

Toxicokinetics:

The pharmacokinetic parameters used to describe systemic exposure (C_{max} , AUC, and C_{av}) are summarized in the following table.

Table (provided by sponsor). Mean pharmacokinetic parameters of gadolinium (Gd) obtained in pregnant rabbits after intravenous administration of 0.3, 0.8, and 2.0 mmol/kg ZK 236018 per kg body weight from day 6 to day 18 of gestation.

Parameters For Gd [unit]	Dose (mmol ZK 236018/kg/Day)					
	0.3/d 6	0.3/d 18	0.8/d 6	0.8/d 18	2.0/d 6	2.0/d 18
C_{max} [mmol/L]	2.73 ± 0.18	2.83 ± 0.23	7.20 ± 1.55	7.46 ± 2.04	13.9 ± 2.14	12.4 ± 1.61
AUC _{0.083-4h} [mmol*h/L]	4.18 ± 0.34	4.15 ± 0.54	8.76 ± 0.90	7.87 ± 0.52	15.5 ± 1.95	13.2 ± 0.28
$C_{av, 0.083-4h}$ [mmol/L]	1.07 ± 0.09	1.06 ± 0.14	2.24 ± 0.23	2.01 ± 0.13	3.97 ± 0.50	3.36 ± 0.07
AUC _{0.083-24h} [mmol*h/L]	n.e.	8.41 [#] ± 0.73	15.8 ± 2.26	13.8 ± 0.96	24.5 ± 4.11	21.7 ± 2.80
$C_{av, 0.083-24h}$ [mmol/L]	n.e.	0.35 [#] ± 0.03	0.66 ± 0.09	0.58 ± 0.04	1.02 ± 0.17	0.91 ± 0.12

The mean of two measurements was used.

n.e. = not evaluable, because the serum level at 24h was below the LOQ of 10 µmol/L

- C_{max} : maximum Gd-concentration in serum observed 5 minutes after drug administration
AUC_{0.083-4h}: area under the concentration versus time curve from the first point in time to 4 hours
 $C_{av, 0.083-4h}$: average serum concentration from the first point in time to 4 hours
AUC_{0.083-24h}: area under the concentration versus time curve from the first to the last point in time
 $C_{av, 0.083-24h}$: average serum concentration from the first to the last point in time

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Mean maximum drug concentrations were observed in serum at 5 min at all dose levels, in a dose-dependent manner. At 0.3 mmol/kg/day, Gd-through levels were below the LOQ, thus, the AUC could not be determined at this dose level. The mean AUC increased under-dose proportionally and non-linearly on day 6 from 0.3-2.0 mmol/kg/day with a 3.7 fold increase in the AUC for a 6.7 fold increase in dose; similar effects were observed on day 18. In addition, an increase of the dose from 0.8-2.0 mmol/kg/day resulted in only a 1.6 fold increase in the AUC. No accumulation of the drug was observed between day 6 and day 18 within the dose range tested.

The terminal elimination phase was not reached at 4 hours post-injection, therefore the half-life, clearance, and volume of distribution could not be evaluated.

An additional time point should have been included in the PK study.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

-No pathological finding was observed at 0.3 and 0.8 mmol/kg/day. Macroscopic examination revealed swellings and/or eschar formation were noted in 5 and 6 dams respectively in the 2 mmol/kg/day group.

-One low-dosed and one high-dosed dam had uni or bilateral dented kidneys. One dam of mid-dose group had multiple dark-red foci (1-2 mm) in the kidneys. These findings were considered spontaneous by the sponsor.

-In the female sacrificed after abortion, the abortus contained 2 fetuses and one placenta. Four fetuses and 5 placentae still remained in the uterus. Fetuses were at a normal development stage according to their age.

-Gravid uterus, carcass, and net weight change from day 6 were not affected with 0.3 or 0.8 mmol/kg/day. At 2 mmol/kg, the gravid uterus was decreased by 25% compared to the control. The sponsor attributed this effect to the severely increased post-implantation loss in this dose group.

-Kidney absolute and relative weights were increased at 2 mmol/kg/day. Although not statistically significant and not observed at the 2 lower dose levels, this change was attributed by the sponsor to the drug.

