

Overall Toxicology Summary

Dextromedetomidine, the dextro isomer of medetomidine, is a potent and selective α_2 -agonist with sedative/hypnotic, hypotensive and analgesic effects. The clinical use is to be in the ICU for postoperative sedation and analgesia reducing anesthetic and narcotic analgesic requirements. The α_2 -agonist activity reduce adrenergic activation, producing a sympatholytic effect, bradycardia and a reduced cardiovascular response to noxious stimuli.

The administration of dexmedetomidine is by intravenous infusion with maximum total daily doses of less than 20 $\mu\text{g}/\text{kg}$ and blood concentrations of less than 30 ng/ml. The maximum recommended human dose (MHRID) is about 17.8 $\mu\text{g}/\text{kg}/\text{day}$ (0.6919 mg/m^2) and the initial recommended loading dose is 1 $\mu\text{g}/\text{kg}$.

Acute Toxicity

The acute toxicity, after rapid iv bolus injection, mice demonstrated a sex difference and the highest non-lethal dose was 5000 $\mu\text{g}/\text{kg}$ in females and 1000 $\mu\text{g}/\text{kg}$ in males; 20,000 and 10,000 $\mu\text{g}/\text{kg}$ in females and males, respectively, after subcutaneous injections. In rats, the highest non-lethal dose after rapid iv bolus was 1000 $\mu\text{g}/\text{kg}$ in both sexes and after subcutaneous injection it was 5000 in males and 1000 $\mu\text{g}/\text{kg}$ in females (vol 23/pg 222). Considering 1000 $\mu\text{g}/\text{kg}$ was non-lethal in mice and rats after iv injection, the ratio of these doses in mg/m^2 and the daily MRHID (loading dose, 1.0 $\mu\text{g}/\text{kg}$) is 120X and 240X, respectively. In comparison with the MRHID of 17.8 $\mu\text{g}/\text{kg}$ for a 24 hour infusion, these non-lethal dose in the mice and rats were still 5X and 9X the human dose on a mg/m^2 basis. The estimated highest non-lethal dose in dogs was about 1000 $\mu\text{g}/\text{kg}$ following rapid intravenous injection and this is approximately 30X the MRHID on mg/m^2 basis.

The clinical signs at high non-lethal doses was the same in both rats and mice; sedation, piloerection, exophthalmia, salivation, tachypnea and clonic convulsions. The signs at lethal doses also included jumping, dyspnea, chromodacryrhea, red urine and red fluid from nose and mouth.

Subacute Toxicity:

The toxicity of repeat dosing was examined in both rats and dogs in studies 4 weeks in duration with im or iv administration. The rats were tested after sc injection for 4 weeks and after intrathecal administration for 2 weeks. The dogs were tested for 2 weeks of intrathecal administration after a single dose pilot study.

The im administration of 20, 100 and 500 $\mu\text{g}/\text{kg}$ to rats produced the pharmacological effects of sedation, piloerection and cloudy corneas in a dose related manner. The two high doses also produced exophthalmus and glucosuria and some hypertrophy of the adrenal glomerulosa. The toxicological effects were seen at 100 and 500 $\mu\text{g}/\text{kg}$ decreased body weight gain and thymus

NDA 21-038

weight and dose-related pulmonary perivascular hemosiderin laden macrophages. The high dose males also had decreased weight of testes, seminal vesicles and prostate. The NOAEL was the 20 $\mu\text{g}/\text{kg}$ dose. The 4 week sc study in rats used the same doses and produced the same effects although there was an elevated alkaline phosphatase level at 100 and 500 $\mu\text{g}/\text{kg}$ and decreased uterine weight at 500. The NOEL was 20 $\mu\text{g}/\text{kg}$ in the males and possibly 100 in the females. The intravenous dosing in rats was at 10, 40 and 160 $\mu\text{g}/\text{kg}$ and there was still the dose-related pharmacological sedation, piloerection and exophthalmos at the high dose. The glucosuria, corneal opacities and elevated alkaline phosphatase at 40 and 160 and increased liver weight for females the high dose. The 10 $\mu\text{g}/\text{kg}$ dose was considered the NOEL. All of these NOELs produced a ratio of less than 1.0 compared to the MHRID in mg/m^2 the daily dose of 17.8 $\mu\text{g}/\text{kg}/\text{day}$.

In dogs, the iv study used doses of 10, 50 and 250 $\mu\text{g}/\text{kg}$ for 4 weeks. The sedation, ataxia, muscle twitches, miosis and slowed respiration rate were dose related pharmacological effects at all doses. The toxicological signs were seen mainly in the high dose animals, elevated ornithine carbamyl transferase, alkaline phosphatase and hepatic apoptotic bodies and serum GGT level were significantly elevated in both MD and HD groups. There was some increased liver weight and decreased thymus weight in the lower doses but 10 $\mu\text{g}/\text{kg}$ could be considered LOAEL and the ratio with MHRID was 0.3. The im study in dogs used the same doses, 10, 50 and 250 $\mu\text{g}/\text{kg}$, but was run once with males and once with females and no pathology reports were supplied for the control and LD animals. The toxicity was similar to the iv study, with elevated alkaline phosphatase, ALT, in the HD males and females and in the MD males. The MD and HD females had eosinophilic inclusions in the hepatocytes. The ALT was over 8-fold in one HD male and the creatinine kinase, aspartate aminotransferase and alanine aminotransferase were all elevated in the high dose males. The NOEL was 10 $\mu\text{g}/\text{kg}$ in both male and female studies. The 50 $\mu\text{g}/\text{kg}$ NOEL dose in the iv study provides a ratio of 1.5 with the MHRID in mg/m^2 , but the 10 $\mu\text{g}/\text{kg}$ dose has a ratio of less than 1. These ratios were in terms of the maximum recommended human dose (MHRID), 17.8 $\mu\text{g}/\text{kg}/\text{day}$.

The intrathecal administration of dexmedetomidine in bolus doses of 1.5, 6 and 24 $\mu\text{g}/\text{rat}$ for two weeks, produced transient dose-related sedation but no histopathology that was difference from saline controls. The pilot study in two dogs indicated that 40 $\mu\text{g}/\text{dog}$ produced transient hindlimb weakness and after 72 μg and 144 $\mu\text{g}/\text{dog}$ there was a marked decrease in heart rate and increased sedation. The sedation was so profound at 144 $\mu\text{g}/\text{dog}$ in 1/2 dogs, that it was not arousable by voice or paw pinch for about one-half hour, starting 20 minutes post-dosing. Dexmedetomidine was administered intrathecally to dogs at the doses of 2, 12 and 80 $\mu\text{g}/\text{dog}$ for 28 consecutive days. The 2 μg dose did not produce any observable changes, the 12 μg dose produced some transient sedation and incoordination. There was incoordination was evident in all dogs at the 80 $\mu\text{g}/\text{dog}$ dose and some demonstrated analgesia. There was no clinical chemistry, hematology or urinalysis changes attributed to dexmedetomidine. The histopathological changes were all attributed to the invasive procedures as this was also evident in control animals. The 80 $\mu\text{g}/\text{dog}$ dose did slightly increase the QT interval in 8/10 dogs and one dog had a 2nd degree AV block. The veterinary

NDA 21-038

cardiologist determined the QT lengthening was minor and not cardiotoxic events.

The acute toxicity studies indicated that the safety ratio of acute toxicity is at least two orders of magnitude greater than a human would be expected to encounter, when compared to the loading dose of 1.0 $\mu\text{g}/\text{kg}$. However, the subacute studies are inconclusive. It is recognized that the ratio values with the MRHID are comparing a dose from a total day of infusion in the ICU with a daily bolus dose in the animals. It would be expected that if the study dose had been infused, then the daily dose comparisons would be more relevant. It is not known if the toxicities observed, liver damage, reduced testes and thymus growth and adrenal glomerulosa hypertrophy, would be reduced if the daily dose were presented in a more gradual fashion, infusion. It is also possible that the extended exposure during infusion, even at lower concentrations, would be more damaging as the tissues have less time without exposure, for repair. In addition, the toxicities observed in the animals studies are the results of 4 week of administration is four times the conceived duration and 14 times the presently proposed duration of treatment.

It is the impression of this reviewer that, although the safety ratios in subacute studies are generally less than unity, the extension of the testing to weeks versus the planned 24 hours, greatly exacerbates any drug induced toxicity. This impression is based, in part, to the fact that the acute toxicities produce safety ratios several orders of magnitude greater than 1. In terms of clinical safety, dexmedetomidine, an α_2 -agonist, has the following possibly adverse pharmacological actions:

- initial hypertension if rapidly injected as an iv bolus. ^{7 bases}
- hyperglycemia - seen transiently in rats, gerbils and rabbits, but not observed in dog studies
- increased GFR and increased "filtered fraction" - seen transiently in rats and rabbits producing glucouria. In dogs no glucosuria was observed by some increased proteinuria was observed in the dog studies. ^{can be reversed}
- hypothermia has been observed in animal studies, although this is controlled in the ICU setting. ^{or not}

The following toxicology effects have all been seen after repeated administration and have not been observed acutely:

- elevated liver enzymes, enlarged livers and eosinophilic inclusions in hepatocytes and have been observed in rats and dogs and after 250 $\mu\text{g}/\text{kg}/\text{day}$ in the dog, apoptotic bodies were also observed. This dose is about 7 fold the MRHID of a daily 17.8 $\mu\text{g}/\text{kg}$, calculated in units of mg/m^2 . ✓
- decreased thymus weight have been observed in rats and dogs and decreased testicular and seminal vesicle weights were observed in rats. ✓
- hypertrophy of the adrenal glomerulosa is observed in both rats and dogs. ✓

Although the ratios of the NOEL in repeated dose dog and rat studies and the MRHID have mostly been below 1, this reviewer feels this was only the result of prolonged administration of high bolus doses and do not portend problems at the present recommended duration of treatment. The lack of

NDA 21-038

toxicology studies using administration by infusion, the clinical use, significantly hinders this evaluation. Another compounding variable is the fact that over 50% of the excreted dexmedetomidine is in the form of metabolites that are not produced in rats or dogs, except for trace amounts. (Metabolic Pathways: pg. 72 (rats), page 100 (human))

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**APPEARS THIS WAY
ON ORIGINAL**

REPRODUCTIVE TOXICOLOGY

[56]

Study title: Fertility study (Segment I study) of Dexmedetomidine in Rats
by Subcutaneous Administration. (#228)

Volume# 40, Page#147:

Study No: and number: TOX 89-001

Site and testing facility: _____

Dates: started January 3, 1989, ended August 18, 1989

GLP compliance: yes (Vol 40/pg 183): QA- Reports Yes (Vol 40/ pg 169)

Lot and batch numbers: Batch 14, series 7.1.88

Protocol reviewed by Division: No (X):

Methods:

- Species/strain: Rats / Sprague Dawley _____
- Doses employed: 0, 6 18, 54 $\mu\text{g}/\text{kg}/\text{day}$; control, LD, MD, HD, respectively.
- Route of Administration: daily subcutaneous injection
- Study Design: Males were injected for 10 week prior to mating and the mating for 2 weeks prior to necropsy. One half of the females were dosed for 2 weeks prior to mating, during mating, gestation until Day 20 of lactation and then sacrificed. The other half to the females were dosed through gestation and lactation, until day 20 post delivery.

Males: 45 days of age at start, about 210 gm, daily injection for ten weeks prior to mating (71 injections).

Females about 75 days of age at start, mean 215 gm, daily injections for 2 weeks prior to mating, during mating, gestation and lactation until sacrifice.

- Number of animals/sex/dosing group: 24 males and 24 females/ dose group

Parameters and endpoints evaluated: Body weights weekly, food and water consumption weekly, clinical signs and mortality daily.

