6 months

Body Weight Range at Dosing Start: 8.06 to 8.96 kg (males); 7.22 to 8.10 kg (females)

RESULTS:

Mortality: The male dog dosed at 0.03 mg/kg (Group 2) was found dead on Day 11. All other test article-treated dogs survived until terminal necropsy on Day 15. Control dogs were taken off the study and returned to the animal pool at the end of the study.

Clinical Signs:

0.03 mg/kg (Group 2)

Clinical observations beginning on Day 4 in the male dog consisted of rapid respiration (severe), hypoactivity, coldness to touch, cyanosis, paleness, dyspnea, scant/no feces and thinness. This dog was found dead on Day 11. For the female dog, clinical observations consisted of paleness and thinness.

COMPREHENSIVE SUMMARY

Clinical Signs (cont.):

0.015 mg/kg (Group 3)

Clinical observations seen beginning on Day 4 in the male dog consisted of thinness, hypoactivity, labored (irregular/shallow) breathing and loose stool. For the female dog, clinical observations beginning on Day 8 consisted of thinness and rapid/irregular respiration.

0.0075 mg/kg (Group 4)

The male dog appeared thin starting on Day 5; however, no adverse clinical signs were observed for the female dog.

Body Temperature: No treatment-related effects on body temperature were seen in any dog at any dose level. However, for the male dog in Group 2 (0.03 mg/kg) that was "cold to touch" on Day 10 and found dead on Day 11, body temperature was not recorded on those days (not scheduled).

Body Weight: The male dog in Group 2 (0.03 mg/kg) lost 1.32 kg (16% decrease) of body weight from Days 1 through 10. The female dog in Group 2 lost 0.84 kg (11% decrease) of body weight from Days 1 through 12 (nadir) and then regained 0.3 kg body weight between Days 12 and 15. The body weight loss in both of these dogs was considered treatment-related. No treatment-related effects on body weight were seen at the 0.015 or 0.0075 mg/kg dose levels.

Clinical Pathology and pO_2: WBC count was increased in the male dog in Group 2 (0.03 mg/kg) on Day 10 compared to the pre-test value. This increase was associated with an increase in absolute and relative neutrophil counts in this dog on Day 10. The increase in neutrophils was also associated with a corresponding decrease in absolute and relative lymphocyte counts on Day 10. Similar effects were seen in the male dog in Group 3 (0.015 mg/kg) on Day 10; however, WBC and neutrophil counts had returned to baseline values on Days 12 and 15.

RBC counts, in addition to HGB and HCT levels, were increased in the male dog in Group 2 (0.03 mg/kg) on Day 10. These increases were, however, considered related to dehydration of this dog, rather than a direct effect of Silvestrol treatment. In addition, the absolute and relative reticulocyte counts were decreased in this dog on Day 10. However, the toxicological significance of the decrease in reticulocyte count is unknown, since no evidence of any bone marrow toxicity was seen in this dog (or any study dog). Reticulocyte counts were also decreased in the male dog in Group 3 (0.015 mg/kg) on Day 10. No treatment-related effects – including effects on WBC, neutrophil or lymphocyte counts – were seen in the Group 2, 3 or 4 female dogs and male dogs in Group 4.

No treatment-related effects on any clinical chemistry parameters were seen in any dog at any dose level.

COMPREHENSIVE SUMMARY

Clinical Pathology and pO_2 (cont.): The only treatment-related effect on blood gas (pO_2) was a decrease in pO_2 level in the male dog in Group 2 (0.03 mg/kg) on Day 10.

The only treatment-related effect on coagulation parameters was an increase in fibrinogen levels in the male dog in Group 2 (0.03 mg/kg) on Days 8 and 10.

Pulse Oximetry: Oxygen saturation ($\%O_2$) levels were decreased in both the male and female dogs in Group 2 (0.03 mg/kg) on Days 8 and 10, and in the female dog on Day 12. Oxygen saturation levels in the female dog returned to normal by Day 15. No effect was seen in Groups 3 or 4.

Respiratory Assessment: An increase in respiratory rate, with an associated decrease in tidal volume and increase in minute volume, was seen in the male dog in Group 2 (0.03 mg/kg) on Days 8 and 10. No changes in respiratory parameters were seen in the female dog in Group 2 or in any dog in Groups 3 or 4.

Gross Pathology: Test article-related gross pathology findings were seen in the lung (mottled) in the male and female dogs dosed at 0.03 mg/kg (Group 2). As noted above, the male dog in Group 2 was found dead on Day 11.