Table of summary of reproduction data (by sponsor)

Parameter	Control (n=20)	0.3 mmol/kg/d (n=20) (3.3X HD)	0.8 mmol/kg/d (n=20) (8.8X HD)	2.0 mmol/kg/d (n=20) (22X HD)	Historical control groups (n=18) -Range for control groups
Corporea lutea					
Total	146	147	143	139	
Per dam	7.3	7.4	7.2	7.0	Per dam 6.7-8.4
Implantation sites					
Total	130	129	125	127	
Per dam	6.5	6.5	6.3	6.4	Per dam 5.4-7.0
Resorptions					
Total	3	20**	17**	35**	
Per dam	0.2	1.0	0.9	1.8	Per dam 0.0-0.9
Early resorptions					
Total	1	19**	16**	33**	Total 0.0-11.2
Per dam	0.1	1.0	0.8	1.7	
Late resorptions					
Total	2	1	1	2	
Per dam	0.1	0.1	0.1	0.1	
Live fetuses					
Total	127	109**	108**	92**	
Per dam	6.4	5.5#	5.4	4.6##	
Dead fetuses					
Total	0	0	0	0	0-2
Pre-implantation loss (mean%)	11.3	13.1	12.0	8.4	6.4-28.6
Post-implantation loss (mean%)	2.5	12.9	14.2	31.9	0.0-16.9

** Significantly different from the controls at $p \leq 0.01$

Including one dam with no viable fetuses

Including three dams with no viable fetuses.

The number of resorptions, mostly early ones, increased from 3 in the control group to 20, 17, and 35 in the 0.3, 0.8, and 2 mmol/kg/d groups respectively. The number of live fetuses decreased from 127 in the control group to 109, 108, and 92 in the low-, mid-, high-dosed animals respectively. Mean % of post-implantation loss increased from 2.5 in the control group to 12.9, 14.2, and 31.9 with increasing doses. These changes were not considered statistically significant by the sponsor. There was no historical control for

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total resorption, therefore this reviewer was not able to determine the statistical significance of these changes.

Offspring (malformations, variations, etc.):

-The mean placenta weight of male fetuses was slightly increased, however this change remained within the normal range of background data combined for males and females, and male fetal body weight was not affected.

-Five malformed fetuses were revealed at the external examination: one dam treated with 0.3 mmol/kg/day had 2 fetuses showing omphalocele (4 and 6 mm) with prolapse of the liver, and in one of them with prolapse of intestine as well. Two dams treated with 2 mmol/kg/day had each a fetus with hydrocephalus, malrotated fore paws, and malrotated hind limbs. Another dam of the same group had a fetus with fontanella severely enlarged and protrusion of the scalp. The sponsor considered these malformations not to be related to the drug substance, claiming that these findings are known to occur spontaneously in this rabbit strain, and also that the fetal incidences of hydrocephalus, malrotated for paws and hind limbs were within the range of background data.

-No macroscopically visible variations were noted at any tested dose level.

-Eight runts were observed at laparotomy, 3 in the control group, 3 at 0.8 mmol/kg, and 2 at 2 mmol/kg/day.

-Fetal skeletal examinations according to Dawson revealed no malformed fetus at any dose level. The number of skeletal retardations -including sternebrae incompletely ossified or not ossified- was slightly decreased in the treated groups compared to the controls.

-Soft tissue examination of the head of the fetuses according to Wilson, revealed one cerebellum absent in one fetus of the control group, and a cleft palate in 2 fetuses each derived from 2 different dams of the high-dose group. In addition, the cerebellum was absent in one of these fetuses. A dilatation of the 4th ventricle was noted in 4, 1, and 2 fetuses of respectively the 0.3, 0.8, and 2.0 mmol/kg/day groups with both fetal and litter incidence significantly increased. Vacuoles, associated in one case with adhesions of the nasal region, were found in the cerebral hemisphere of 2 high-dosed fetuses. The malformations and variations were not considered by the sponsor to be treatment related the latter claiming that these findings were known to occur spontaneously in this rabbit strain.

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Summary table of malformations in the fetuses

	Control	0.3 mmol/kg (3.3X the human dose)	0.8 mmol/kg (8.8X the human Dose)	2.0 mmol/kg (22X the human dose)	Historical controls -Range for control groups mean % -(Mean %)
Number of fetuses	127	109	108	92	2015 fetuses 17 groups
Omphalocele with prolapse of liver and/or intestine		2* 1.83%			0.0-1.0 (0.19)
Hydrocephalus with malrotated fore paws and hind limbs				2** 2.17%	0.0-1.1 (0.06)
Fontanella severely enlarged (protrusion of the scalp)				1 1.09%	0.0-1.8 (0.1)
Cerebellum absent	1 0.79%			1 1.09%	0.0-2.4 (0.13)
Cleft palate				2** 2.17%	0.0-0.8 (0.04)
4 th cerebral ventricle dilated		4** 3.67%	1 0.93%	2** 2.17%	0.0-3.8 (0.21)
Nasal region adhesion				1 1.09%	
Cerebral hemispheres: 2 vacuoles				2** 2.17%	

*Fetuses from same dam

** Fetuses from different dams

Bold numbers indicate data outside the historical range.