-At necropsy, uterine content and corpora lutea on Day 20 of gestation with one-half of dams and the remaining half at Day 20 of lactation. Viability and weight of young on days 1, 4, 14 and 21 postnatal.

Results: Males

- Clinical signs: sedation and piloerection, dose related
- Mortality: No mortality during study.

NDA 21-038

- Body weight: reduced weight gain. In LD group there was no significant change in weight gain, but at MD and HD groups there were significant reductions accumulated weight gain, 10% and 15%, respectively.
- Food consumption: there was a slight, but significant reduction in food intake in the HD group. This was only during the first week of the 10 weeks of injections.
- Water intake: The water intake significantly increased during weeks 2 and 4 in the LD group, weeks 2, 3 and 4 in the MD and 8 of last 9 weeks in the HD group. The increase of 37% was seen during week 10 and as the sponsor indicates this is reflecting the increased diuresis with dexmedetomidine.

Fertility in Males

- In-life observations: No treatment induced change in copulatory or fertility indices. However, one control male and 2 LD males showed no evidence of mating by vaginal smears of the female. In the LD group, 6/24 males were unsuccessful in mating and produced no pregnancy, as compared to 1/24 in MD and in HD groups and 0/24 in controls.
- Terminal and Necropsy evaluations: The terminal absolute weights of the seminal vesicles and epididymis was significantly reduced in the MD and HD groups, but this probably reflected the reduced weight gain as the relative weights were not reduced and the testes weight as percent body weight was significantly increased.

Females

- Clinical signs: As with the males, there was dose related sedation and piloerection.
- Body weight: The significant changes of weight gain in the MD and HD at 2 weeks pre-mating. In the 20 days of gestation, only the HD had a significant reduction in weight gain and this was an 11% reduction. During lactation there was no significant differences in body weight gains.
- Food consumption: There was no significant change in food consumption at any dose.
- Water intake: The females in the HD group drank significantly more water than control animals the second week of pre-mating, and day 1 and day 6 of gestation, 16-18%..

Fertility and Early Embryonic Development in Females

Terminal and Necroscopic evaluations:

F₀ Dams: The fertility index did not vary significantly between treatment groups. No differences in mean number of corpus lutea, implantations, preimplantation losses, early or late resorptions, live or dead fetuses or malformations at any dose. The postimplantation losses were significantly more than control in the MD and HD groups (pg231). There were no increases in fetal death were found in the teratology stage and the sponsor suggests that the loss occurred in late gestation or during delivery before the first observation and the dams had eaten the young. The sponsor suggests that the low body weight and delayed development may have caused the deaths (pg 196). Examination of the data (Vol 40/pg 231) indicates that although the number of living fetuses is not significantly

NDA 21-038

different, the number of implantation sites - number of live births is significantly greater in the MD and HD than control, number of postimplantation losses. The number of postpartum deaths is not different between treatment groups.

There was a dose related decrease in uterine weight, but this was not statistically significant. However, the post-implantation losses were significantly more in the MD and HD groups than in controls and this resulted in a dose related increase in fetal losses between implantation and recorded births.

Early Embryonic Development in Offspring

Fetal body weights were significantly reduced in MD (-13%) and HD (-28%). The mean litter weights were significantly reduced in the MD group only Day 1 of lactations. The HD groups was significantly lighter than control all days measured, 1, 4, 14 and 21 (-15%). Pg230

The ossification of the fetuses appeared to greatly reduced in the HD animals, ribs, metacarpals, metatarsals and caudal and lumbar vertebra (Vol 40/Pg 228). The only other parameter of physical development measured that may have provided evidence of delayed maturation was the delayed appearance of surface righting reflex, number of failures for control, LD, MD, HD; was 3, 9, 7, 16. In addition there appeared to be a decreased wire-hanging ability in the HD group. However, both tests were during lactation, but this may have been the presence of dexmedetomidine in the dam's milk. There were no delays in other reflexes or sexual maturity, descent of testicles and vaginal opening, pinna opening, opening of the eyes, response to sound.

Prenatal and postnatal development, including maternal function, F₁ generation.

In-life observations:

In the F₁ groups of rats scheduled for mating, the statistically significant reduced body weight in the HD males lasted through lactation and into week 12(-6.5%), and although insignificant weeks 13-15, the body weight was 5% below control groups (p235). In the F₁ females, the MD was statistically lighter than controls through most of the 15 week and at week 15 (-5.5%) and HD females were significantly lighter than controls the entire 15 weeks, 12% less on week 15. When body weight gain was summed weeks 3-15 or 4-15, there was no significant difference between controls and any dose group. It is not possible to make a conclusion of the effects of dexmedetomidine in the F₁ generation to maturity as the subjects represented in the tables were preselected and not the complete F₁ generation.

Fertility in Males:

There was no difference in fertility between dose groups. Unlike F₀ generation, there was no difference in seminal vesicles or epididymis weights in the MD and HD groups versus control.

Fertility and Early Embryonic Development in Females

There was no difference in fertility between dose groups and there were no significant differences in post-implantation losses, living offspring, implantation sites, sex ratios or weight of

NDA 21-038

pups Day 1 or Day 4. The uterine weights and fetal examination of possible teratogenic effects were not presented.

Summary

Dexmedetomidine hydrochloride was injected sc at doses of 6, 18 and 54 µg/kg/day. The study included Segment I, Segment II and Segment III portions.

In the Segment I portion, 10 treatment weeks prior to mating and 2 weeks of mating in males and 2 treatment weeks prior to mating and two weeks of mating in the females. The body weight gain was significantly reduced in the MD and HD groups males and the HD females. The food consumption was significantly reduced only in HD males, but water intake was significantly increased during the initial weeks of treatment in LD and MD males and in the HD females, reflecting a diuretic effect of dexmedetomidine. There were statistically significant reductions in absolute weight of seminal vesicles and epididymis in the MD and HD males, but this reflected the decrease body weight gain and the relative weights were not affected. There was no significant or dose related change in male or female fertility. Therefore 54 µg/kg/day can be regarded as a safe dose in terms of Segment I, fertility in males and females.

Segment II portion of the study found no significant changes in number of corpora lutea, implantations, preimplantation losses, early or late embryonic deaths, number of males or females or sex ratio or living implants. However, the MD and HD young were significantly lighter than controls, -13% and -28% respectively. There was significant increase in post implantation losses and the sponsor suggests that these were due to late gestational losses or loss at birth when the light young with delayed development were eaten by the mothers, prior to examination. There were no teratogenic effects seen at any dose and the LD, 6 µg/kg/day, is the dose with no adverse effects on fetuses, fetal development and fetal survival.

Segment III

The body weight of the HD young rats were still significantly 15% below controls on Day 21. Although there were no obvious teratogenic effects, there were many skeletal sites which were less ossified in the fetuses of the HD group than in the control group, but other signs of maturation, eye opening, pinna unfolding, descent of testes, vaginal opening, were not significantly different from the control group. There was no difference in pregnancy or fertility between treatment groups. The litter size, deaths during lactation and sex ratios were not different between groups.

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NDA 21-038

[57]

Study title: Pre- and Post-Natal Study (Segment III study) of Dexmedetomidine in Rats by
Subcutaneous Administration (#230)

Study No: TOX 90-017:

Site and testing facility: _____

Volume 41/page 001

GRP compliance: yes (Vol 41/pg21, 36)

Study start date: Sept 18, 1990

QA- Reports Yes (X) (Vol 41/pg21)

Lot and batch numbers: Batch QT0231

Formulation assays: 98.9-110.3% of target concentration

Protocol reviewed by Division: No

Methods:

- Species/strain: Rat / Sprague Dawley outbred
- Doses employed: 0, 2, 8 and 32 $\mu\text{g}/\text{kg}/\text{day}$
- Route of Administration: subcutaneous injection
- Study Design: The females were injected daily in the morning from Day 16 of pregnancy to weaning, Day 25 postpartum. ICH guidelines say Day of implantation, Day 6, is the initiation of treatment, but this study was pre-ICH
- Number of animals/sex/dosing group: 22 pregnant females / dose group

- Parameters and endpoints evaluated:

F₀ = sedation, piloerection, exophthalmos

sd1 = slowly moving, slight sedation

sd3 = asleep but awake when disturbed

p¹ = piloerection slight

sd2 = awake sedated

sd4 = asleep cannot be awoken

p² = piloerection marked

F1:

A. Physical development was recorded on the day indicated

- 1) Pinna unfolding (Day 2) - detachment of the edge of the pinna.
- 2) Hair growth (Days 3 and 4) and fur growth (Days 6,7 and 8) - macroscopic observation of hair and fur growth..
- 3) Tooth eruption (Days 9, 10, 12) - eruption of upper incisors through the gum.
- 4) Eyes opening (Days 13, 14,16, 17 and 18) - separation of the upper and lower eyelids. Recorded positive, when both eyes were open.
- 5) Testes descent (Days 14, 15 and 16).
- 6) Opening day of the vagina - inspected from Day 30 until opening observed.

NDA 21-038

B. Neuromuscular function

- 1) Surface righting reflex (Days 2, 3 and 4) - ability to turn to prone position from supine position.
- 2) Air righting test (Day 21) - ability to land in prone position when dropped
- 3) Wire hanging Days (16, 17, 18) - forelimb hanging longer than 5 seconds

C. Hearing and visual function

- 1) Hearing ability (Day 21) - response to a sharp noise.
- 2) Visual function (Day 21) - see a solid surface ahead and to move onto it.
- 3) Pupillary reflex (Day 21) - direct and indirect

D. Activity

Activity - locomotor activity; movements of the litters were recorded for 18 hours. Activity measurement of various litters was always performed on the same day after weaning.

E. Learning

A water-filled Y-maze was used to evaluate learning ability of the offspring on Day 33 postpartum. The times taken by each animal to swim through the maze in six successive trials was measured. A maximum of 60 seconds was allowed for each trial. Any animal exceeding this time was removed and was considered to have failed the test. Maintained improvement in swimming time was taken as an indication of learning. Test was repeated on the fourth day after first trial and maintained improvement was considered as indication of memory.

F. Reproductive Performance

At five weeks of age, 1 male and 1 female picked at random from each litter. At week 13-15 animals were paired, avoiding mating of siblings. Vaginal smears were made every morning and if there was no sign of spermatozoa within one week, the female was placed with another male for a maximum of another week.

Results:

F₀- Clinical signs:

Slight sedation was observed in the MD group, and occasional piloerection postpartum in the nursing phase. The HD group was sedated or sleeping 30 minutes post dosing and occasional piloerection was observed.

- Mortality: no maternal deaths occurred during the study.

NDA 21-038

- Body weight: Only the HD females had a significant reduction in bodyweight gain between initiation at Day 16 and Day 20. The HD females gained significantly less than the controls during the dosing from gestation Day 16 to Day 20, but were never significantly lighter than controls. The post-partum weights or weight changes were not different in the HD versus the control.

- Terminal and Necroscopic evaluations: No treatment related effects were observed at autopsy.

- Fertility and Early Embryonic Development in Females -

The fertility of all groups and the length of gestation were the same. The number of pups per litter was slightly (insignificantly) reduced in the HD group (11.0 from 12.4), but so were the number of implantation sites (12 to 14). There was no difference between groups in the gestation index, viability index or lactation index.

- Embryo-fetal Development: On Day 1 postpartum: The mean fetal weights were significantly less in the HD group than in controls litters (5.7 vs 6.6 g). The significance remained for both males and female pup. In the middose group, 8µg/kg/day, the females were significantly lighter on Day 4. On Day 4, litters greater than 8 pups were randomly culled to this maximum. The HD pups remained significantly lighter through the 25th day and the MD group was also significantly lighter during this time. On Day 25, the MD pups weighed 6.6% less than control and the HD pups 12% less.

