

-At a range within 1X to 10X the maximum therapeutic concentration, phenprocoumon did not change the 1/T1 effect of MS-325 both at 0.1mM and 0.6mM; 0.4% to 2.0% decrease in 1/T1 was noted at 20 MHz and 0% to 2.6% at 64 MHz.

-At 100X the maximum therapeutic dose, the decrease in 1/T1 varies from 7.3% to 9.5% at 20 MHz and from 4.3% to 5.3% at 64 MHz.

Reviewer's comments:

Agree with sponsor's conclusions: at clinically relevant concentrations (1X to 10X the maximum therapeutic dose).phenprocoumon does not change significantly the 1/T1 effect of MS-325

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief summary

In most of the PK studies, the sponsor combined all MS-325 data obtained with _____ and sodium formulations, with the assumption that different formulations will behave identically, in all species, and for any parameters measured. However, this assumption is based on results of Study 105 where the equivalency was determined solely for the albumin binding affinity of MS-325 in rabbits, although it was shown that binding was different across species.

b(4)

1. Excretion in breast milk

MS-325 was shown to be excreted in the breast milk. The amount of radiolabeled MS-325 excreted (as measured by gamma counting) showed wide variations between animals. Since Vasovist has been shown to be eliminated from the human body at a much slower rate than in rat, []

b(4)

2. Biodistribution

Highest uptake was found in kidney, liver, spleen, and heart in monkey. By 72 hrs, 99% of the activity was eliminated and the remaining activity could be detected in kidney, bladder and bowel loops.

In another study, 14 days following IV administration of ¹⁵³Gd-MS-325 to monkeys, the radioactivity content of heart, spleen, kidney, liver and bone (femur) were evaluated. Taken together, 0.18-0.22% of injected activity was recovered in these organs, of which 0.07-0.11 was recovered from the bone.

In rats, 24 hrs post-injection, the highest radioactivity was found in large intestine, muscle, kidney, and fat. Combined total activity in these organs was less than 2%. By Day 7 post-injection, 0.4% of the radioactivity was recovered.

3. Metabolism

HPLC analysis of urine, bile and blood from rats and monkeys administered IV injection of radiolabeled MS-325 showed one peak with gamma activity, at the same retention time as a standard sample of MS-325. No metabolites were detected. Similarly, TLC plates of male rat bile and urine show gamma activity at the same R_f as a standard sample of MS-325, along with some gamma activity detected at the origin. Furthermore, MS-325 is not metabolized by human liver microsomes at concentrations of up to 3.6 mM (~8X the concentration reached in circulation for the intended clinical dose) after 60 min.

4. Pharmacokinetics

(Summary table prepared by sponsor)

Species	Study Number	Dose ($\mu\text{mol/kg}$)	$T_{1/2\alpha}$ (hr)	$T_{1/2\beta}$ (hr)	$AUC_{0-\infty}$ ($\text{hr} \cdot \mu\text{mol/L}$)	V_{dss} (L/kg)	Cl (L/hr/kg)
Rat	301	25	0.014±0.002	0.38±0.05	58±4	0.254±0.040	0.432±0.031
Rat	301	100	0.015±0.003	0.39±0.05	259±50	0.236±0.034	0.397±0.069
Rabbit	302	100	0.05±0.01	1.97±0.38	2198±665	0.137±0.016	0.049±0.013
Monkey Covance	6754-127	30	0.07±0.02	2.86±0.11	984±125	0.120±0.012	0.032±0.004
Monkey CRL	PTR2003-40	30	0.18±0.06	4.07±0.10	1190±40	0.144±0.006	0.025±0.001
Monkey CRL	PTR2003-40	100	0.21±0.04	3.88±0.06	3194±70	0.167±0.004	0.031±0.001

In rats, rabbits and monkeys, the plasma kinetics of MS-325 follow a bi-exponential model with a relatively short distribution half-life, and an elimination half-life that is longer than that of marketed gadolinium compounds. Distribution half-life was approximately 5' for most species, and elimination half-life ranged from approximately 30 min in rat to 3-4 hrs in monkey. This is considerably shorter than human elimination half-life, which was shown to be approximately 16 hrs. Because MS-325 binds to albumin, its renal clearance was slower and the distribution volume relatively smaller.

3.3.2 Distribution

Report 300: Whole body retention and elimination of unformulated MS-325 in rats.

Unanesthetized rats were injected via tail vein with either MS-325 at 0.10 mmol/kg or Gd-DTPA. Animals were sacrificed at either day1 or day7 post-injection by CO₂ inhalation followed by cervical dislocation.