(Table prepared by reviewer)

Reviewer's comments:

Increase in the kidney weight in the dams was the only maternal toxicity sign observed in the high-dose group. Eight runts were noted at laparotomy, 3 in the control group, 3 at 0.8 mmol/kg/day, and 2 at 2 mmol/kg/day. The mean implantation loss was increased in all treated groups. The loss appeared more severe, perhaps because of the very low value found in the control group. On the other hand, the low- and mid-dosed groups fall within the background range, and only in the 2 mmol/kg/day group does the change seem relevant as a treatment related effect. In this group, the gravid uterus weight, as well as the mean number of fetuses, was decreased. Various malformations were noted: Omphalocele in 2 low-dosed fetuses, hydrocephalus, malrotated fore paws, and malrotated hind limbs in 2 high-dosed fetuses, and a severely enlarged fontanella in one high-dosed fetus. Cleft plate was noted in 2 high-dosed fetuses from different dams, absent cerebellum was noted in one aforementioned high-dosed fetus and in one control fetus. The malformations were observed at 22X the clinical dose, and the only malformation noted in the low dose group, seemed to be marginal, since no dose effect

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could be established. In view that the teratogenic effect observed at 0.3 mmol/kg was not dose-dependent, the NOAEL for the malformations may be established at 0.8 mmol/kg. NOEL for embryotoxicity could not be established because of lack of historical reference for total resorptions.

It is not clear how these malformations are induced. One possibility would be that the drug is dissociated resulting in the free gadolinium ions release, which in turn compete with the endogenous zinc. Zinc is known to be indispensable for fetal development, in particular for the ossification process. Furthermore significantly higher levels of zinc (at least 2 fold the control levels) have been detected in human urine at about 24 hours following administration of the drug, which may be a sign that zinc ions have been released following Gd dissociation from Vasovist.

Pharmacokinetic data show a tri-exponential decrease of Gd-serum levels down to the last sampling time point with an initial distribution phase lasting less than 0.5 hours, a second distribution phase of less than 4 hours. The terminal distribution phase could not be established due to the lack of an appropriate number of data. The sponsor should have included additional time points between the 4 hrs and 24 hrs time points, since it has been shown in a previous study, that the elimination half time in the rabbits was 2 to 3 hours. The under-proportionally and non linear increase in AUC is likely due to the binding of the drug to albumin, increasing dosage resulting in albumin saturation and increase in the free fraction in the serum

Prenatal and postnatal development

Study title: Study for effects on the pre- and postnatal development (including maternal function) in rats with ZK 236018 (SH L03588A; Epix Medical, MS-325) after daily intravenous administration to the dams of the F₀-generation from day 6 of gestation to day 21 postpartum.

Key study findings:

- Stillbirths were observed in the F0 generation: 3 at 0.03, 3 at 0.21, and 2 at 1.5 mmol/kg/day. In the F1 generation, 3, 6, and 1 stillbirths were noted in the groups treated with 0.03, 0.21, and 1.5 mmol/kg/day (0.16, 1, and 8 times the human dose based on BSA) respectively. This effect does not represent a safety issue.
- A dose-dependent increase in kidney weights was observed, and tubular and urothelium vacuolation was noted in all groups at a dose-dependent degree.
- Maternal NOEL: Not established, kidney vacuolation occurred at all doses.
- F1 generation NOAEL: 1.5 mmol/kg/day (8 times the clinical dose).
- F2 generation NOAEL: 1.5 mmol/kg/day.

Study no.: 14707-01
Volume #, and page #: 5, 10226
Conducting laboratory and location: T

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Date of study initiation: 09/13/2001
GLP compliance: Yes and ICH guideline 4.1.2 compliance.
QA reports: yes (X) no ()
Drug, lot #, and % purity: Gadofosveset trisodium, 3095p19, 99.4%.

Methods

Doses: 0.03, 0.21, and 1.5 mmol/kg/d, respectively 0.16X, 1.1X, and 8X the clinical dose based on body surface area. A control group received 0.9% saline.

Species/strain: Female rat/Sprague-Dawley

Number/sex/group: 20/dose

Route, formulation, volume, and infusion rate: Intravenous, aqueous solution, 10mL/kg, bolus.

Satellite groups used for toxicokinetics: 10/dose

Study design: Female rats were treated once daily from implantation (6th day of gestation) until weaning (21st day of lactation). After spontaneous delivery, the F1 generation animals were examined and their development assessed using parameters such as body weight gain, morphological landmarks, and functional tests. The dams that did not deliver spontaneously were laparotomised on the 3rd day after the calculated day of delivery. One male and one female rat of each litter and group (F1-generation; with a body weight nearest to the mean litter weight) were raised to maturity and mated at the age of 13 weeks. Inbreeding was not carried out. The males were sacrificed after the mating period. After spontaneous delivery, the pups (F2-generation) were weaned and their development was observed using parameters such as body weight gain. The dams that did not deliver spontaneously were laparotomised on the 3rd day after the calculated day of delivery. The dams and pups were sacrificed after 3 lactation weeks, dissected and examined macroscopically.