The testing of developmental endpoints did not show any significant difference between controls and any dose group in pinna unfolding, eye opening, surface righting, hair or fur growth, testes descent in males and vaginal opening in females, tooth eruption, auditor function, visual function, pupillary reflex, or air righting reflex. The only significant difference was on wire-hanging on postpartum Days 16, 17 and 18. A smaller percentage of the HD males and females were able hang on to the wire on the initial day of testing. The body weight in the first 8 weeks after nursing was significantly less in the MD males on weeks 3, 5 and 6. The HD males were significantly lighter than controls every week. The HD females were lighter only on week 7 and the MD females did not differ significantly from controls.

Reproduction in F₁ generation:

The mating performance did not differ between treatment groups. This was true of the day of sperm in vaginal smear, number not pregnant, males not mating, copulation index and fertility index.

The female body weights and body weight gains during pregnancy did not differ significantly between treatment groups and the fetal body weights and placental weights also did differ between treatment groups. The number of corpora lutea did not differ significantly, however, the mean number of living fetuses were significantly reduced in the HD females (11.5 from 13.9) and the mean number of early implantation losses was increased (0.4 to 0.9). Examination of the fetuses (F₂) did not show any macroscopic group differences.

Summary

Pregnant females received daily subcutaneous injections with dexmedetomidine hydrochloride at doses of 2, 8 and 32 µg/kg from Day 16 to Day 25 postpartum. The 8 µg/kg/day dose slightly reduced F₁ body weight gain during nursing and this decreased bodyweight continued after weaning in the males until week 6. The 32 µg/kg/day dose decrease bodyweight gain in the F₀ dams during pregnancy but not during lactation and the absolute bodyweights never differed significantly from controls. In the F₁ generation the bodyweights were reduced after delivery and only in the males after weaning for every week measured. There was little effect on maturation although the lighter HD males and females had significantly more difficulty wire-hanging. There was no differences in mating performance. The number of dead fetuses was slightly, but significantly increased and the number of living fetuses slightly, but significantly decreased in the HD group, F₂ generation. The 8 µg/kg/day was a NOAEL as the only significant difference from control group rats was reduced bodyweight and this was not different by week 8.

The reproductive toxicity of dexmedetomidine at the HD, 32 µg/kg/day, to the F₀ during gestation may have developmental consequences on the F₁ generation, as they were lighter at birth, and the males continued to be lighter even after weaning. The only significant developmental difference was a reduced number of HD animals able to lift hindfoot or body onto a wire they were hung on by their forepaws. The deficit was for only one day, but it was statistically significant with both the males and the females on Day 16. Although the F₁ generation mated successfully, the number of living implants was significantly decreased and number of early dead implants significantly increased above controls.

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[58]

Study title: Examination of the Influence of MPV-1440 HCL on the Pregnant Rabbit and the Foetus by Intravenous Administration. (#231)

Volume #42/page 001

Study No: Pjt. #5139/89

Site and testing facility:

Study Dates: July 17, 1989 - November 21, 1989

GLP compliance: yes (Vol 42/pgs-11, 85)

QA- Reports Yes (x)

Lot and batch numbers: PE 058 L1, PE 056 L1, PE 055 L1

Protocol reviewed by Division Yes () No (x):

NDA 21-038

Methods:

- Species/strain: Rabbit/Himalayan (locally bred)
- Doses employed: 0, 6, 24 and 96 µg/kg/day, control, LD, MD, HD, respectively.
- Route of Administration: intravenous, iv
- Study Design: Daily injections from Day 6 to Day 18 of pregnancy. Cesarean section on Day 29 of gestation and examination of uterus and fetuses.
- Number of animals/sex/dosing group: 12/ treatment group

Results:

- Clinical signs: LD demonstrated slight sedation and very slight meiosis after injection and this lasted in several dams for 20 minutes. In the MD group, the dams assumed the abdominal position and showed sedation and meiosis for 1 to 2 hours. The sedation and time in the abdominal position was increased in the HD group and meiosis lasted in one dam for 6 hours.

The bodyweight gain, graphically(pg 55), appeared to be more in the HD and MD groups than in control, but the tables of daily weights did not appear significantly different. The sponsor provided no tabulation of weight gain or statistical analysis.

- Mortality: There were no deaths in any treatment group.
- Consumption: Graph of daily food consumption did not suggest any treatment effect. (Vol 42/Pg 56): Water consumption was not affected, according to the sponsor.

Segment II in Rabbit

- Embryo-fetal Development: There were no drug related fetotoxicity or teratogenicity. The number of corpora lutea, implantation sites, total fetuses, percent in left and right, number of placenta, number of resorptions and mean number per dam did not vary significantly between treatment groups and control dams. Other parameters are in the following table:

	Control	6 mcg/kg	24 mcg/kg	96 mcg/kg
Pre-implantation losses- %	23.3	22.9	27.8	20.6
Post-implantation losses-%	16.5	7.1	20.5	11.1
runts	0	0	0	1
total malformations	2	1	0	1
fetuses with variations	43	29	28	26
variation rate	65.2	37.2	45.2	36.1
mean Fetus body weight	36.6	34.3	38.5	38.5
mean placenta weight	5.03	4.56	5.34	5.45

NDA 21-038

There was no evidence that dexmedetomidine hydrochloride at 6, 24 or 96 mcg/kg/day had any adverse effects on the rabbit fetus when administered iv from day 6 to 18 of pregnancy. The sponsor contrasts this to the effects in rats and cites publications of another α_2 agonist, clonidine, which had adverse effects in the rat but not the rabbit ^{1,2}.

References:

- 1). Pizzi WJ, Ali SF and Holston RR *Behavioral evaluation of rats prenatally exposed to the adrenergic agonists and lofexidine* Neurotoxicology 9(3):559-66 (1988)
- 2). PDR, 1995

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[59]

Study Title: Abbott-85499 Drug Metabolism Report No.31
Lacteal excretion and fetal tissue distribution of radioactivity following a single subcutaneous dose of [³H]dexmedetomidine HCl (Abbott-85499. 1) in the rat (248)

Study No: 6161-175: R&D/97/565 (Study No. Covance 6161-175)

Vol #44, and page #149:

Conducting laboratory and location: _____

Date of study initiation: September 1997

GLP compliance: yes

QA- Reports: Yes

Methods: 33 female Harlan Sprague Dawley rats were separated into 2 groups. Group 1, 12 timed pregnant rats at Day 18 of gestation. Group 2 was 21 lactating females, about 10 days postpartum. The rats were 11.5 to 12 weeks of age and 241 to 365 grams.

Dosing: Subcutaneous dosing with labeled dexmedetomidine at 0.015mg/kg.

Drug, lot#, radiolabel, and % purity: Medetomidine HCl, with tritium on the bridge methyl group, was synthesized by Amersham and the dexmedetomidine isomer was separated at Abbott by chiral chromatography. (Lot #55585-ST-108; 72.6 mCi/mmol) Unlabeled dexmedetomidine, Lot #295260-0-AX, was added to the labeled dexmedetomidine HCl to provide a solution of 75 mCi/ml and 0.015 mg/ml.

Formulation/vehicle: dexmedetomidine, labeled and unlabeled, was dissolved in normal saline

Observations and times: Group 1 received a subcutaneous injection of 0.015 mg/kg of labeled dexmedetomidine and 3rats/time point were sacrificed 1, 8, 24 and 72 hours post administration. Group 2 lactating females were injected subcutaneously with 0.015 mg/kg of labeled

NDA 21-038

dexmedetomidine and milk and blood samples were collected, 3 rats/time point, 0.5, 1, 2, 4, 8, 24 and 72 hours postdosing. The pups in Group 2 were removed 4 hours prior to sampling and the milk was collected by a specially designed milking machine after sc oxytocin stimulation of milk expression.

Results:

In Group 1 animals, the placental transfer and fetal levels of dexmedetomidine were measured. The maximum concentrations occurred at one hour postdosing, except for maternal adrenals, amniotic fluid, fetal blood and kidneys, which reached measured maximum levels at 8 hours postdosing. The highest maternal concentrations were observed in the adrenal, lungs, livers and kidneys. The fetal concentrations were highest in blood, liver and kidneys. The radioactivity decreased with time and at 72hrs post, not detectable levels were seen in maternal brain or heart or fetal heart or kidneys. The tissue/maternal plasma ratios were greater than 1 at at least one time period for all tissues and the whole fetus at hours 1, 24 and 72. The following tables were extracted from the submission (Vol 44/pg 155, 158):

[³H]Dexmedetomidine-related radioactivity

	<u>Maternal</u>		<u>Fetal</u>	
	C _{max} (ng eq/g)*	T _{max} (h)	C _{max} (ng eq/g)*	T _{max} (h)
Lungs	187.8	1.0	2.1	1.0
Kidneys	62.1	1.0	4.8	8.0
Liver	61.2	1.0	5.1	1.0
Brain	7.5	1.0	2.7	1.0
Heart	6.2	1.0	2.0	1.0
Blood	1.9	1.0	7.2	8.0
Adrenals	275.7	8.0	-	-
Ovaries	26.5	1.0	-	-
Placenta	8.5	1.0	-	-
Uterus	3.0	1.0	-	-
Plasma	2.3	1.0	-	-
Amniotic Fluid	0.4	8.0	-	-
Whole Fetus	-	-	3.2	1.0

*expressed as the free base

NDA 21-038

**Mean Concentrations of Radioactivity in Blood, Plasma and Maternal and Fetal Tissues
for Pregnant Female Rats following a Subcutaneous 0.015 mg/kg Dose of
[³H]Dexmedetomidine.HCl**

Tissue	Mean Concentration (ng Eq Base/g (hours after dose)			
	1	8	24	72
Maternal				
Adrenals	105.72	275.73	267.27	105.72
Amniotic Fluid	0.45	0.76	0.45	0.07
Blood	1.93	1.51	0.24	0.04
Brain	7.82	1.00	0.16	nd
Heart	6.17	1.62	0.25	nd
Kidneys	62.08	12.18	0.99	0.10
Liver	61.24	34.76	2.14	0.29
Lungs	187.77	59.46	3.21	0.41
Ovaries	26.47	3.69	0.91	0.10
Placenta	8.54	2.39	0.47	0.07
Plasma	2.34	1.97	0.28	0.03
Uterus	2.99	1.70	0.24	0.04
Fetal				
Whole Fetus	3.22	1.78	1.01	0.05
Fetal Blood	2.36	7.16	0.14	0.06
Fetal Brain	2.65	0.99	0.19	0.03
Fetal Heart	1.96	1.26	0.06	nd
Fetal Kidneys	4.67	4.78	0.57	nd
Fetal Liver	5.13	2.18	0.40	0.07
Fetal Lungs	2.11	1.38	0.29	0.03

nd = not detected

Group 2 examined the lacteal excretion of labeled dexmedetomidine following a maternal dose of 0.015 mg/kg. The plasma levels and milk concentrations reached maximum 4 hours postdosing and declined with time. The milk/plasma ratios were less than 1, 0.48 to 0.87, at all time intervals. The following table was extracted from the submission (Vol 44/pg 160):

Mean Concentrations of Radioactivity in Milk and Plasma, and
Milk: Plasma Concentration Ratios for Lactating Rats following a Subcutaneous
0.015 mg/kg Dose of [³H]Dexmedetomidine.HCl

Hours After Dose	ng EqBase/g		Milk:Plasma Ratio
	Plasma*	Milk	
0.5	1.45	0.88	0.59
1	1.74	1.12	0.65
2	2.44	1.56	0.64
4	3.31	1.57	0.48
8	1.93	1.34	0.70
24	0.18	0.16	0.87
72	0.03	nd	nd

*Average of premilk and postmilk values
All numbers have been rounded off to two decimal places
nd = not detected The last column was misslabeled in the submission.