Compounds tested

¹⁵³Gd-labeled MS-325 (100mM) formulated with sodium. Lot TJM24-196

¹⁵³Gd-labeled MS-325 (100mM) formulated with — Lot TJM24-132

b(4)

Results

-Mean recovery for radiolabeled compounds ranged between 90% and 102%.

-¹⁵³Gd-labeled MS-325 was 98.1% eliminated from the body within 24 hours, with 80.7% renally excreted and 17.4% enterohepatically excreted. At 7 days post-injection, greater than 99% of MS-325 was eliminated from the body, with 76% renally excreted and 23.6% excreted in the feces.

-Mean corrected % I.D./organ was 0.666 for large intestine, 0.351 for muscle, 0.382 for kidney, and 0.299 for fat. The lowest %I.D.s were found in the brain and the lymph with 0.0006%, and 0.0018% at 24 hr post-injection. By 24 hours, most of the intestines had eliminated the drug, which resulted in the high fecal excretion by day 7 post-injection.

Reviewer's comments

MS-325 is known to affect renal function due to its main excretion by the kidney. The drug is claimed to not cross the BBB, which is concordant with the findings here. On the other hand, the relevance of the high concentration of the drug found in the muscle is not clear. One possibility is that the drug lipophilicity facilitates its penetration in fatty tissues and muscle. The impact may not be significant since only 0.4% of the drug remains in the body at Day7 post administration.

Study 303: Unformulated MS-325 biodistribution and excretion in monkeys.

The objective of this study was to determine the distribution and elimination kinetics of MS-325 in Male cynomolgus monkeys (n=4-5/dose).

Compounds tested

¹⁵³Gd-labeled MS-325 at 0.10 mmol/kg (1.1X the intended clinical dose), formulated with [] 1 min IV injection including 0.5 mL saline flush. Lot TJM24-186A.

¹⁵³Gd-labeled MS-325 at 0.10 mmol/kg (1.1X the intended clinical dose), formulated with [] 0.5 minute IV injection including 2 mL saline flush. Lot TJM83-60.

¹⁵³Gd-labeled MS-325 at 0.025 mmol/kg (0.27X the human clinical dose), formulated with sodium; 0.5 minute IV injection including 2 mL saline flush. Lot TJM24-201.

b(4)

Biodistribution of ¹⁵³Gd-labeled MS-325 (approximately 100 μ Ci/mL) was determined in monkeys for at least two hours post-injection. Excretion of ¹⁵³Gd-labeled MS-325 in urine and feces was determined for 0-24, 24-48, and 48-72 hours post-injection.

Results

Biodistribution: Studies were conducted in 2 laboratories. Three monkeys received ¹⁵³Gd-labeled MS-325 IV injection at 0.10mmol/kg [] Five monkeys received 0.025 mmol/kg and 1 monkey 0.10 mmol/kg [] Percent of ¹⁵³Gd-labeled MS-325 in tissue was estimated from the region of interest analysis of the images. Five minutes post-injection, the kidneys, ureters, and bladder were visible indicating renal excretion. The heart was also visible and percent of MS-325 in heart were very similar to those obtained for the kidney. At time points between 0-2 hours, the liver is visible in most cases, whereas the GI system is not in any case.

Elimination: At both doses tested, MS-325 was mainly excreted via urine. In the 0-24 hour time period, most of the renal elimination had occurred, whereas the peak for feces

b(4)

elimination occurred between 24 and 48 hours. By 72 hours, ~92% and 95% I.D. was eliminated by urine and 8% and 3% by feces for 0.025 mmol/kg and 0.10 mmol/kg respectively.

Table 1. MS-325 elimination kinetics in Cynomolgus monkey (by sponsor).

Time period	0.025 mmol/kg (n=2)		0.10 mmol/kg (n=3)	
	Urine (%I.D.)	Feces (%I.D.)	Urine (%I.D.)	Feces (%I.D.)
0-24 hours	85.1±6.43	0.200±0.1697	91.9±1.09	0.157±0.0603
24-48 hours	5.59±4.497	4.44±1.754	2.79±1.049	1.71±0.516
48-72 hours	0.935±0.4313	2.95±1.018	0.487±0.0651	0.770±0.3045
0-72 hours	91.6±1.50	7.59±2.602	95.2±0.78	2.64±0.641