Parameters and endpoints evaluated: uterus was examined macroscopically and the implantation sites were determined according to Salewski in F₀- and F₁-generations. The kidneys of all dams of F₀ generation were weighed and examined histologically.

Statistic evaluation: The Dunnett test and the student t-test were used for statistical analysis of intergroup variations of mean values.

Dose (mmol/kg)	0.03	0.21	1.5	NOAEL 1.5
Human multiple dose (based on BSA)	0.16	1.1	8	8

Results

F₀ in-life:

-At 1.5 mmol/kg/day, absolute and relative food consumption reduction of up to 10% compared to control, were noted between gestation day 9 and 15.

-Reproduction data, overall litter performance and survival indices were not influenced with any tested dose. Three stillbirths were noted in each 0.03 and at 2.1 mmol/kg/day

group, and 2 were noted in the highest dose group. No pup was malformed at any dose level.

F₀ necropsy:

-At 1.5 mmol/kg/day, necropsy revealed pale kidneys in 17 of 20 dams.
-Absolute and relative kidney weights were increased, compared to the controls, by 8-11% and 45-47% at 0.21 and 1.5 mmol/kg/day respectively. Tubular vacuolation was observed in all groups in a dose-dependent fashion. In addition, vacuolation of the urothelium of the renal pelvis was noted in 1 low-dosed dam, and in most of the dams in the mid- and high dose groups.

F₁ physical development:

-The mean pup body weights and the mean entire litter weights were marginally, but not significantly, decreased at 1.5 mmol/kg/day. The sponsor points out a lack of dose-dependency, and concludes that this effect is not drug-related.
-Morphological development was not affected by any of the dose level in the dams observed until weaning.
-All functional tests and the open-field test revealed similar results in the control group and in any of the treated groups observed until weaning.

F₁ behavioral evaluation:

No effect was noted on behavior, external appearance and feces.

F₁ reproduction:

-Reproduction indices including birth index, viability index, lactation index, and overall survival index, were not influenced by treatment at any dose level. Lactation index and overall was slightly increased in the 2 highest dose groups, and survival index was also slightly increased at 1.5 mmol/kg/day. These effects have probably no biological relevance.
-At 0.21 mmol/kg/day, the mean number of implant sites was significantly decreased. This effect was isolated.
-Three, 6, and 1 stillbirths were noted in the groups treated with 0.03, 0.21, and 1.5 mmol/kg/day respectively. This effect was not dose-dependent, however it was observed in the treated but not the control group.
-No pup was malformed.

F₁-dams and their male partners examination at termination:

-No significant effect was noted in the fertility and in the number of implantations. There were no macroscopically visible findings in any group, except for one of the F₁-dams treated with 0.21 mmol/kg/day, and which revealed pale kidneys at necropsy. This effect is likely not drug related.

F₂ findings:

-F₂ reproduction indices including birth index, live birth index, viability index, lactation index, and overall survival index were not affected by treatment.

- Sex distribution was slightly different in the high dose group, with an increase in the number of males and a decrease in females. According to the sponsor, this effect is due to the relatively high respectively low number of females and males in the control group. However, since this effect was observed only in this group, one cannot rule out a drug related effect. Having said that, this effect, even if drug related, was slight, and would not represent a safety issue.
- Litter weight was decreased in low and mid dose, as well as body weight in group 3.

Reviewer's comments:

The F1-generation mean pup body weights and the mean entire litter weights were marginally, but not significantly, decreased at 1.5 mmol/kg/day. Although the sponsor points out a lack of dose-dependency, this effect may be drug-related. Indeed, there was no effect at low doses, however, a dose-dependent effect may have been seen, had higher doses been tested.

In the F0 generation 3 stillbirths were noted in each of the 0.03 and the 2.1 mmol/kg/day group, and 2 were noted in the highest dose group. In the F1 generation, 3, 6, and 1 stillbirths were noted in the groups treated with 0.03, 0.21, and 1.5 mmol/kg/day respectively.

This effect seems to be drug related since it was observed at a relatively high incidence in 2 different generations, but in neither of the corresponding control groups. However, this effect does not represent a major safety issue. No malformation was observed in any of the pups.