Summary:

The subcutaneous administration of labeled dexmedetomidine, 0.015 mg/kg, to pregnant rats in the 18th day of gestation was used to examine placental transfer and fetal disposition and the injection to lactating females examined the lacteal secretion. The mean maximum concentrations in both dam and fetal tissues was observed at 1 hour postdosing except for maternal adrenals, amniotic fluid and fetal blood and kidneys peaking 8 hours postdosing. The maternal tissue/plasma and whole fetus/plasma ratios were all greater than 1 in at least one time point, suggesting some accumulation. The lacteal excretions were maximum at 4 hour postdosing and milk/plasma ratios were all less than 1, indicating no accumulation in the milk.

Dexmedetomidine is excreted in the milk of rats, and the lack of accumulation of dexmedetomidine in rat milk does not predict this lack of excretion or accumulation in human milk as milk constituents are species specific and drug accumulation is also.

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SUMMARY REPRODUCTIVE TOXICITY

Segment I testing of dexmedetomidine in rats [56] at 6, 18 and 54 µg/kg/day starting dosing of the females 3 weeks prior to mating and males 10 weeks prior to mating. No treatment induced changes in fertility in males or females at the highest dose tested, 54 µg/kg/day sc (NOAEL), and the ratio with the MRHID, on a mg/m² basis, was less than 1 (0.5). There were significant

NDA 21-038

reductions in body weight gain at 18 and 54 $\mu\text{g}/\text{kg}/\text{day}$ in F_0 in both males and females and reduced weights of seminal vesicles and epididymis in the males. Fetal toxicity, reduced fetal weight and increased post-implantation losses, was observed at 18 and 54 $\mu\text{g}/\text{kg}/\text{day}$. The high dose group fetuses had more incomplete ossification of metatarsals and metacarpals than controls. The NOAEL was 6 $\mu\text{g}/\text{kg}/\text{day}$ and the ratio with the MRHID, on a mg/m^2 basis, was much less than 1 (0.05).

This study was extended with one half of the dams giving birth and treatment injections were continued through nursing. The HD dams were significantly lighter than controls at the end of gestation, but only 6% and there was no significant difference by the end of lactation. The pup bodyweights were significantly below control at birth in both 18 and 54 $\mu\text{g}/\text{kg}/\text{day}$ groups, but only the HD group was below control past postnatal Day 1 and remained below through week 10 postnatal. The sponsor has not marked any significant differences in the developmental tests of the F_1 rats, but the 54 $\mu\text{g}/\text{kg}/\text{day}$ group appeared to have more failures of surface righting, 16 failures versus 3 in controls; and wire-hanging, 21 failures versus 13 controls and delayed eye opening, 6 versus 1 control pup. Twelve litters were examined in both control and HD groups. There were not any significant, F_2 generation drug induced differences in the number of living or dead fetuses, implantation sites, post implantation losses or any reproductive measure. The NOAEL can be considered the 18 $\mu\text{g}/\text{kg}/\text{day}$ treatment and the ratio of this NOAEL dose and the MRHID was below one (0.18) on a mg/m^2 basis.

Segment II study in rats (#229, original review) dosed the dams, Day 5 through Day 16 of gestation, with 2, 20 or 200 $\mu\text{g}/\text{kg}/\text{day}$. There was no teratogenicity at any dose and only the high dose of 200 $\mu\text{g}/\text{kg}/\text{day}$ was fetotoxic. Although there was reduced maternal weight at the 20 $\mu\text{g}/\text{kg}/\text{day}$ group, there was no fetal toxicity and the NOAEL was 20 $\mu\text{g}/\text{kg}/\text{day}$, a ratio value of less than one (0.18) with the MRHID. In the rabbit [58], tested at 6, 24 and 96 $\mu\text{g}/\text{kg}/\text{day}$ the NOAEL was observed at the highest dose tested, 96 $\mu\text{g}/\text{kg}/\text{day}$ and the safety ratio was about 2 (1.7) on a mg/m^2 basis. The AUC in the rabbits at 96 $\mu\text{g}/\text{kg}/\text{day}$ was 40.7 $\text{ng}\cdot\text{hr}/\text{ml}$ and the ratio with the AUC at MRHID was approximately 1. No significant maternal weight changes were reported in this rabbit study.

Segment III [57] testing was conducted in the rat, 2, 8, or 32 $\mu\text{g}/\text{kg}/\text{day}$ sc from Day 16 of gestation to Day 25 postpartum. The MD and HD groups had reduced birth weights significantly. The bodyweights of the 8 and 32 $\mu\text{g}/\text{kg}/\text{day}$ litters remained significantly below control through 6 to 8 weeks after lactation. In the HD group, both sexes had difficulty on the wire-hanging test, days 16, 17, 18 and although mating and pregnancy of F_1 were not different from controls, the number of F_2 dead fetuses increased and the number of living fetuses decreased significantly. The NOAEL was the low dose, 2 $\mu\text{g}/\text{kg}/\text{day}$, and the safety ratio, on a mg/m^2 basis, was much less than 1 (0.012).

All three studies presented data indicating that dexmedetomidine produced fetal toxicity and two also indicated postnatal toxicity. The sc injection to both sire and dam prior to, during and post-mating [56], reduced F_1 bodyweight postpartum in the 18 and 54 $\mu\text{g}/\text{kg}/\text{day}$ groups and this reduced bodyweights of the pup was also observed in the second experiment [57] at 32 $\mu\text{g}/\text{kg}/\text{day}$ when

NDA 21-038

dexmedetomidine was administered sc to the pregnant rats on days 16 through 25 of gestation. The developmental delays observed in the F₁ generation at 32 $\mu\text{g}/\text{kg}/\text{day}$ group in the second experiment appeared to have also occurred at 54 $\mu\text{g}/\text{kg}/\text{day}$ in the first experiment.

Maternal/Fetal Transfer

The maternal/placental transfer of dexmedetomidine to the fetus and presence of dexmedetomidine in the milk of lactating females was measured after maternal injections of dexmedetomidine on the 18th day of gestation. The maternal and fetal tissue levels mostly peaked within one hour and both maternal and fetal tissue/plasma levels exceeded 1 at some time point suggesting some tissue accumulation. The milk of the lactating females did not indicate accumulation of dexmedetomidine, but species differences in lactation and metabolism do not allow prediction of dexmedetomidine disposition in human milk.

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**APPEARS THIS WAY
ON ORIGINAL**

GENETIC TOXICOLOGY

[60]

Study Title: Dexmedetomidine Hydrochloride. Bacterial Mutation Assay (#232)

Study No: FSG 17/930219

Study Type: *in vitro*

Volume # 42 and Page #82:

Conducting Laboratory: _____

Date of Study Initiation/completion: January 11 to February 5, 1993

GLP Compliance: yes

QA- Reports Yes (X)

Drug Lot Number: ST0531, _____

Methodology:

- Strains/Species/Cell line: *S. Typhimurium*; TA 1535 *his G46 rfa uvB*
TA 1537 *hisC3076 rfa uvB* TA 1538 *hisD3052 rfa uvB*
TA 98 *hisD3052 rta uvB pKM101* TA 100 *hisG46 rfa uvrB pKM101*
- Dose Selection Criteria:
- Basis of dose selection: At 5000 mcg/plate, dexmedetomidine was toxic (incomplete bacterial lawn) to all strains and 1500 mcg/plate was chosen as the top dose. Five doses at half log intervals
- Range finding studies: done at 5, 50, 500, 5000 mcg/plate
- Test Agent Stability: Not determined in this test series
- Metabolic Activation System: S-9 fraction from Aroclor 1254 stimulated rat livers
- Controls: - Vehicle: water
- Negative Controls: sterile water
- Positive Controls: ENNG - N-ethyl-N'-nitrosoguanidine; 9-aminoacridine, 2-fluorene, 2-aminoanthracene
- Incubation and sampling times: incubation for 3 days at 37C
- Doses used in definitive study: 5, 15, 50 150 500 and 1500 mcg/plate
- Study design:
 - Counting method: Seescan Automatic Colony counter
 - Cytotoxic endpoints: incomplete bacterial lawn
 - Statistical methods: statistics only if treatment mean > 2x control
 - Criteria for Positive Results: must be at least 2X control revertant colonies.

Results:

Dexmedetomidine was tested in - *S. Typhimurium*; TA 1535 *his G46 rfa uvB*, TA 1537 *hisC3076 rfa uvB*, TA 1538 *hisD3052 rfa uvB*, TA 98 *hisD3052 rta uvB pKM101*, and TA 100 *hisG46 rfa*

NDA 21-038

uvrB pKM101, with and without SP activation. The doses used were 5, 15, 50, 150, 500, and 1500 mcg/plate. The high dose of 5000 mcg/plate was cytotoxic to all strains and the 1500 mcg/plate dose was toxic to TA 98 and TA 100. No evidence of mutagenicity was observed. The strain TA 1538 is no longer recommended and this has been corrected during further testing.

Summary:

There were no significant increases in revertant colonies at any dose of dexmedetomidine, with or without S9 and the positive controls did produce significant increases, with and without S9.

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[61]

Study Title: Bacterial Reverse Mutation Assay of Dexmedetomidine Hydrochloride in
Escherichia Coli. (#233)

Study No: R&D/95/360: TX95-093

Study Type: Ames; agar overlay plate test

Volume # 42 and Page #107:

Conducting Laboratory: Abbott Laboratories, Abbott Park, IL, 1995.

Date of Study Initiation/completion: April 1995 - July 1995

GLP Compliance: yes

QA- Reports Yes (x) No () : pg 119

Drug Lot Number: #032940 Batch # 002. Assays of used concentrations varied for 94.6 to 200% of intended dose. Only the lowest doses of 0.01, 0.03 and 0.10 mg/ml were more than 110%, 200% and 133% respectively.

Methodology:

- Strains/Species: E. Coli WP2 μ v_rA-
- Metabolic Activation System: S9 fraction from livers of Aroclor-1254 treated rats.
- Controls:
- Vehicle: water
- Negative Controls: water
- Positive Controls: N-ethyl-N'-nitrosoguanidine. 10 mcg/plate in non-activated assay. 2-Aminoanthracene, 50 mcg/plate in activated assays.
- Comments: the positive controls were dissolved first in DMSO and then diluted.
- Exposure Conditions:
 - Incubation and sampling times: the petri dishes were incubated for 48 hours at 37°C.
 - Doses used in definitive study: doses studied were 1, 3, 10, 30, 100, 300, 1000, 2000 and 5000 mcg/plate

NDA 21-038

- Analysis:

- No. plates/replicates analyzed: 3 replications at each dose
- Counting method: not included
- Cytotoxic endpoints: reductions in mean number of revertants
- Statistical methods: none used as no dose produced even a doubling of revertants from negative control groups.

Results:

- Study Validity: Appears valid with both negative and positive controls with expected values.
- Study Outcome: No indications of increased revertants at any dose. The dose 1000 mcg/plate reduced the revertants by 50% from control levels. The doses of 2000 and 5000 mcg/plate demonstrated toxicity as the number of revertants were much less than negative controls. The positive controls had many times the number of revertants as the control, (32x S9- and 7.4x S9+).

Summary

Dexmedetomidine was not mutagenic in the reverse mutation assay with E. Coli WP2uvrA-, either activated with S9 or without. The doses tested were 1, 3, 10, 30, 100, 300, 1000, 2000 and 5000 mcg/plate and the three higher doses were cytotoxic.

