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OND/ODE-1/dcrdp(HFD-110)

Subject : Overview of Pre-clinical Reproductive Toxicology Studies of Ranolazine (NDA 21-526)

Sponsor : CV Therapeutics, Inc.

This is an overview of pre-clinical reproductive toxicity studies, and other relevant toxicity studies, performed by Syntex Research Instit. of Toxicol. Sci.. (Palo Alto, CA). They involve the rat and/or rabbit to evaluate effects of Ranolazine on fertility, organogenesis, and neonatal behavior. Contentious issues include impaired fertility (male/female rat), in-utero development tox (skeletal malformations in rat; embryotoxicity in rabbit); and neonatal developmental toxicity. It focuses on study design, especially systemic exposures relative to clinical drug levels; and on interpretation- interpretability of results *vis a vis* behavior in contemporary control cohorts. I also sought evidence of testis pathology in multiple standard sub-chronic/chronic rat toxicity tests to confirm that encountered at high dose (HD) of 300mg/Kg in the rat fertility test , and will convey at the outset that I could not find such – in either rat or multiple sub-chronic/chronic dog toxicity studies.
For a different perspective, I also sought to identify relative potency for targeted pharmacologic activity vs. depressed fertility toxicity i.e., a veterinary safety ratio in the rat. Such was indeterminite (ranolazine was not tested in a rat model of angina; and the pharmacodynamic activity underlying any anti-anginal activity is moot).
Pre-clinical reproductive and chronic toxicity studies have been comprehensively reviewed, and archived (9/2/2003), by Elizabeth Hausner, D.V.M.

Summary:

A. Male Rat Fertility:

[First off. it should be noted that in this fertility trial pregnancy rate in untreated females offered to untreated males was only 70% vs essentially 100% historical incidence, which immediately raises a questions regarding the status of breeders and/or the breeding conditions.].

Evidence of 30% depressed male fertility at high dose (HD) derives from a single study at 0, 5, 40, and 300 mg/Kg involving 20 males /40 females per breeding cell. At the HD,5 males and 4 females died prior to initial breeding (this drug is lethal in the rat even though it affords only 1-2 X the human AUC burden based on other rat PK studies. Pregnancy rates in the 30

control and the 25 HD females with evidence of mating was 98% and 64%, respectively. The surviving HD males were re-dosed at the HD in two successive follow-on trials (133 days; 156 days +32 days recovery: each involving untreated females) with the same results, namely 30% reduction in conception rate in the untreated females. So it is really three trials with the same male actors.

Regarding maternal reproductive performance as informed by mid-gestation caesarian: pregnancy rate in control and HD was 100 and 67%, respectively, confirming the impairment in survivors seen at term via natural delivery.

Testis histopath: Retrospectively, based on individual rat impregnating performance, four HD male rats with testicular lesions were identified which if sterile, would mathematically account for the same (30%) impairment in the initial trial and two follow-on trials. Accordingly. I searched for testis toxicity in other animal studies. Dr. Hausner reports that the "histopath for the standard 3-month rat study was incomplete with no incidence or summary tables; and that histopathology results for the 6 month rat study were not susceptible to easy interpretation. While the ability of those rat toxicology studies to confirm testicular histopathology is moot. , I do note that testis weight in those studies was unaffected, and that testis histology is evidently normal in a 1-year rat study.

B. Embryotoxicity and Teratology:

1.From Fertility study:

In the fertility study above, incidence of pregnancy with at least 1 resorption was 75% in all treated dams, but fully 50% in control. However, there was no drug-associated reduction in mean litter size, mean no.of total resorptions, or mean no.of implantations. at Caesarian,

II. Segment II teratology studies : These were performed in rats and rabbits at up to maternotoxic high dosages of 400 and 150 mg/Kg, respectively, administered during the critical period of fetal embryogenesis followed by Caesarian at end of term. The HD is an $LD_{35\%}$ and $LD_{25\%}$, in rats and rabbits, respectively. In my judgement, these tests were still informative since there were 15 litters available for analysis in rat and 13-15 in rabbit – even though 16 to 20 is recommended by ICH). I see no evidence of selective fetal toxicity in these studies, indeed there is absence of terata even at the egregiously toxic HD:

Rat: Fully 24-52% of litters from controls had delayed or reduced ossification (in this case, pelvic, cranial and possibly sternebral) and mis-shapen sternebrae vs 33-80% of litters from HD dams. Such "lesions" are relatively common .and not considered to be manifestations of teratogenesis. In my opinion, excesses in these "lesions" are a non-issue. Moreover, all other bones were evidently normally ossified. Whether this is or is not an important lesion, it is noted that this ossification "anomaly" occured at probably approx. 1.5X the human AUC. Minimum live litter size was decreased at MD and HD, but this could reflect <u>drug-unrelated</u> decreased ovulation at these dosages (min, corpora lutea way down at MD and HD).

Fetal weight was significantly decreased, and incidence of pregnant dams with 100% still-born fetuses egregiously increased, at HD. However, the HD approaches an $LD_{50\%,}$ with cyanosis and convulsions, in this study – hardly the profile of selective embryocidal toxicity. One may even have expected to see frank terata at such a systemically toxic dosage. There were none.

Rabbit: First off, as noted, I consider 13-14 litters in all cohorts to be evaluable, and not egregiously below the suggested limit. The **implantation index** [(implantations/corpora lutea) x100] was indeed significantly reduced at the MD(45 mg/Kg) and the HD (150 mg/kg), but this

is related in part to, strangely enough, a "dose -related" increase in number of corpora lutea (this cannot be drug-related as they are treated after ovulation). Furthermore, there was no impairment of gestation survival index [(live fetuses/total fetuses)X100] , or resorption index [(total resorptions/implantations) X100], or live litter size. Fetal weight was unaffected, even at the lethal HD.Reduced ossification of sternebrae is again a non-issue - it occured in 54% of control litters and 77% of HD litters. These results do not reveal any selective fetal toxicity in my opinion.

C. Neo-natal development: In the above fertility trial summarized above , delayed neonatal development per standard landmarks (e.g., eye-opening) was clearly evident . Development did "catch-up" to control by the last monitoring period. Mean pup weight was non-dose-relatedly reduced, but only by 5% on day 4 and 3% on day 21 (HD vs control). Reduced neonatal survival was statistically significant and dose-related, but not appreciable: mean survival index being 95 % in treated groups vs. 100% in control up through weaning, attributable to excess neonatal mortality only in the post-natal days 1-4 interval.

In a Segment III trial ,which are typically specifically designed to look at neonatal behavior, there were, inexplicably, no developmental landmarks monitored. However, survival and mean body weight of pups from dams treated at up to 200 mg/Kg were indistinguishable from control. (Recall that the 300 mg/kg dosage is lethal in females and males).

A. Fertility (study 116-R-86-43285-PO-RMF)

[This study informs rat fertility and neonatal development. It is the only trial of fertility, as rabbits are not used for such testing]

Pregnancy rates: Fertility study 116-R-86-43285-PO-RMF involved 20 males offered to 40 females per mating cohort; 0, 5, 40, or 300 mg/Kg rats total per cohort; and with both males and females treated. It provided persuasive *prima facie* evidence of appreciably (30%) impaired capacity of males to impregnate at the convulsant lethal high dose (HD) of 300 mg/Kg, (i.e., impregnation was 64 % and 93% of HD and control females, respectively ($P<0.05$.) who presented with evidence of having mated. [I note that only 75% of control co-habited females had evidence of mating, which is appreciably lower than the ca. 100% incidence usually encountered in such studies.] The dose-response possibly begins at 40 mg/Kg,(approx.10% impairment), the next lowest dose tested. This is based on a reduction in gravidity of female consorts both at Caesarean sacrifice(where, from each cohort, the first 12-15 dams with evidence of mating were sacrificed at mid-gestation) , and as evident from pregnancy status in the surviving females allowed to litter at term.

Sponsor asserts that "the pregnancy rate in treated rats given 300 mg/kg/day was within the range seen in primiparous control rats". Documentation of that assertion.was not provided.