3.4.7 Local tolerance

Report 206: Acute intravenous and perivascular irritation study of MS-325 in rabbits

Key study findings: Intravenous injection of MS-325 to rabbits produced no significant irritation. Perivascular injection of MS-325 in rabbits produced mild irritation of the skin and subcutaneous tissue which was slightly greater than in the control treated sites

Study no: 95-1377
Volume #, and page #: 5, 11010
Conducting laboratory and location: T

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Date of study initiation: 02/05/1996
GLP compliance: No
QA reports: yes (X) no ()
Drug, lot #, radiolabel, and % purity: MS-325-DPI (abbreviates as MS-325), Lot 512016.
Formulation/vehicle: Formulated with sodium, 730 mOsM/kg/0.9% saline

Methods:

The test article or the control was administered to 6 New Zealand White rabbits via vascular (marginal ear vein) injection at a dose volume of 1 mL in the right ear, and via perivascular (parallel to the marginal ear vein) of 0.5 mL in the left ear of each animal.

Dosing:

The study included 12 rabbits (6 males+6 females) divided into 2 dose level groups. The animals were at least 8 weeks of age at the start of the study, weighing between 1.9 to 2.4 kg. A single dose of 0.250 mmol/mL was administered to each animal, followed by 1 to 4 days of observations.

Observations and times:

Viability checks were conducted twice daily. The sites of injection were examined at 24 hrs (all animals), 48, 72, and 96 hrs (animals not sacrificed at 24 hrs). Histopathology for inflammatory response was performed at the injection site, at 0.5 cm and at 1.5 cm proximal to injection site. Right ear and left ears were preserved in 10% neutral buffered formalin and saved.

Results:

No animal died on study.

Intravenous injection: Erythema and/or edema were observed at 24 hours in 2/6 treated animals. Similar observations were made in the control group. Microscopic examination revealed slight reversible irritation at the injection site and the sites proximal to injection sites, in both the treated and the control rabbits, suggesting that the effect was not drug related. The changes limited to the treated group at 24 hours were slight fibrin deposit on the vein intima and subcutis, and slight epidermal hydropic degeneration and necrosis. The number of tissue changes as well as the severity of the lesions in treated animals declined at 96 hrs.

Perivascular injection: All treated animals exhibited erythema and/or edema 24 hours post-dose. This effect was persistent in 2 out of 3 animals through 96 hours. One animal in the control group exhibited erythema. Microscopic examination revealed generally moderate irritation on both control and treated animals, however the irritation was more severe and more persistent in the treated group, indicating an irritancy potential of perivascular injection. The changes considered to be drug related were fibrin deposit in the subcutis, more severe and persistent subcutaneous edema, epidermal hydropic degeneration and necrosis, and persistent subcutaneous heterophil/lymphoid cell infiltrates.

Reviewer's comments:

No significant drug-related irritation was detected with vascular administration of the drug article. This study detected mild irritation of the skin and subcutaneous tissue, indicating that local irritancy potential is likely to occur after accidental extravasation of Vasovist at 0.250 mmol/mL. However, the animals were not observed until resolution of the local reactions. However, the decrease in the severity of the signs indicates that the effect is likely reversible.

The test article is unformulated. The clinical (commercial) formulation contains an excess of ligand (fosveset), which was not tested for irritancy potential either separately or as part of the final formulation. However, in an acute toxicity study, the ligand did not induce any irritation-type effect in rats, unlike the drug substance, so the final formulation is likely to cause similar events than those described in this study.

3.4.8 Special toxicology studies

Report 204: In-vitro hemolytic potential and blood compatibility testing of MS-325 in human whole blood and plasma. Lot 512016

The objective of this study was to evaluate the hemolytic potential of MS-325 and its physical compatibility with human plasma.

Study design: Human blood samples were collected from volunteers who have not been exposed to any drugs or alcohol for at least two weeks prior to the test.

(i) Hemolytic assay: MS-325 (0.250 mmol/ml) was gently mixed, and incubated for 45 min at 37°C, with whole human blood at the following ratios: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 resulting respectively in final concentrations of 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2 mM (Human concentration: 0.43 mM). Deionized water (1:1) was used as positive control, and 0.9% saline solution (1:1) as negative control. The concentration of hemoglobin, indicative of a hemolytic process, in the supernatant was measured using a wavelength of 376 nm.

(ii) Compatibility test: MS-325 was mixed with plasma at ratios of 1:1, 1:2, 1:3, 1:4, and 1:8 (MS-325: plasma). The samples were examined for visible evidence of precipitation or turbidity, immediately or after 10 min incubation. Thrombin (1:1) was used as a positive control, and 0.9% saline (1:1) was used as a negative control.

Results: MS-325 did not produce a significant percentage of hemolysis of whole blood at any of the ratios tested. Percent hemolysis ranged from 0.2% (1:1) to 0.4% (1:8) compared to 0.2% in the control sample. No precipitation or coagulation was produced following treatment with MS-325 at any of the ratios tested. The sponsor concludes that MS-325 has no hemolytic potential in whole blood and that it is compatible with the human plasma.