Safety parameters: ECG, blood pressure, creatinine clearance, clinical chemistry, hematology, and blood gases that were monitored in the [] did not show any significant changes for monkeys under anesthesia, as stated by the laboratory veterinarian in an appendix. One monkey of the low dose group had only 3% I.D. in the bladder -compared to 30% in other animals- during the first 2 hours post-injection. This was attributed by the sponsor to the animal decreased blood pressure (MAP~53 mm/Hg) believed to be induced by deep anesthesia. After the animal was taken off anesthesia, the GFR increased and within 24 hours post-injection, about 81% of MS-325 was eliminated.

b(4)

Reviewer's comments:

The biodistribution data based on ROI were not corrected for attenuation. Thus, because ¹⁵³Gadolinium is a weak gamma emitter, soft tissue may have affected scintigraphic imaging in this study, which may explain the large fluctuations, observed in the individual monkey organ ROIs. In spite of the limitations in signal quantification, the imaging provides qualitative evidence on the mechanism of MS-325 distribution. PK data were collected and reported in Study # 309.

Study RTAZ-0114: Limited tissue distribution and mass balance of ¹⁵³Gd-MS-325 following a single intravenous administration to non-naïve cynomolgus monkeys. Study conducted by [] GLP, test article Lot JG392-19 and JG392-18.

b(4)

The objective of this study was to determine the mass balance and the tissue distribution of ¹⁵³Gd-MS-325 following a single intravenous administration to non-naïve cynomolgus monkeys.

Study design: The study consisted of 2 groups of 2 male and 2 female cynomolgus monkeys per group. $^{153}\text{Gd-MS-325}$ was administered as a single intravenous injection at doses of 0.03 and 0.1 mmol/kg (0.3X and 1.1X the human dose). Urine and feces were collected pre-dose, 0-6 and 6-24 hours post-dose, and then over 24-hour intervals through 336 hours (Day 15) post-dose. Blood samples were collected at specified time points up to 24 hours post-dose for plasma samples analysis by gamma counting. Hematology and serum chemistry analyses were performed on blood samples collected pre-dose and at 1, 24, 48, 96, and 336 hours post dose. Urinalysis (glucose, pH, protein) was performed on urine samples collected for all intervals. The animals were euthanized on Day 15, and bone (femur), bone marrow (femur), kidney (cortex and medulla), heart, liver, lungs, and spleen were collected from each animal. Samples were analyzed by gamma counting for radioactivity content.

Results

MS-325-Equivalents of Radioactivity in Blood, Plasma, and Plasma Filtrate

Mean concentrations in blood, plasma, and plasma filtrate for 0.1 mmol/kg group (Group 2) were greater than for 0.03 mmol/kg group (Group 1) with lower levels in blood compared to plasma by a factor ranging from 0.5 to 0.8, indicating partitioning of MS-325 to the plasma. Plasma filtrate concentrations of MS-325 were 2 to 3 fold lower than the plasma concentrations during the first hour post-injection, and 7 fold and up after 4 hours. There was a slightly greater fraction of plasma radioactivity associated with the filtrate for Group 2 than for Group 1 at the first 2 or 3 sampling times.

MS-325-Equivalents of Radioactivity in Tissues

Mean concentrations of MS-325 in selected tissues at 336 hours post-dose were similar for males and females of Group 1, and were slightly greater for males of Group 2. In the Group 1, MS-325 concentration in kidney (cortex) for females was more than twice that for males, whereas mean concentration in the lungs was 2 fold higher in males than females. In the Group 2, mean concentrations for tissues were greater than for Group 1 by factors reflecting the dose difference. The radioactivity present in the selected tissues represented a very small fraction of the administered dose. The highest mean concentrations for males and females of both groups were in kidney (medulla and cortex), and liver, with the exception of kidney (cortex) for males of Group 1. Spleen contained the next highest concentrations and the lowest concentrations were in heart for both groups. Bone (femur) contained the largest fraction of total dosed radioactivity, 0.07 to 0.11%, for males and females of both groups. The mean total recoveries of radioactivity in the selected tissues ranged from 0.18 to 0.22%, indicating that very low quantities of gadolinium remained in these organs at 14 days post-injection. Similar results were obtained in a previous distribution Study # 303

Elimination of $^{153}\text{Gd-MS-325}$ -Related Radioactivity

Excretion of MS-325 occurred primarily via urine with mean recoveries of radioactivity in urine ranged from approximately 77% to 86% and recoveries in feces ranged from approximately 7% to 10%. Excretion via urine was rapid and appeared to be complete by 48 hours post-dose while the overall elimination was completed by 96 hours post-dose.