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[62]

Study Title: Dexmedetomidine Hydrochloride: Mammalian Cell Mutation Assay
(#234)

Study No: FSG 19/931143

Study Type: *in vitro*

Volume # 42 and Page #130: 5-21-130

Conducting Laboratory

Date of Study Initiation/completion: April 21 to July 12, 1993

GLP Compliance: yes, pg 133

QA- Reports: Yes

Drug Lot Number: batch ST 0531

Methodology:

- Cell line: L5178Y (TK +/-)
- Dose Selection Criteria:
 - Basis of dose selection: toxicity and precipitation in medium
 - Range finding studies: preliminary toxicity tests @ 37.5, 75, 150, 300, 625, 1250, 2500, 3750, 3990 mcg/ml
- Test Agent Stability: not evaluated
- Metabolic Activation System: S9 fraction from livers of Aroclor-1254 treated rats.

NDA 21-038

- Controls:
 - Vehicle: DMSO 1% v/v final concentration
 - Negative Controls: solvent
 - Positive Controls: With S9, 2-methylcholanthrene, w/o S9, ethyl methane sulfonate
 - Comments:
- Exposure Conditions:
 - Incubation and sampling times: The treatment with dexmedetomidine was 3 hours. After 24 and 48 hour of incubation in HEPES, the concentrations were adjusted to 2×10^5 cells/ml during the mutant phenotype expression period. After 48 hours 3 plates per sample were made on soft agar, 200 cells/plate (to determine cell viability) or 10^6 cell per plate in selective medium (to determine mutant frequency). Incubation for 12-13 days and the colonies counted by image analyzer.
 - Doses used in definitive study: 10, 20, 40, 80, 50, 200, 250, 300 mcg/ml
 - Study design: soft agar methodology
 - Analysis: colony counts > 100 μ m diameter
 - No. plates analyzed: 2/dose
 - Counting method: _____
 - Cytotoxic endpoints: growth suspension, cell survival
 - Genetic toxicity endpoints/results: mean mutant frequency (MMF), relative total growth (RTG)
 - Statistical methods: weighted ANOVA
 - Criteria for Positive Results: 1) a significant > 2x increase in MMF, over solvent controls: 2) an indication of a dose-response 3) repeatability

Results:

- Study Validity: study was valid
- Study Outcome: The preliminary toxicity testing found concentrations of 625 mcg/ml or above produced precipitation. The RTG was severely decreased between 150 and 300 mcg/ml both with and without S9. The tests were done twice and there was considerable variability in both controls and treatment groups. The only statistically significant increase in mutant frequency was observed in the initial test with +S9 at the dose of 40 mcg/ml, an increase of about 30%. The 80 mcg/ml dose had mutant frequency the same as control and the repeat of the test with +S9 produced no significant increases. Therefore the results did not meet criteria for positive results on either dose-response or repeatability. The positive controls were from 5x to 7x the solvent control.

Summary

Dexmedetomidine was not mutagenic in this assay in mammalian L5178Y (TK +/-) cells.

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NDA 21-038

[63]

Study Title: Dexmedetomidine Hydrochloride. Metaphase Chromosome Analysis of Human lymphocytes Cultured in vitro. (# 235)

Study No: FSG 18/931206

Study Type: in vitro

Volume # 42 and Page #168:

Conducting Laboratory:

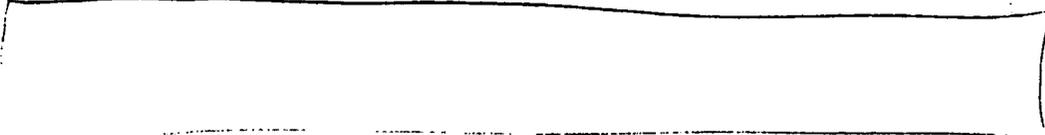
Date of Study Initiation/completion: 1994

GLP Compliance: yes

QA- Reports Yes (x)

Drug Lot Number: ST0531

Methodology:

- 
- Range finding studies: The doses of 5.9, 11.7, 23.4, 46.9, 93.8, 187.5, 375, 750, 1500 and 3000 mcg/ml were chosen to start.
 - Test Agent: dexmedetomidine HCl was soluble to 444 mg/ml in water, but when placed in culture medium, 1% v/v, a precipitate formed. The concentration was adjusted to 300 mg/ml which produced only a slight precipitate at a final concentration of 3 mg/ml
 - Metabolic Activation System: S9 fraction from livers of Aroclor-1254 treated rats.
 - Controls:
 - Vehicle: water for dexmedetomidine HCl
 - Negative Controls: water
 - Positive Controls: cyclophosphamide (+ S9), ethyl methansulfonate (- S9)
 - Exposure Conditions: The cultures without S9 were incubated for 18 hours and those with S9 were incubated for 3 hours, centrifuged and washed and resuspended and incubated for another 15 hours.
 - Doses used in definitive study: a test was run at 25, 50, 100, 200, 250, 300, 350, 400 and 500 mcg/ml, with and without S9.
 - Study design: Two hours prior to cell harvest, the mitotic activity was arrested by addition of colchicine to each culture. After two hours, the cells were centrifuged, resuspended in hypotonic solution and then spun again to pellets. The pellets were fixed with a methanol glacial acetic acid mixture for at least 2 hours. The pellets were aspirated, resuspended in a small volume of fixative and 2 or 3 drops were placed on a slide and allowed to air dry. The slide was stained with Giemsa and air dried.

NDA 21-038

A second test was run with the doses of 12.5, 25, 50, 100, 125, 150, 175, 200 and 250 mcg/ml without S9 and 50, 100, 200, 250, 300 and 400 mcg/ml with S9. The latter test was doubled with the standard 18 hour reading and a 32 hour reading.

- Analysis:
- No. slides analyzed: two slides per dose and 4 control slides. This was done for both with and without S9.
- Counting method: in a light microscope at 160X, the proportion of mitotic cells per 1000 was recorded. Then the slides were coded and metaphase figures were identified at 160X and examined at 1000X using oil immersion.
- Cytotoxic endpoints: doses which decreased mitotic index by 40-80%.
- Genetic toxicity endpoints/results: The number of aberrant metaphase figures were recorded and compared with control groups.

Results:

- Study Validity: The tests do appear valid, with positive and negative controls as expected. The validity of the interpretation of the results by the study director may be disputed.
- Study Outcome:

First test: There were no viable cells in the -S9 groups at doses of 375 to 3000 mcg/ml. The mitotic cells were 3% of control at 187.5 mcg/ml, 64% at 93.8 mcg/ml and 104% at 46.9 mcg/ml. The cultures treated with + S9 had relative mitotic indexes of 113%, 84%, 99%, and 6% at doses of 46.9, 93.8, 187.5, 375 mcg/ml, respectively. At a repeat of this test, the doses were 25, 50, 100, 200, 250 300, 350, 400, 500 mcg/ml and the relative mitotic index was 117%, 70%, 66%, 21% and 2% at doses of 200, 250 300, 350, 400 mcg/ml.

Cultured human lymphocytes - 18 hour harvest - Test 1					
Dose - -S9 mcg/ml	relative Mitotic Index	mean % aberrant cells -gap	Dose - +S9 mcg/ml	relative Mitotic Index	mean % aberrant cell -gap
Water	100%	0.5	Water	100%	2.25
Dexmedetomidine 5.9	103		Dexmedetomidine 25	106	
11.7	122	0.5	50	91	
23.4	124		100	113	1.5
46.9	104	0.0	200	117	1.5
93.8	64	2.0	250	70	
187.5	3		300	66	6.0*
750	0		350	21	
1500	0		400	2	
ethyl methane sulfonate		35***	cyclophosphamide		20***

NDA 21-038

Cultured human lymphocytes - Test 2 - 18 hour harvest					
Dose - -S9 mcg/ml	relative Mitotic Index	mean % aberrant cells -gap	Dose - +S9 mcg/ml	relative Mitotic index	mean % aberrant cell -gap
water 10mcl/ml	100%	1.25		100%	0.5
Dexmedetomidine 12.5	78	0.5	Dexmedetomidine 50	84	
25	98		100	86	0.0
50	96	0.5	200	63	0.0
100	56	0.5	250	56	
125	36		300	42	1.5
150	56		350	30	
175	0		400	—	
ethyl methanesulfonate		10***	cyclophosphamide		13.5***

Cultured human lymphocytes - Test 2 - 32 hour harvest					
Dose - -S9 mcg/ml	relative Mitotic Index	mean % aberrant cells -gap	Dose - +S9 mcg/ml	relative Mitotic Index	mean % aberrant cell -gap
water 10mcl/ml	100%	2.25		100%	1.5
Dexmedetomidine 12.5	59	1.5	Dexmedetomidine 50	87	3.0
25	62	2.5	100	119	
50	68	3.5	200	124	2.0
100	71		250	108	
125	44		300	106	
150	72		350	57	4.5*
175	6		400	—	
ethyl methanesulfonate		10.5***	cyclophosphamide		37.5***

Summary:

The study director discounted the statistically significant increases in chromosomal aberrations at 300 mcg/ml in the initial 18 hour test and in the 32 hour test at 350 mcg/ml because the latter result

NDA 21-038

was within the historic range of controls and the initial one was "just outside the historic control range". The historical controls, over a 10 year period representing examination of 181,897 cells, was a mean of 0.84% and a range of 0 to 5.25. The 6% of the cells with aberrant chromosomes (test 1, 18 hrs at 300 mcg/ml, +S9) is considered positive by the reviewer. The 4.5% at 32 hours (+S9) was within the historic range of control values, but is still considered confirmation of the previous results as the historical control mean is less than 1.

The cellular toxicity of dexmedetomidine is represented by a very steep dose-response curve and the compound appears to be clastogenic only after S9 metabolism and in a very narrow range of doses.

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[64]

Study Title: DEXMEDETOMIDINE: PRELIMINARY DOSE RANGE FINDING STUDY
FOR MOUSE MICRONUCLEUS TEST

(#200)

Study No: tox 95007/1

Vol #31, page #125:

Conducting laboratory and location:

Date of study initiation: August 1995

GLP compliance: No

QA- Reports Yes () No (X):

Methods: NMRI male mice, 25 with 5/test group.

Dosing: A single iv injection of dexmedetomidine hydrochloride (Batch 002) in saline, doses of 0, 100, 200, 500, 1000 ug/kg by rapid bolus into tail vein.

Drug lot#, % purity: _____ .C

Observations and times: The mice were observed for clinical signs twice a day for four days and the rectal temperature was taken 1, 3, 5, 24, 28 and 95 hours after dosing.

Results:

Sedation was observed in all animals treated with dexmedetomidine. The duration of sedation was less than 5 hours at 100 and 250 ug/kg and more than 5, but less than 23 hours at 500 and 100 ug/kg. The body weights were taken at the beginning and at the end of 4 days and there may have been a slight decrease in weight gain in the two high dose groups. The mean maximum decreases in rectal body temperature are presented in the table below:

Mean Maximum Changes in Body Temperature	
treatment (mcg/kg) (n = 5/group)	body temperature change - °C
Saline control	- 3
dexmedetomidine 100	- 5
dexmedetomidine 250	- 8
dexmedetomidine 500	- 11
dexmedetomidine 1000	- 11

There were no deaths during the 4 days of observation postdosing.

Summary:

The MTD may be greater than 1000 ug/kg as there were no deaths at any dose and the body temperature decrease was not completely dose related as 500 and 1000 both had maximum drops of body temperature of about 11 °C. This suggests the ability to regulate body temperature was inhibited and that the mice of both groups just equilibrated with room temperature. No micronucleus study was done.

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[65]

Asanami S, Shimonok. Hypothermia Induces Micronuclei in Mouse Bone Marrow Cells. Mutation Res 1997;393:91-98.

Vol #31, and page #117: (#199.)