Reviewer's comments: Agree with the conclusions of the study.

Report PTR 2003-01: Effect of MS-35 on mast cell degranulation.

The objective of this study was to investigate the effect of MS-325 on adverse allergic-like reaction.

Assay design: The mast cell degranulation, used as an in vitro indication of histamine release, was assessed by measuring β -hexoaminidase activity. MS-325 at doses of 0.25,

0.5, 2.5, 5, 25, and 50 mM and a positive control A23187, a calcium ionophore, at doses of 0.5, 1, 2.5, 5, and 10 μ M were incubated for an hour with a rat mast cell line and the percent degranulation was measured.

Results: MS-325 showed less than 6% degranulation at all concentrations. Moreover, at this slight increase, no dose-response could be established. In addition, incubation with the positive control A23187 resulted in a concentration-dependent release up to 5 μ M with a maximum of approximately 58% at 5 and 10 μ M.

Reviewer's comments: The doses of MS-325 tested were approximately 0.5, 1, 6, 12, 60, and 120X the plasma concentrations that would be achieved with the intended clinical dose of 0.03 mmol/kg. Therefore, at doses greater than 100 times the clinical dose, no appreciable effect was observed on mast cell degranulation, suggesting that MS-325 is not likely to cause a IgE mediated allergic reaction.

Report 205: Guinea pig maximization test with MS-325 (Method of Magnusson and Kligman). Conducted by [redacted] Lot 512016 MS-325-DP1 (0.251 mmol/mL, 730 mOsM/kg)

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The objective of this study was to evaluate the allergic contact sensitization potential of MS-325 in guinea pigs. Male and female Dunkin Hartley Guinea pigs were used: n=15/sex for sensitization study and n=10/sex for irritation controls.

Study design: The test consisted of an attempted induction of sensitization followed by a challenge to evaluate the sensitization. MS-325 was administered at 5% for the intradermal induction on Day 1, and at 100% for the topical induction on Day 8 (10/sex). On Day 22, the challenge treatment was administered topically at 100%. The susceptibility of the animals (n=5/sex) was demonstrated using dinitrochlorobenzene (DNCB) a positive control. On Days 24 and 25, the response was evaluated: redness at the challenge site, which was clearly greater than that seen in the irritation control animals, was considered an allergic response. In order to differentiate dermal reactions produced by irritation from dermal reactions specific to sensitization, 10 animals (5/sex) treated concurrently during induction with only vehicle and/or Freund's Complete Adjuvant (FCA)/water emulsion, were subjected to the same challenge procedures as the animals which received test material during the induction steps.

Results:

-All 10 animals treated with DNCB at 0.1% exhibited dermal responses at challenge with an incidence index of sensitization to DNCB of 100%, and severity indices of 1.6. On the other hand, irritation control animals were free of clear dermal responses, with severity indices of 0.2 and 0.1. This response shows the susceptibility of animals to sensitization.
-All 20 animals challenged with MS-325 as well as the 10 irritation control animals were free of dermal responses. Incidence index of sensitization to MS-325 was 0%. In addition, severity indices at both days were 0 for both MS-325 and irritation control animals.

The sponsor concludes that MS-325 did not exhibit a potential to produce dermal sensitization in guinea pigs.

Reviewer's comments: Agree with conclusions.

Report 5149-44205: Determination of potential immunomodulating effects of MS-325 by means of the plaque-forming cell assay and lung macrophage phagocytosis test after 18 days of intravenous injection of male Wistar rat. Lot 3095p19

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Study design: MS-325 at 0.03, 0.5 and 2 mmol/kg (0.16, 2.7, and 10X the human intended dose) or vehicle was administered daily to male rats in a constant volume of 8 ml/kg during 18 days. Fourteen days after the first treatment, animals received an IV injection in the tail vein with 5×10^8 sheep red blood cells (SRBC). Functionality of the T cell-dependent immune response was evaluated by means of the Plaque Forming Cell (PFC) assay (8 animals/group).

At necropsy, hematology was performed and spleen, lungs and kidneys were isolated and weighed. In addition, cells were collected by lung lavage (5 additional animals/group), and the phagocytizing capacity of the adherent cells was assessed ex vivo on day 18. In all 13 animals/group, clinical signs, weekly body weight and feed intake, and examination at necropsy for gross macroscopic changes were recorded.

Results:

Death: One high dose animal died in the necropsy room just before necropsy, due to unknown reasons.