Histopathology: For the dogs dosed at 0.03 mg/kg (Group 2; male was found dead on Day 11), microscopic findings were noted in the lung [infiltration, neutrophilic, septal, hemorrhage (male only), single cell necrosis (male only), accumulation of fibrin (male only), fibrosis (male only), hypertrophy of pneumocytes type 2 (male only)]; bronchial lymph node [lymphoid depletion (male only)]; skin (necrosis, sweat gland); spleen [white pulp depletion (male only)]; and thymus [atrophy (male only)]. For the dogs dosed at 0.015 mg/kg (Group 3), microscopic findings were noted in the lung [infiltration, neutrophilic, septal (male only)]. For the dogs dosed at 0.0075 mg/kg (Group 4), microscopic findings were noted in the lung [infiltration, neutrophilic, septal (male only)].

Plasma Elimination Kinetics: Kinetics for the drug were linear over the dose range studied. AUC and C_{max} increased in a roughly dose proportional manner. As would be expected for a drug with a terminal half-life of approximately 2-3 hours, the drug did not accumulate when dosed as in this study, as evidenced by the similarity between Day 1 and Day 12 AUC and C_{max} values.

CONCLUSION: Intravenous bolus injection of Silvestrol (NSC-783538) to male and female dogs for two 5-day dosing cycles resulted in mortality at the 0.03 mg/kg level and toxic effects on the respiratory system at 0.03, 0.015 and 0.0075 mg/kg. Given that a male animal was found dead and that inflammatory infiltration was noted microscopically, male dogs may be more sensitive to the toxic effects of Silvestrol than females.

DOSE RANGE-FINDING AND PHARMACOKINETICS STUDY OF ROCAGLAMIDE (NSC-326408) IN BEAGLE DOGS

ABSTRACT

This study was performed to determine the toxicity profile and plasma elimination kinetics of Rocaglamide (NSC-326408) in beagle dogs. NSC-326408 was administered to male and female dogs (one per sex per group) at dose levels of 0 (vehicle), 0.03, 0.09 and 0.18 mg/kg/day in a vehicle of 20% 2-hydroxypropyl beta-cyclodextrin (HPBCD) in sterile water. NSC-326408 or vehicle was administered by intravenous bolus injection once daily for 5 days followed by 2 days of no treatment, followed by a second cycle of once daily administration for 5 days followed by 2 days of no treatment. Dosing of each treatment group occurred sequentially, one week apart. The dose level for Group 3 (0.09 mg/kg/day) was determined based on observations from Group 2 (0.03 mg/kg/day); likewise, the dose level for Group 4 (0.18 mg/kg/day) was determined based on observations from Groups 2 and 3. [Note: On Day 1 for the high dose group, dogs were dosed at a level of 0.27 mg/kg, but exhibited clinical signs of toxicity (emesis, salivation, pale mucous membranes, coldness to touch, diarrhea). Therefore, the dose was reduced to 0.18 mg/kg for remainder of the study, starting on Day 2.] Experimental endpoints consisted of moribundity/mortality and clinical observations; body temperature; body weight; clinical pathology parameters (hematology, clinical chemistry, blood gas and coagulation); pulse oximetry; respiratory parameters; and necropsy and histopathological evaluations. Plasma and urine samples were provided to the Sponsor for determination of drug levels and calculation of pharmacokinetics parameters.

Clinical signs of toxicity consisted primarily of emesis and salivation in both the male and female dogs at the 0.09 and 0.18 mg/kg dose levels. In addition, both dogs at the 0.18 mg/kg dose level exhibited signs of possible hepatotoxicity (as indicated by increased alkaline phosphatase levels) and inflammation (as indicated by increased fibrinogen levels). Male dogs in the mid and high dose groups and female dogs in the high dose group also demonstrated transient decreases in platelet counts. Increases in plasma cholesterol were seen in the high dose group in both sexes. No evidence of hepatic, bone marrow, or other toxicity was identified during microscopic examination of tissues. No evidence of respiratory toxicity was seen at any dose level.

In conclusion, intravenous bolus injection of Rocaglamide (NSC-326408) to male and female dogs for two 5-day dosing cycles resulted in emesis at the 0.09 and 0.18 mg/kg dose levels. Elevations in alkaline phosphatase, cholesterol, and fibrinogen, and transient decreases in platelet counts were seen in both sexes receiving drug at 0.18 mg/kg.