Because of the impaired fertility, surviving HD sires (P1) were not sacrificed, but, rather, were re-tested twice in two consecutive longer term trials, each of which revealed 30% impaired ability to impregnate untreated females after 133 days of re-instated high-dosing (study 176-R-87) , and, again, after 156 days high-dosing followed by a 32-day recovery (study 195 -R-87). In both follow-up studies , 100% of control males impregnated females vs. 69% of HD males, establishing -and confirming - that it was male fertility which was evidently compromised at the HD.

Regarding effects on female fertility, there was no cohort of treated females exposed to untreated males. Accordingly, I do not know whether an increase in incidence of gravid females with resorptions noted at Caesarian of all treated dams, (about 75% with at least one resorbed fetus vs. 50% of controls) reflects impaired maternal reproductive performance , or, conceivably, an effect on sperm to reduce embryo viability. Dr. Hausner did not note that this "impairment" is mitigated by essentially unchanged Resorption Index [(total resorptions/implantations)X100] and Implantation Index [(implantations/corpora lutea) X100]. Accordingly, there was no significant decrease in litter size.

Neonatal development: In the initial trial, development delays – eye opening; vaginal opening; negative geotaxis and reduced survival – were seen in all drug-treated groups. However, I note that neonatal development informed by such landmarks had "caught-up" to controls by the last monitoring period. The reduced neonatal survival was statistically significantly, but not appreciably, impaired : mean survival index being 95 % at in treated groups vs. 100% in control up through weaning, attributable to excess neonatal mortality in the post-natal days 1-4, and no exacerbation subsequently through weaning. Dr. Hausner notes, correctly, that "Mean pup weights in the HD group were decreased compared to control at all points of determination and reporting"; but the decrement was only 5% on day 4 and 3% on day 21. No neonatal monitoring was performed in the second or third follow-up trials to confirm these unimpressive and/or reversible changes in behavior of neonates exposed to ranolazine *in utero* and *via* lactation.

Parental toxicity: The HD was clearly toxic, convulsant, and evidently lethal: 7 males and 4 females died prior to or subsequent to mating due, according to Sponsor, " principally to aspiration and/or malintubation , possibly related to excess salivation and convulsions". The HD of 300mg/Kg is close to 400 mg/Kg which in teratology study AT3758 (See below)is also convulsant and an $LD_{35\%}$..

Histopathology of treated males: It was determined retrospectively from individual fertility data, that five repeatedly used HD males contributed to the reduced fertility in the main study and its two "extensions". Autopsy performed on 4 of these 5 rats at the end of study three revealed atrophied testes and/or epididymides. Three of these HD males (all of which, by then, had been exposed to ranolazine for approx. 1 year followed by a 1-month drug-free recovery), presented with epididymal atrophy and virtual aspermia (2 rats)or hypospermia(1 rat). Four of these 13 HD males surving the 1 year HD regimen had atrophied seminiferous tubules vs. 2 of 20 control males.

Dr. Hausner did the math and confirms that sterility in the 4 identified rats could account for the 31% decrease in fertility in the last two consecutive re-dosing trials

It is indeterminate when in the course of the three consecutive trials these lesions in 4 rats occurred, as there were no biopsies, and no HD sacrifices until after the end of the third re-trial followed by a month recovery. If these four damaged rats account for the HD impairment of fertility, then the lesions were present by the end of the initial 80 day day dosing which preceded the first testing of fertility, and neither abated - nor were additional rats affected - over a subsequent 9 months of re-challenge (i.e., two follow-on trials) in view of the identical 30% impairment in all three trials.

Testis weight/histology in other rat and dog tox. studies:

I looked for evidence of testicular injury in standard chronic rat (and dog) toxicity studies where testis weight (both absolute and as %body wt) and histology are routinely monitored.

Rat: Dr. Hausner's review does not identify the testis as an affected organ in 3, 6, or 12 month rat toxicity studies done at up to 200 or 500 mg/Kg, except that in the 3 month study mean testis weight was unchanged at 250 mg/Kg and actually slightly increased (11%) at an egregiously systemically toxic 500 mg/kg. dosage. Regarding the veracity of these "non-findings", Dr. Hausner reports that the "histopath for the 3-month study was incomplete with no incidence or summary tables. Histopathology results for the 6 month rat study were not susceptible to easy interpretation". However, at least testis weights were given, and data revealed no atophy as I just noted. Clearly, histopath. was provided for the 1 year rat study at up to 200 mg/Kg, where clear drug-associated changes were noted in adrenals, pituitary and lungs, but no mention of excess testicular pathology.