PFC-assay: Measured parameters associated with the PFC-assay, i.e. cell viability, viable cells per spleen, PFC per million spleen cells and per whole spleen were not significantly affected by the test substance treatment, although a small increase in number of PFC per 1×10^6 cells was observed in mid and high dose groups. The total number of PFC per spleen also did not show significant differences. A small increase in number of PFC per million was observed in mid- and high-dose groups. Dosing with cyclophosphamide (CPS) at 50 mg/kg bw resulted in severe effects including an almost complete absence of plaque-forming cells (expressed both per 10^6 cells and per spleen), consistent with previous experience for this model, confirming the sensitivity of the model to immunomodulation.

Phagocytosis function: was tested ex-vivo in adherent cells from lung lavage fluid. Fluorescent microspheres were added to the cell cultures and the number of phagocytized intracellular microspheres was counted (<3, 3-20, >20). After modeling of the quantitative data, it was concluded that the chance on very positive cells (containing more than 20 microspheres) decreased with increasing dose: the number of negative cells was ~50, 60, and 70% for control, low and mid-dose, and high dose respectively. Ten percent of the cells were very positive, in control and the 2 low dose groups, only 4% was very

positive in the highest dose group. However, no definitive conclusion about a NOAEL could be drawn based on these quantitative data alone. A remarkable morphological feature in the cell cultures used for the phagocytosis assay was the severe vacuolation in all cells derived from high dose animals. Morphological changes in the appearance of the call were visible.

Clinical observations:

-On Day 3, all the high dose treated animals offered resistance and wheezed upon injection. One animal of that group showed transient uncoordinated behavior on Day 3, and paralyzed hind legs on Day 8, at which time point, another animal in the group had tensed hind leg muscles and was transiently lethargic

-Macroscopic findings at necropsy: MS-325 induced grossly observed discoloration at the site of injection in all high-dose animals. Body weights were up to 10% lower in high dose animals from day 4 onward. In these animals food consumption was lower throughout the study. These changes were considered to be treatment-related. At low and mid-dose levels, the body weights did not differ substantially from the control group.

-In the high dose group significant decreases were observed in red blood cell count, hemoglobin and packed cell volume, whereas a significant increase was observed in thrombocytes. CPS-treated animals displayed similar changes except for the thrombocytes, which were decreased. No effects on red blood cell count were observed in low and mid-dose animals. In white blood cell counts at necropsy, a decreased absolute number of lymphocytes and an increased number of neutrophils were observed in high dose animals. The percentage of neutrophils was increased in mid and high dose groups, whereas percentage of lymphocytes was decreased at all doses. These effects are considered to be treatment-related, and were not observed in low and mid-dose animals.

-In high dose animals a significant, dose-dependent increase in absolute and relative kidney weight was observed. Moreover, relative weights of lung and spleen were elevated in the high dose group as well. This change is considered to be treatment-related, and was not observed in low and mid-dose animals.

Microscopic examination:

-Presence of vacuolation of proximal tubular epithelial cells in the kidneys of all high- and mid-dose animals; the severity of vacuolation increased with the dose

-Accumulation of vacuolated macrophages in the alveolar lumina of the lungs of all high-dose animals, whereas it was not observed in low and mid dose groups.

-Vacuolated macrophages, predominantly in the red pulp, in the spleen of all high-dose animals, and not in low and mid dose groups.

The sponsor concluded that the T cell-dependent antibody response was not affected by the treatment with MS-325. In combination with the microscopic observation of severe

vacuolation in alveolar lumina of the lungs in high dose animals and not in low and mid dose groups, it can be concluded that macrophage function and morphology will be affected at the high dose level.

Overall, for the immunotoxicological evaluation of this study it can be concluded that:

1) There was no effect of MS-325 on the T cell-dependent immune response as tested in the Plaque-Forming Cell assay at all doses tested (NOAEL >2.0 mmol/kg).

2) Ex-vivo testing of the phagocytosis function of lung lavage cells showed that the chance on very positive cells (containing more than 20 microspheres) decreased with increasing dose. In combination with the microscopic observation of severe vacuolation both in lavage cells and in alveolar lumina of the lungs in high dose animals, a NOAEL can be set on 0.5 mmol/kg.

Reviewer's comments:

Since in a previous 2-week study in rats male animals showed a higher incidence and severity of vacuolated macrophages in lung, spleen and liver only male animals were used in this study. I agree with sponsor's conclusions.

3.5 OVERALL CONCLUSIONS AND RECOMMENDATIONS

See EXECUTIVE SUMMARY (Page 1)

Signatures (optional):

Reviewer Signature _____ Siham Biade _____

Supervisor Signature _____ Concurrence Yes ___ No ___

3.6 APPENDIX/ATTACHMENTS

None

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/s/

Siham Biade
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PHARMACOLOGIST

Adebayo Lanionu
12/10/04 08:52:01 AM
PHARMACOLOGIST

Supervisory Pharmacologist Memo

NDA: 21-711
Drug: Vasovist™
Sponsor: Epix Medical

Gadofosveset trisodium (Vasovist) is a trisodium salt of a gadolinium (III) complex of a substituted diethylenetriaminepentaacetate (DTPA) ligand proposed for Magnetic Resonance Angiography (MRA) imaging.