Study: The study examined the effects of reserpine induced hypothermia on the induction of micronuclei in mouse bone marrow cells. When the body temperature was maintained in a heated room, there was no increase in micronuclei, however, when the body temperature decreased to less than 33°C for 40 hours, micronuclei increased significantly. The number of relatively large micronuclei (diameter > or equal to 1/4 diameter of cytoplasm) amounted to about 50 percent of the induced micronuclei. The doses of 50, 100 and 200 mg/kg of reserpine reduced the body temperatures 9.5, 8.7 and 10.1 degrees centigrade and induced significant increases in the number of micronuclei by 3.3x, 3.2x and 2.6x, respectively. If the body temperature decrease was limited to 2 to 3 degrees, there were no significant increases in micronuclei.

Summary:

Although the sponsor has cited this publication to explain the significant increase in micronuclei

NDA 21-038

after a dose of 5000 ug/kg of dexmedetomidine, there are several reasons that the situations are different. The primary difference is the duration of hypothermia. Reserpine did not increase the micronuclei significantly in 24 hours although the body temperature decrease was at a maximum at 24 hour post dosing and the significant increase in micronuclei did not appear until 48 hours postdosing and continuing at 72 and 96 hours post. The hypothermia after dexmedetomidine was gone by 24 hour post after 100, 250, 500 and 1000 ug/kg in the range finding study and after 40, 100 and 250 ug/kg doses in study PT97112100025. The 5000 ug/kg dose did appear to increase the duration of hypothermia, 5/6 <30°C at 24 hours and 3/6 <30°C at 48 hours, but there was no correlation between the reduction in body temperature at 48hr and the appearance of increased proportions micronuclei among the polychromatic erythrocytes. Although a subgroup of mice given 5000 ug/kg of dexmedetomidine was warmed to prevent the severe drop in body temperature, no data on micronuclei abundance were presented.

A second difference was the significant appearance of large micronuclei in the reserpine treated mice, representing about 50% of the increase. This was not cited in the report of the positive effects of dexmedetomidine.

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[66] :

Study Title: Erythroid Hypoplasia in Bone Marrow Induced by Hypothermia in Mice.
(#198)

Study No: TOX 95-007/2,

Volume #31 and Page # 75 : 5-10-75

Conducting Laboratory: _____

+ Abbott Laboratories, Abbott Park, IL

Date of Study Initiation/completion: February, 1996/April, 1996

GLP Compliance: No

QA- Reports: No

Drug Lot Number: Batch 002 _____

Methodology:

- Strains/Species: NMRI mice; male 20 in part 1: male mice 17, females 3 in Part 2.
- Dose Selection Criteria:
 - 5000 mcg/kg of dexmedetomidine hydrochloride
- Controls:
 - Vehicle: normal saline
 - Negative Controls: normal saline
 - Positive Controls: none
- Comments: This study was non-GLP and evidently the examination for micronuclei did not take place in the laboratory in Finland, but in Abbott Labs in the USA.

NDA 21-038

- Exposure Conditions: In Part 1; 10 male mice were injected iv with normal saline and 10 were injected with dexmedetomidine at 5 mg/kg. One half of each group was left at room temperature, $22^{\circ}\text{C}\pm 1$ (normal temp) and the other five of each group was placed on a heating pad kept at 37°C (heated).

Part 2 was the same except that there were 3 females and 2 males in the saline, normal temperature, group rather than the all male population of all other groups.

- Study design: General observations were made and body temperatures were taken (only in test 2) before dosing and hours 1, 3, 5, 8, 24 and 48 post-dosing. At 48 hours post dosing the animals were sacrificed and the marrow of the right femur was extracted and used to produce slides.
- Analysis: Generally about 2000 erythrocytes were examined and counted as normochromic erythrocytes, NCE's, or polychromatic erythrocytes, PCE's. Only tables of individual data are presented in the report. This segment was done by Abbott personnel and evidently only from the mice without supplemental heating.
- No. slides/animals analyzed: one - right femur marrow
- Genetic toxicity endpoints/results: micronucleated PCE's were presented only for the normal temperature group, those unheated.
- Statistical methods: Fisher's Exact Test

Results:

- Study Validity: this was non-GLP and it is not clear where the micronuclei slides were prepared or read. However, since the study was done by the sponsor and indicates possible mutagenic effects of dexmedetomidine, the results will be considered valid until a complete GLP study, using the doses and temperature controls cited here, is submitted.

- Study Outcome: Four of the treated mice without supplemental warming died. The sponsor supplied a table (V31/pg81) which compared only the controls and dexmedetomidine treated mice which remained at room temperature without any supplemental heating. There was no report of the micronucleated PCE's in the heated group to make any comparison. The mean percent micronucleated PCE's was 0.14% for control and 0.5% in the mice treated with 5mg/kg of dexmedetomidine hydrochloride, significantly different, $p < 0.0001$. There were no positive controls cited in the report.

Summary:

The sponsor suggests that the increased micronucleated PCE's in the dexmedetomidine group were due to the hypothermia experienced by the mice at the high dose, but do not present any data indicating the dose of 5 mg/kg would not increase the micronuclei PCE's in heated mice. The sponsor cited an article which used reserpine to induce hypothermia, and in that study the animals which received supplemental heating did not increase the percentage of micronucleated PCEs. The mice that endured 40 hours of low body temperature did have an increased percentage of micronucleated PCEs. The present study only indicates that 5mg/kg was positive in the mouse

NDA 21-038

micronucleus test.

#####

[67]

Study Title: Micronucleus Test of Dexmedetomidine Hydrochloride in Mouse Bone Marrow.
(#201)

Study No: PT971 12100025

Volume # 10 and Page #149

Study Type: *in vivo*

Conducting Laboratory: _____

Date of Study Initiation/completion: January 15/24, 1997

GLP Compliance: yes

QA- Reports: Yes

Drug Lot Number: Batch 002

Methodology:

- Strains/Species: Mouse/ NMRI _____
treatment group.
- Dose Selection Criteria: The use of the MTD is required and this was previously done at 5000 mcg/kg. However, the sponsors explain extensively (pg 160-162) that this high dose produced such hypothermia that this could be the cause of the significant increase in micronuclei (V10/pg75).
- Basis of dose selection: The sponsors stated that the doses chosen, 40, 100, and 250 mcg/kg, were high enough (V 10/pg 162) as this would represent estimated C_{max} levels up to 50 times the human C_{max} , but package insert data shows that it could be a maximum of 33.3 times the human C_{max} . This was not an acceptable basis of dose selection.
- Range finding studies: none
- Test Agent Stability: solution made immediately before injection.
- Controls:
 - Vehicle: normal saline
 - Negative Controls: normal saline
 - Positive Controls: cyclophosphamide in distilled water, 40 mg/kg
- Exposure Conditions: The mice were injected iv on Day 1.
- Study design: Mice were sacrificed either 24 or 48 hours after injection. Body temperatures were recorded prior to injection, 3, 24 and 48 hours post injection. The femurs were both removed from each mouse and a smear was made of the marrow flushed from the bone with fetal calf serum and centrifuged.
- Analysis:
 - No. slides/animal analyzed: 2 slides/animal, 1 for right femur and second for left femur.
 - Counting method: 1000 polychromatic erythrocytes (PCE) were counted and

NDA 21-038

scored for micronucleated cells. The ratio of PCE's to normochromic erythrocytes (NCE's) was also counted in 1000 cells.

- Statistical methods: The results were tested by an ANOVA calculation.
- Criteria for Positive Results: $p < 0.05$

Results:

- Study Validity: The tested doses were well below the MTD and the test was not considered valid. The previous dose ranging study, reviewed above, found no deaths at 100, 250, 500, or 1000ug/kg doses.
- Study Outcome : There were no dexmedetomidine related changes in PCE/NCE ratios, number of PCE's /animal, total number of micronucleated PCE's or relative number of micronucleated PCE's. There were significant increases in all these parameters in the positive controls, cyclophosphamide and there was usually a sex difference with the males being more sensitive to the clastogen.
- Sedation: The low and medium doses of dexmedetomidine induced sedation for 2 to 3 hours after injection. The high dose group was sedated more than 5 hours and less than 22 hours.
- Temperature changes: The body temperatures were unchanged 24 and 48 hours after drug administration. However, the body temperatures 3 hours after dexmedetomidine administration are presented in the following table;

Changes in body temperature - 3 hours post intravenous injection		
treatment mcg/kg	body temperature change - males	body temperature change - females
Saline control	- 2.2	- 0.6
dexmedetomidine 40	- 1.7	- 1.4
dexmedetomidine 100	- 6.9	- 5.7
dexmedetomidine 250	- 11.2	- 12.0

Summary:

The study is not considered valid because the high dose used was less than the MTD or the limit dose. The high dose did lower body temperature 11 to 12°C and caused sedation for more than 5 hours and less than 22. The 11°C drop in body temperature was also observed in the range finding study to occur at 500 and 1000 ug/kg, without lethality. The drop in body temperature was as great as observed previously at the dose of 5000 mcg/kg and no increase in micronucleated PCE's was observed in the present study. This suggests that decreased body temperature is not necessarily the cause of dexmedetomidine induced increase in micronucleated PCE's.

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GENOTOXICITY SUMMARY

Dexmedetomidine hydrochloride was negative in the following tests of mutagenic potential: Ames reverse mutation assay with *S. Typhimurium* ; the reverse mutation assay with *E. coli*; and in the *in vitro* mouse lymphoma assay. In the *in vitro* chromosomal aberration assay in human lymphocytes, dexmedetomidine was positive with metabolic activation in two tests, 300 and 350 $\mu\text{g/ml}$, but was negative without metabolic activation. The high dose (5000 $\mu\text{g/kg}$) in the *in vivo* Micronucleus Test in mice was positive, indicative of clastogenic activity.

The reviewer's conclusions were supported by the CDER Genotoxicity Committee, See attached consult, Appendix 2.

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**APPEARS THIS WAY
ON ORIGINAL**

NDA 21-038

SPECIAL TOXICOLOGY STUDIES

A. Irritancy

[68]

Study Title: Local intramuscular Irritation Study of MPV-1440 (dexmedetomidine) in Rat. (#217)

Study No: TOX 90-004/1

Vol #36. and page #247:

Conducting laboratory and location: _____

Date of study initiation: February 1990

GLP compliance: not stated

QA- Reports: Yes

Species/Strain: Rats/Sprague Dawley - approximately 400 g at initiation

Number/sex/dose: 5/group

Methods: Single dose followed by 4 days of observation and sacrifice with histopathology of muscular injection site.

Dosing: Anesthetized rats with chloral hydrate ip, were injected intramuscular into biceps femoris with dexmedetomidine at 100, 200, 1000, 3000 mcg/ml in saline, lidocaine at 20 mg/ml (positive control) or saline. All injections at 0.1ml/rat, a single dose. The approximate doses were 250, 500, 2500 and 7500 mcg/kg of dexmedetomidine hydrochloride. The clinical formulation is also saline.

Drug, lot# and % purity: dexmedetomidine batch 028 L1, 027 L1, 026 L1, 012 L1 - all reanalyzed in

Observations and times: Observed for 4 days, weight, behavior recorded and after sacrifice at end of 4th day, the injected muscles were removed, fixed in formalin, stained and examined for histopathology.

Results: There was a dose related increase in the duration of the chloral hydrate sedation. The saline and lidocaine rats recovered consciousness 3 hours after dosing. The 100 mcg/ml dose recovered in 4 hours, the 200 mcg/ml dose in 5 hours, the 1000mcg/ml dose was still slightly sedated after 5 hours and the 3000 mcg/ml were also slightly sedated after 5 hours and some exophthalmos and piloerection were observed.