Dog: :It is also evident from Dr. Hausner's review of chronic conventional toxicity studies that the dog testis is not consistently affected vis a vis weight or histology, in 3, 6, or 12 month toxicity studies also done at up through systemically toxic dosages (up to 80 mg/Kg). At 60 mg/Kg, testis weight was slightly increased in a 3-month study, decreased 25% in a 6 mo. study, and unchanged in a 12-mo. study. Her review is silent on any testis histopathology, which presumably was absent**.]**

B. Teratogenicity/embryotoxicity:

As noted in the summary,Rat and a Rabbit Segment II teratogenict y studies were performed at up to maternocidal dosages. I believe that sufficient number of litters were available (13-15), even at the HDs, to be revealing, and saw no evidence of any teratogenic activity or selective embryocidal activity of veterinary importance to project any clinical concern. This drug is toxic in both rats and rabbits at AUC exposures (at least in the rat, and probably rabbit as well) approximating clinical (we saw the same thing with ACE inhibitors in the rabbit tests of their teratogenic potential.). To get a handle on their inherent reproductive liability, a veterinary safety ratio of , say, an $Fertility_{-30\%}$ / $ED_{30\%}$ might be revealing. I could not develop such surrogates. Dr. Hausner just received reports CVT303.064-P and CVT303.062-P on anti- beta and anti-alpha adrenergic activities , respectively, of ranolazine in conscious rats. Perhaps data theirein could afford such safety ratios.

Drug exposures and projected safety multiples: In the fertility study**,** No blood levels were provided or referenced to compare to AUC of 33,700 ng.hr/ml in humans who received 1000 mg of ranolazine t.i.d. for 5 days. However, in a 6 month rat toxicity study, dosages of 50 or 150 mg/Kg /day for 3 months afforded AUCs of approx 14,000 and 64,000 ng.hr/ml., respectively, in the males. Accordingly, if we assume that the threshold level for impairing male fertility was 40 mg/Kg (not enough dosages were tested to firmly establish such) then rats may begin to reveal impaired fertility at approx 0.5X the human AUC. What can more confidently be said is that important impairment of male rat fertility was clearly observed at 300 mg/Kg which affords approx. twice the human AUC – not a very re-assuring safety multiple.

[It should be noted, at least in passing, that impairment of fertility was not progressive. Impairment was still approx. 30% after about 1 year HD (300 mg/Kg)exposure when , based on other rat studies, the $AUC_{0\text{-}24\ hr}$ is expected to be approx 300,000 ng.hr/ml or 9X the human exposure (200 mg/Kg afforded 200,000 ng.hr/ml in the 1 year standard tox. study)]

Therapeutic ratio : I could not calculate a toxic dose/pharmacodynamic dosage in the rat because the drug was not tested in an in vivo rat model of angina pectoris. Furthermore, as the pharmacodynamic basis underlying therapeutic effect is moot, I could not identify a surrogate marker for anti-anginal activity.

CONCLUSIONS:

In a study of male rat fertility, ranolaziner impaired fertility at the lethal HD, which likely reflected no or reduced sperm counts in 4 rats. No other chronic rat or dog toxicity study identified any testicular toxicity as informed by organ weight, although Dr Hausner notes that histopath tables were not forthcoming in all potentially relevant toxicity studies. Regarding teratogenicity, there was none, even at maternocidal dosages. Regarding embryotoxicity, there were reductions in impantation indices but not, as far as I can determine, when corrected for increases in ovulation . There were decrements in fetal weight and neonatal developmental delays, especially at the HD.

The evaluation of any reproductive toxicity in the context of maternal and paternal toxicity, and at or within a few-fold of human AUC exposures, The extent to which such reproductive toxicity is selective is indeterminate, in my judgement, due to an excessive interval between mid and high dosages which precluding determination of toxicity thresholds.