DOSE RANGE-FINDING AND PHARMACOKINETICS STUDY OF ROCAGLAMIDE (NSC-326408) IN BEAGLE DOGS

COMPREHENSIVE SUMMARY

Study Title: Dose Range-Finding and Pharmacokinetics Study of Rocaglamide (NSC-326408) in Beagle Dogs

Test Article: NSC-326408

Vehicle: 20% HPBCD (2-hydroxypropyl-beta-cyclodextrin) in sterile water

Dose Groups:

Group Number	Treatment	Dose Level (mg/kg/day)
1	Vehicle	0
2	NSC-326408	0.03
3	NSC-326408	0.09
4	NSC-326408	0.18*

* *On Day 1, dogs in Group 4 were dosed at a level of 0.27 mg/kg, but exhibited clinical signs of toxicity. The dose was reduced to 0.18 mg/kg for remainder of the study, starting on Day 2.*

Dose Route and Administration: Intravenous bolus injection once daily for 5 days followed by 2 days of no treatment, then another cycle of once daily administration for 5 days followed by 2 days of no treatment (observation period).

Dosing of each treatment group occurred on a sequential basis, and was separated by one week. The dose level for Group 3 was determined based on observations from Group 2; likewise, the dose level for Group 4 was determined based on observations from Groups 2 and 3.

Species: Canine (purebred beagle)

Number/Sex of Animals: 4 males and 4 females (1 dog per sex per group)

Age at Dosing: Approximately 6 months

Body Weight Range at Dosing Start: 9.44 to 10.16 kg (males); 7.72 to 8.40 kg (females)

RESULTS:

Mortality: All test article-treated dogs survived until terminal necropsy on Day 15 (Groups 3 and 4) or Day 16 (Group 2). Control dogs (Group 1; dosed with vehicle only) were taken off the study and transferred to the animal pool on study Day 29 (Study Day 15 for Group 4).

DOSE RANGE-FINDING AND PHARMACOKINETICS STUDY OF ROCAGLAMIDE (NSC-326408) IN BEAGLE DOGS

COMPREHENSIVE SUMMARY

Clinical Signs:

0.03 mg/kg (Group 2): No clinical observations were noted in the male dog receiving drug at 0.03 mg/kg/day. Clinical observations in the female dog receiving drug at 0.03 mg/kg/day were limited to emesis on Day 11 only.

0.09 mg/kg (Group 3): In the male dog receiving drug at 0.09 mg/kg/day, salivation was observed on Days 3, 5, 8 and 10-12, and emesis was seen on Days 1-5, 10 and 12. Clinical observations in the female dog receiving drug at 0.09 mg/kg/day were limited to salivation on Day 10 only.

0.18 mg/kg (Group 4): After receiving one dose of drug at 0.27 mg/kg, both dogs exhibited salivation, emesis (phlegm) and pale mucous membranes. The male dog also exhibited shaking/shivering and diarrhea; the female dog was cold to the touch. On the basis of this toxicity, the dose was lowered to 0.18 mg/kg for the remainder of the study. In the male dog, administration of drug at 0.18 mg/kg/day induced emesis on Days 2-5 and 9-10, salivation on Days 2-5 and 10-12, and diarrhea on Day 9. In the female dogs, administgration of drug at 0.18 mg/kg/day induced emesis on Days 2-5 and salivation on Days 3-5 and Day 10.

Body Temperature: No treatment-related effects on body temperature were seen in any dog at any dose level. Body temperature was not scheduled for measurement and was not recorded on Day 1 for the female dog that was "cold to touch" following administration at the 0.27 mg/kg dose.

Body Weight: No treatment-related effects on body weight were seen at any dose level.

Clinical Pathology and pO_2: No treatment-related effects on pO_2 were seen in any dog at any dose level. Increased alkaline phosphatase levels and fibrinogen levels were seen in both the male and female dog in Group 4 (0.18 mg/kg) on Days 3 through 15; both sexes in this dose group also demonstrated transient decreases in platelet counts.

Pulse Oximetry: No effects on oxygen saturation ($\%O_2$) levels were seen at any dose level.

Respiratory Assessment: No effects on respiratory rate, tidal volume or minute volume were seen at any dose level.

Gross Pathology and Histopathology: Hemorrhage at the site of injection was seen at several time points in dogs in the mid and high dose groups. No test article-related microscopic findings were seen at any dose level.

CONCLUSION: Intravenous bolus injection of Rocaglamide (NSC-32640 8) to male and female dogs for two 5-day dosing cycles resulted in emesis at the 0.09 and 0.18 mg/kg dose levels. Elevations in alkaline phosphatase, cholesterol, and fibrinogen, and transient decreases in platelet counts were seen in both sexes receiving drug at 0.18 mg/kg.