The weight gain was slightly reduced by the two high dexmedetomidine doses. The lidocaine caused a moderate degree on muscle damage, both regenerating and necrotic muscle fibers. The dexmedetomidine solutions of 100 and 200 mcg/ml caused no muscle damage and the higher doses of 1000 and 3000 caused slight damage. At the 3000 mcg/ml dose superficial hemorrhages (3 rats) and pale areas (2 rats) were observed at the injection site.

NDA 21-038

histopathology of muscle	control (n=5)	dex @ 100 µg/ml (n=5)	dex @ 200 µg/ml (n=5)	dex @ 1000 µg/ml (n=5)	dex @ 3000 µg/ml (n=5)	lidocaine @ 20 µg/ml (n=5)
regenerating	2	0	0	5	3	5
adjacent granulation	0	0	0	0	1	5
necrotic fibers	0	0	0	0	0	3

Summary:

The intramuscular injection of dexmedetomidine hydrochloride in concentrations of 100 and 200 mcg/ml did not cause muscular damage in rats. The formulation to be marketed contains 118 mcg/ml of the salt in saline and states in the package insert that this must be diluted with 0.9% sodium chloride solution to achieve the required concentration. The dilution is the same for both the loading dose and the infusion, 2 ml of dexmedetomidine solution and 48ml of saline, for a concentration of 4 mcg/ml of the base. Higher doses of 1000 and 3000 mcg/ml did induce some moderate muscle damage, 3/5 with some regenerating muscle fibers and 1/5 with slight granulation in adjacent tissue.

#####

[69]

Study Title: Acute Arterial Irritation Evaluation of Dexmedetomidine Hydrochloride in Rabbits. (#218)

Study No: TE95-284

Vol #36, page #276:

Conducting laboratory and location: Abbott Laboratories: Abbott Park, IL

Date of study initiation: October, 1995

GLP compliance: Yes

QA- Reports: Yes (pg 287)

Methods:

- Species/strain: 10 male New Zealand White rabbits
- Route of Administration: middle ear vein infusion; 1ml of solution at 0.1 ml/minute, by butterfly, 25 ga. needle attached to syringe pump.
- Doses: normal saline or dexmedetomidine hydrochloride, 4 mcg/ml in normal saline
- Number of animals/sex/dosing group: 5 males
- Drug, lot#, _____

NDA 21-038

- Observations and times: Injected ear examined daily and animals sacrificed 4 days post injection and macro and microscopic examination of the tissue at and around the site of injection.

Results: No irritation observed in either saline or dexmedetomidine injected ear veins.

Summary:

Dexmedetomidine hydrochloride, at 4 mcg/ml, was non-irritating to the ear vein of rabbits in a 10 minute infusion at the rate of 0.1 ml/minute. The formulation to be marketed contains 118 mcg/ml of the salt in saline and states in the package insert that this must be diluted with 0.9% sodium chloride solution to achieve the required concentration. The dilution is the same for both the loading dose and the infusion, 2 ml of dexmedetomidine solution and 48ml of saline, for a concentration of 4 mcg/ml of the base, 4.72 mcg/ml of the salt. -

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B. Hemolytic potential

[70]

Study Title: Hemolysis test on Eight Formulations of MPV- 1440. (#219)

Study No: TOX-91032; Lab No. 11541

Vol #36, page #298:

Conducting laboratory and location: _____

Date of study initiation: November, 1988

Methods: 5 ml of each dexmedetomidine solution was placed in water bath at 37°C for 30 minutes then 100mcl of diluted human blood (oxalate stabilized) was added, mixed gently and incubated for another 60 minutes in water bath and then centrifuged. Supernatant was transferred to cuvette and absorbance measured at 545 μ . Distilled water was the positive control and normal saline the negative control. Three replications were employed.

Concentrations: 1.25, 2.5, 5, 10, 20, 100, 200, 3000 mcg/ml.

Drug, lot#, % purity: _____

Results: 3000 mcg/ml caused 98% hemolysis and was the only concentration to cause any hemolysis.

Summary:

No mention of the solvent in the study, but would assume normal saline as distilled water induced hemolysis. The C_{max} in humans during the treatment, loading dose 1 mcg/kg and infusion at 0.7 mcg/hr, was 2.4 ng/ml and the recommended infusion concentration is approximately 4mcg/ml.

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NDA 21-038

C. IMMUNOTOXICOLOGY:

[71]

Study Title: Evaluation of Dexmedetomidine, Administered as the Hydrochloride Salt, for Passive Cutaneous Anaphylaxis in Guinea Pigs. (#220)

Study No: TF95-009

Vol. #36, pg343

Site and testing facility: Abbott Laboratories: Abbott Park, IL

GRP compliance: yes

QA- Report Yes (x) No ():

Lot and batch numbers: Batch #002

Methods:

Male Hartley guinea pigs were used, 36 from _____ randomly assigned the 6 treatment groups, 6/group. See following table:

The subjects were

Test Group	No. animals	Test Material	Dose (mg/animal)		Dose volume (ml/animal)
			sensitization sc	challenge iv	
T0	6	0.9% saline	0	-	2
T1	6	egg albumin	1	-	2
T2	6	dexmedetomidine	1	-	2
T3	6	dexmedetomidine	-	0.1	1
T4	6	egg albumin	-	1	1
T5	6	dexmedetomidine	-	0.1	1

The sensitization injections were done daily for 3 days a week for 2 weeks. Two weeks after the last sensitization injection, the animals in groups T0, T1 and T3 were anesthetized and blood samples were obtained by cardiac puncture. The blood was centrifuged and the serum was pooled for each treatment group. The samples were diluted with normal saline to concentrations 1/16, 1/64, 1/256 and injected intradermal into subjects of T3, T4 and T5. The backs of these subjects were previously shaved and each guinea pig received one injection of each dilution. Four to five hours after the intradermal injections, each subject received an intravenous challenge dose of dexmedetomidine (T3, T5) or egg albumin (T4) and immediately afterward an iv injection of Evan's blue dye.

The subjects were euthanized 30 to 45 minutes post challenge dose, skin of the back reflected and

NDA 21-038

the diameter of the blue dye spots at injection site were measured. The spot diameters were converted to a grading index from 0 to 4, for diameters of less than 5mm to greater or equal to 20 mm, respectively.

Results:

The animals receiving dexmedetomidine injections showed the pharmacological effects of sedation, prostration and piloerection. The PCA reactions were only with the egg albumin group and the severity depended upon the concentration of the antiserum injected. The guinea pigs injected with serum from dexmedetomidine sensitized animals did not show any passive cutaneous anaphylactic reactions. There was some variation in the analysis of the concentration of duplicates from dexmedetomidine solutions used for sensitization, from 63 to 109% of the theoretical. The investigators were at a loss to explain the variation, but records maintained indicated the solutions were correctly prepared. This should not have impaired the results of the PCA test.

Summary

The animals receiving serum from animals sensitized to egg albumin demonstrated dose related passive cutaneous anaphylaxis (PCA), however there were no reactions from guinea pigs sensitized to dexmedetomidine.

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[72]

Study Title: Evaluation of Dexmedetomidine, Administered as the Hydrochloride Salt, for Delayed Contact Hypersensitivity in Guinea Pigs (Draize Method).

(#221)

Study No: TF95-011

Vol #36, and page #381:

Conducting laboratory and location: Abbott Laboratories: Abbott Park, IL

Date of study initiation: February 1995

GLP compliance: yes

QA- Reports Yes (x) No ():

Methods:

The test animals, 22 male guinea pigs, Hartley _____ were randomly divided into three treatment groups. The positive and negative control groups contained 6 subjects each and the test group with dexmedetomidine hydrochloride was composed of 10 animals. The negative control was physiological saline and the positive control was 0.05% 1-chloro-2,4-dinitrobenzene (DNCB) and 0.0591% dexmedetomidine hydrochloride. Sensitization injections were given Monday, Wednesday and Friday for a total of 10 injections. The injections were intradermal in backs in an area previously clipped clear of hair. The dose volume was 0.1

NDA 21-038

ml/animal for the first injection and 0.2 ml/animal for the remaining nine injections.

Two weeks after the tenth sensitization injection, the skin in the back for the challenge injection was clipped free of hair and 0.1 ml of the respective sensitizing solutions were injected intradermally. The area around the injections were evaluated 24 and 48 hours after the sensitization injections and after the challenge and graded for erythema and edema, both on a 0 to 4 point scale (Draize Method). The Dermal Irritation Score (DIS) was a combination of 24 and 48 hour points divided by 4 and considered positive if the DIS exceeded the mean sensitization scores by 1.0 or more. The analysis of the dexmedetomidine hydrochloride solutions confirmed the intended concentrations.

Results:

The animals injected with dexmedetomidine displayed the expected pharmacological effects, sedation, ataxia, ptosis and prostration. There were no significant differences between treatment groups in body weights during the experiment. The challenge was positive in 3/6 of the positive control although very slight to well defined erythema was observed in all 6 animals upon the challenge and many sensitization injections. There were 0/6 positive in the negative control group and 0/10 in the dexmedetomidine test group.

Summary

Dexmedetomidine hydrochloride did not induce delayed contact hypersensitivity in the guinea pigs.

#####

D. Toxicity of Impurities

[73]

Study Title: Subacute Toxicity Study of Levomedetomidine Hydrochloride by Daily Intravenous Administration to Female Rats for Two Weeks (#225)

Study No: TOX91-031

Vol #38. and page #306:

Conducting laboratory and location: _____

Date of study initiation: May 1991

GLP compliance: No

Methods:

The rats were dosed daily, iv tail vein, for two weeks except for the high dose group, 10 mg/kg, which was only dosed for one week.

NDA 21-038

The macroscopic external and internal appearance of the tissues was noted and any abnormalities recorded. The following organs from all animals at the scheduled sacrifices were dissected out and weighed: both adrenals, brain, heart, both kidneys, liver, lungs, mesenteric and submandibular lymph nodes, pituitary, spleen, thymus and uterus. The relative weight of organs was calculated by dividing the organ weight (kg) by the bodyweight (kg) at autopsy and multiplied by 100.

Dosing:

- species/strain: Rats/Sprague-Dawley outbred
- #/sex/group or time point: All females, 5/treatment group
- age: 80 days at start
- weight: approximately 210 g
- dosage groups in administered units: 0, 0.3125, 0.625, 1.25, 2.5, 5, 10 mg/kg. Treatment groups 1 through 7 respectively.
- route, form, volume, and infusion rate: daily injections into tail vein, 1ml/kg volume.

Drug lot# and % purity: Batch #PT4212,

Formulation/vehicle: solutions in normal saline

Results:

Morbidity and Mortality: No mortality was observed, but the high dose group #7, 10 mg/kg, developed severe necrosis at the injection site and were sacrificed after one week of dosing.

Clinical signs: Piloerection was observed after dosing in Groups 5-7 with dose related severity. The doses of 5 and 10 mg/kg caused irritation at the site of injection.

Body weights, Food consumption, Ophthalmoscopy: No treatment related differences

Hematology: The highest dose, 10 mg/kg(#7), decreased RBC count and HBG, PCV, MCHC and increased MCV. In the 5 mg/kg group (#6), the MCHC was also significantly decreased. In group #7, the WBC differential revealed decreased lymphocytes and an increase in neutrophils. Reticulocytes were significantly increased in the 1.25 and 5.0 mg/kg groups, but this was not considered drug related.

Clinical chemistry: In this parameter, only the blood glucose was measured and this was unaffected by drug treatment.

Organ Weights: There were some reductions in organ weights in the high dose group, but since this group was sacrificed one week early, these results are in question. The relative organ weights revealed an increased relative liver weight in the 5 mg/kg and 10 mg/kg groups. The spleen, kidney, adrenal and ovary weights were also significantly increased in the high dose group and the uterus relative weight was significantly decreased. The early termination of the latter group could account for some of these differences.