It is used in adults with suspected or known vascular disease. In Gadofosveset trisodium, the DTPA ligand is substituted by a phosphodiester moiety, which confers the albumin binding property of the drug, and supposedly prolongs plasma half life and increases the relaxation rate of water protons in plasma. The proposed dose is 0.03 mmol/kg in adults.

Dr. Siham Biade reviewed the preclinical Pharmacology and Toxicology section of NDA 21-711. She concluded that the studies conducted supported safety and efficacy from preclinical Pharmacology/Toxicology perspectives, and recommended approval of Vasovist. This secondary review was based on Dr. Biade's review; please see Dr. Biade's review for details.

Cardiovascular (including QT_c, hERG potassium channels, and cardiac action potential assessments), and CNS safety evaluations were considered adequate. For renal safety studies, Dr. Biade identified certain deficiencies including the equivocal nature of studies evaluating intravenously administered Vasovist in rodent models of renal impairment that may partly reflect inadequate methodological development. However, she noted that the sponsor conducted clinical studies in renal impaired patients that obviate the need for additional preclinical evaluation in this disease model. Dr. Biade did not identify safety issues that would require the conduct of additional preclinical studies. MRI efficacy as measured by the pharmacodynamic parameter 1/T1 (relaxation rate) was demonstrated.

Vasovist is not metabolized in rats and monkeys. Pharmacokinetics studies in rats, rabbits and monkeys showed that the plasma kinetics follow a bi-exponential model with a relatively short distribution half-life and an elimination half-life that is longer (reflecting protein binding) than that of other marketed gadolinium compounds. In monkeys and rats, the kidneys eliminate 85-98% of administered Vasovist by 24 hours. Fecal elimination accounted for between 8-17% by 72 hours. Vasovist is excreted in milk of the lactating rat.

Definitive toxicology (acute and repeat-dose) studies were conducted in monkeys and rats. These studies were adequate. Types of toxicities described in the acute toxicity study in monkeys included focal accumulation of vacuolated and foamy macrophages in lungs, cytoplasmic vacuolation of the reticuloendothelial cells at 11X, and 32X human dose (HD), NOEL was 2.2X HD (all dose multiples based on body surface area comparison). For the rat acute toxicity study, there were dose-dependent incidence and severity of tubular alterations in kidneys, NOAEL was 2.7X HD. In both monkeys and rats, the

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principal findings from the repeat-dose toxicity studies were vacuolation of the kidneys, lungs and reticuloendothelial systems that were reversible in some cases after 28 day recovery period. NOEL for the monkey study was 1X HD, whereas no NOEL was established in the rat study. Perivascular injection in rabbits produced irritation of the skin and subcutaneous tissue. Vasovist did not demonstrate a significant hemolytic potential. Immunological studies did not show a significant effect on mast cell degranulation, dermal sensitization, or T cell-dependent antibody response. A full battery of genetic toxicology studies was conducted. Vasovist was not mutagenic in these studies.

Reproductive toxicology studies were conducted in rats and rabbits. Vasovist reduced the number of spermatids per gram of testicular issues without affecting male or female fertility indices when administered to rats at 8X HD daily for 4 weeks. It was not teratogenic in rats at up to 11X HD, but increased the number of skeletal variations at this dose multiple. In rabbits, Vasovist at 3X HD increased the number of post-implantation loss and resorption, and decreased the number of live fetuses, but was not teratogenic in the same species at 9X HD (a dose multiple that was considerably higher than evaluated for previously approved gadolinium compounds. At a much higher dose multiple (22X HD) that was not evaluated for other approved gadolinium compounds, Vasovist produced teratogenic effects. Prenatal and postnatal development of F1 and F2 generation pups were not affected by Vasovist. Dr. Biade recommended labeling changes to reflect the results of these studies.

Dr. Biade concluded that the preclinical package of Vasovist was complete, and that the studies conducted support the safety and efficacy of Vasovist from preclinical pharmacology/toxicology perspectives. **She recommends approval of the NDA and suggested changes in the label that would more appropriately reflect findings from preclinical studies.**

I concur with Dr. Biade's recommendations.

Adebayo Laniyonu, Ph.D.

Supervisory Pharmacologist

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/s/

Adebayo Lanionu

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PHARMACOLOGIST

I concur with Dr. Biade's recommendations