Gross pathology: Except for the injection site inflammation mentioned above, there were no treatment related pathologies.

NDA 21-038

Histopathology: The injection site dermal-epidermal ulcers were evident in 1/5 at 5 mg/kg and 4/5 rats at 10 mg/kg. Zona fasciculata hypertrophy in adrenals was observed in 2/5 and 3/5 in the two highest dose groups. In the liver, there were no hepatocellular vacuolization in rats from 0, 0.3125 and 0.625 mg/kg, there were vacuoles evident in the higher doses and 4/5 and 5/5 in the two highest dose groups. Extramedullary hematopoiesis in the spleen was noticed in the two highest dose groups, slight to minimal in severity. The highest dose also appeared to induce stromal atrophy in the uterus.

Summary

The doses of 0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/kg/day were administered intravenously to female rats for two weeks by tail vein. The intravenous injection in doses of 0.3125 and 0.625 mg/kg/day to female rats for two weeks, appeared to have no toxicological effects and 1.25 mg/kg appeared only to have one parameter differing from controls. This was the appearance of minimal periportal hepatocellular vacuoles in 2 of the 5 rats and the actual toxicological significance of these findings are in question. Compound related effects, hepatocellular vacuolization, zona fasciculata hypertrophy and extramedullary hematopoiesis, were observed in the 5 and 10 mg/kg groups. Considering that the maximum percent of this contaminant is 1% and the daily recommended dose of dexmedetomidine is 17.8mcg/kg/day, the safety factor is 570x on a mg/m² basis using the 0.625 mg/kg dose in this study. However, there was no full panel clinical chemistry and no data on liver function enzymes, sites of dexmedetomidine toxicity.

#####

[74]

Study Title: MPV- 1441 (Levomedetomidine Hydrochloride) Subacute Toxicity Study by Daily Subcutaneous Administration to Rats for Four Weeks. (#226)

Study No: TOX 88-029

Vol # 39, page #1:

Conducting laboratory and location: _____ id

Date of study initiation: November 1988

GLP compliance: No

QA- Report Yes No (X)

Dosing:

- species/strain: Rats / Sprague-Dawley
- #/sex/group: 10/sex/group
- age: not stated
- weight: first week the males were about 190g and the females 160 g.
- dosage groups in administered units: The doses were 0, 20, 100, 500 and 2500 mcg/kg/day.
- route, form, volume, and infusion rate: Daily subcutaneous injections in the mornings for four consecutive weeks. The dosing volume was 1 ml/kg.

NDA 21-038

Drug, lot#, radiolabel, and purity: _____

Formulation/vehicle: All in normal saline; no post verification

Observations and times:

Clinical signs: twice per day

Body weights, Food and Water consumption: weekly

Hematology, Clinical chemistry: 5/sex/group at necropsy

Results:

Clinical signs: No mortality and no drug related behavioral changes

Body weights, Food consumption, Hematology, Clinical chemistry: No drug related changes

Organ Weights - Absolute and Relative: No drug related changes

Gross pathology: At the site of injection, subcutaneous hematomas appeared in all of the rats, male and female at the high dose of 2.5 mg/kg and only one half of the rats injected with 0.5 mg/kg and 10 to 20% of control injections.

Histopathology: In accord with the injection site hematomas, subcutaneous necrosis was evident in all the high dose animals and no rats in the controls or at lower dose of levomedetomidine hydrochloride.

Toxicology Summary:

No target organ toxicity or drug related toxicity was observed at 2.5, 0.5, 0.1, or 0.02 mg/kg, subcutaneous in rats. The only drug related toxic effects of levomedetomidine hydrochloride (MPV-1441) seen in this high dose group rats, 2.5 mg/kg sc, was irritation (hematoma and subcutaneous necrosis) at the site of injection. Some hemorrhages and hemosiderin-laden macrophages at the injection site were also observed in the 0.5 mg/kg group.

#####

[75]

Study Title: Toxicity Study of MPV-1441 (Levomedetomidine Hydrochloride) by Daily Intravenous through Intravenous Administration to Dogs for 28 Days. (#227)

Study No: TOX 96-002

Vol #39-40, and page #249-146:

Conducting laboratory and location: _____

Date of study initiation: February 1996

GLP compliance: yes

QA- Report: Yes

NDA 21-038

Methods:

- species/strain: Dogs / beagle
- #/sex/group: 3/sex/group
- age: 5-6 months
- weight: 6.5 - 8.7 kg
- dosage groups in administered units: 0, 0.4, 2.0, 10.0 mg/kg, groups #1, #2, #3 and #4 respectively.
- route, form, volume, and infusion rate: iv in foreleg, daily for 4 weeks in Groups #1, 2, 3; Group #4 males were dosed for 3 days and females for 2 days. The dosing volume was 0.25 ml/kg for all preparations.

Drug, lot#, and % purity: batch 10056X

Formulation/vehicle: normal saline as solvent. The analysis of the formulations indicated all were between 90 and 103% except for the first day when pipetting error resulted in the Group 2 males receiving 0.66 mg/kg rather than the intended 0.4 mg/kg and Group 3 males received 2.54 mg/kg versus the intended 2.0 mg/kg.

Results:

Clinical signs: No drug related effects were seen in groups 1, 2 or 3. However, the high dose group displayed salivation, tremors, diarrhea, vocalization, piloerection, redness in the eyes and transient aggression. Local irritation was seen later at the injection site of the high dose and a reddish urine was seen in this group. The dosing of group 4 was terminated after 2 (females) or 3 (males) injections.

Body weights No significant drug related changes in bodyweight were observed.;

Food consumption: The dog were all offered 400 grams of food per day and drug related changes were not observed.

Ophthalmoscopy: No ophthalmic changes were evident.

Electrocardiography: The EKG was taken prior to study initiation and 1 and 24 hours post dosing during week 3. The results reported were based on the recordings of limb lead II. Statistically significant differences were seen in P (low dose), PR (low dose), ST (mid dose), QT (mid dose) and R (low dose). All were considered normal variations and not due to treatment effects. One mid dose dog demonstrated an increased PR-interval, one hour after dosing. There were no EKG abnormalities observed.

Heart Rate: The mid dose, 2.0 mg/kg, produced a slight but statistically significant decrease in heart rate one hour after dosing

Blood Pressure: No drug related changes were observed..

Hematology: Although there were several statistically significant differences between the controls and the low dose group; WBC count, Hb, PCV(38.45 vs 35.47), lymphocytes; these were considered normal variations and not drug effects. The same was considered for the eosinophils in the mid dose group (0.92 vs 3.0).

NDA 21-038

Clinical chemistry: The serum creatinine and urea values were elevated in the males at the third day of dosing in the high dose group. This recovered during the drug free days and was probably drug related. There were statistically significant differences in ion concentration, blood glucose and enzyme levels, but all were within normal ranges and were considered normal variations.

Urinalysis: The only significant difference was a decrease in the osmolality in the low and mid doses. The investigators stated this was normal range and was not treatment related.

Organ Weights, Gross pathology: No treatment related changes.

Bone Marrow: There was a slight but significant increase in myelocytes in the low dose group males and this was considered normal variation by the investigators.

Histopathology: Although minimal perivascular fibrosis was seen in 4/6 of the control dogs at the site of injection, the severity of fibrosis increased to slight at the low (1/6) and mid doses (3/6) and severe at the high dose (3/6). The high dose group were the only dogs with severe vascular fibrosis (5/6). The histopathology at the site of injection was the only histopathology that was treatment, levomedetomidine, related.

Toxicokinetics: Samples were obtained from all dogs prior to dosing and 0.5, 1, 2, 3, 5, 7 and 24 hours after the initial dosing with levomedetomidine and again during week 4. The results are presented in the following table:

Dosage Group (mg/kg/day) intravenous	Day 1		Week 4	
	C _{max} (ng/ml)	AUC _(0-24hr) (ng.hr/ml)	C _{max} (ng/ml)	AUC _(0-24hr) (ng.hr/ml)
Males				
0.4 ^a	153	356	97	194
2.0 ^b	978	2300	705	1470
10	4743	15035	c	c
Females				
0.4	117	275	105	193
2.0	828	2587	695	1822
10	5000	18317	c	c

^a Day 1 miss dosed at 0.66 mg/kg in males

^b Day 1 miss dosed at 2.54 mg/kg in males

^c Males sacrificed Day 3, females Day 2

Summary:

The iv high dose dogs displayed salivation, tremors, diarrhea, vocalization, piloerection, redness in the eyes and transient aggression and were terminated after 2 or 3 injections, Days. The mid dose, 2.0 mg/kg slightly decreased the heart rate and slightly increased the PR-interval during EKG recordings one hour post administration. There were no drug related bodyweight changes, food consumption, ophthalmic pathology, organ weights or relative organ weight changes. The low dose, 0.4 mg/kg of levomedetomidine hydrochloride is considered the NOAEL. Although there were statistically significant differences from control in sporadic hematology, clinical chemistry and urinalysis parameters, the differences were within the normal range, did not show any dose dependence and often were sex specific, all indicating spurious findings.

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Summary of Special Toxicology Studies

Irritation

Intramuscular injection of dexmedetomidine in the rat caused no muscle damage at concentrations of 100 and 200 $\mu\text{g/ml}$, but at 1000 and 3000 $\mu\text{g/ml}$, did induce muscle damage. In the rabbit ear vein, dexmedetomidine at 4 $\mu\text{g/ml}$ and 0.1 ml per minute for 10 minutes did not produce intravascular irritation, but it is difficult to compare the 10 minute infusion to 24 + hours of iv infusion to be used in the clinic.

Allergic hypersensitivity

Dexmedetomidine did not induce either Delayed Contact Hypersensitivity or Passive Cutaneous Anaphylaxis in the guinea pig. The results show that dexmedetomidine has a low potential for inducing hypersensitivity.

Toxicity of impurities

Levomedetomidine, the principal impurity in the clinical drug product, was tested in rats and dogs for 4 weeks. In rats, sc injections at 20, 100, 500 and 2500 $\mu\text{g/kg/day}$ did not differ from control results in clinical chemistry, hematology, body weights and food consumption. The histopathology of the injection sites revealed subcutaneous hematomas with increasing frequency as dose increased and the highest dose induced subcutaneous necrosis. In an iv study of 4 weeks in rats, findings at 312.5 and 625 $\mu\text{g/kg/day}$ were not different from controls, but at 1250 $\mu\text{g/kg/day}$ there were findings of periportal hepatocellular vacuoles which increased with dose. In addition to the hepatic vacuoles, the hypertrophy of the adrenal zona fasciculata, splenic extramedullary hematopoiesis and severe dermal ulcers at the injection site were observed in the high dose rats, 5000 and 10,000 $\mu\text{g/kg}$.

NDA 21-038

In the dog study, four weeks of daily iv injections of 400, 2000, or 10,000 $\mu\text{g}/\text{kg}/\text{day}$ produced pharmacological and toxicological changes. The high dose dogs displayed salivation, tremors, diarrhea, vocalization, piloerection, redness in the eyes and transient aggression and were terminated after 2 or 3 injections (days). Local irritation was seen later at the injection site of the high dose (vascular fibrosis) and a reddish urine was also seen in this group. The low dose, 400 $\mu\text{g}/\text{kg}$, was considered the NOAEL and the AUC was about 300 ng.hr/ml on Day 1 and 194 ng.hr/ml at the end of 4 weeks. This impurity is to be less than 1% of the marketed product and the factor of 800X does not indicate that humans will be exposed to any toxicity of this isomer.

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**APPEARS THIS WAY
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