Mean total recoveries of in all matrices through 336 hours post-dose for males and females were 92.00% and 87.84%, respectively in Group 1, and 87.87% and 93.49% in Group 2. The tissue analyses and mass balance data indicate that radioactivity was almost completely eliminated from the body by 336 hours post-dose, with little apparent retention in tissues.

Serum chemistry/hematology/urinalysis

-In one female of Group 1, the triglycerides (TRG) were elevated at 48 and 96 hours, by approximately 8-10 fold, but were back to normal at 336 hours. However, there were no intermediate time points. In Group 2, ALT and AST were elevated in males, at 24, 48, and 96 hours, but returned to normal values as well at Day 15. There was an apparent elevation of TRG for both Group 2 males through the time-course. The sponsor attributed these fluctuations to food consumption.

-Glucose was detected in urine of one male of Group 1, with values ranging from trace to 250 mg/dL. Urine pH in predose was 8.0 to 8.5 except for the first sampling interval where a slight decline in pH, 6.0 to 8.5, was noted. All pH values return to pre-dose values within 24-48 hours.

Reviewer's comments: This study demonstrated that the free fraction of MS-325 contained in the filtrate is lower than in the plasma. The amount of the free fraction is dose-dependent, and with the drug being continuously eliminated, it was expected that this fraction would decrease accordingly. MS-325 binds to albumin and at equilibrium, where the binding sites are saturated, the free fraction is expected to increase, and to a greater extent in the high-dose group. The distribution in the tissues shows that the drug has an affinity for the kidney but also for the liver, which was determined in the elimination study showing that the drug is eliminated both through the urine, and through the feces. Most of the data are consistent with the findings of Study # 303. The selection of the organs is limited, and was apparently based on anticipated results. The examination of other organs, including the brain, would have been of value, to determine if the drug crosses the blood brain barrier.

3.3.3 Metabolism

Report 320: Metabolism of unformulated MS-325 in rat bile, urine, and blood following intravenous administration.

The objective of this study was to determine if MS-325 is metabolized in Sprague-Dawley rat bile, urine or blood following IV administration of 0.025 mmol/kg MS-325 (0.14X the human dose) using high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Lots TJM24-196 and TJM83-72, formulated with sodium, were administered to male and female rats, respectively, for the study of blood and urine, and Lot TJM24-189, formulated with [] was administered to males for the study of the bile.

b(4)

MS-325 was injected at 21 μ Ci/mL for males, and 47 μ Ci/mL for females, via tail vein.

Bile was collected on anesthetized rats, for 15 min pre-injection, and in 1.5-2 hours in intervals of 30 minutes. Urine was collected and centrifuged at 5000 rpm for 5 minutes to remove any particulates. Bile and urine were analyzed using HPLC and TLC. Arterial blood was collected via catheter on anesthetized rats, at 1min and 20 min post-injection. Plasma separated by centrifugation, was protein denatured, and after a 2nd centrifugation, the supernatant was subjected to HPLC.

Results

-The HPLC analysis of injected urine, bile and blood from male rats, as well as female rats chromatograms obtained at determined time points post-dose, showed one peak with gamma activity, at the same retention time as a standard sample of MS-325. No metabolites were detected.

-The TLC plates of male rat bile and urine show gamma activity at the same R_f as a standard sample of MS-325. In addition, some gamma activity was detected at the origin. This effect was observed also when blank bile and urine were spiked with labeled MS-325. The sponsor concludes that matrix effects from the bile and the urine salts were responsible for these findings.

Reviewer's comments: The sponsor injected female rats twice as much radioactivity as males received, with no apparent rationale. Because the bile is expected to be more concentrated in any potential metabolite, than the feces, the sponsor studied the hepatobiliary excretion by collecting the bile. No metabolites were detected using HPLC or TLC. The presence at the origin of the TLC plates of some activity should have been further investigated by identifying the source of gamma activity, in view that this event may signal the presence of free gadolinium in the sample.

Report 321: Metabolism of unformulated MS-325 in monkey blood, urine, and feces following intravenous administration.

The objective of this study was to determine if any metabolic products exist in male Cynomolgus monkeys urine and feces following IV administration of 0.10 mmol/kg MS-325 (1.1X the human dose) using high performance liquid chromatography (HPLC). Lots TJM24-186 and TJM24-187, formulated with [] were used. b(4)

This study did not test MS-325 formulated with sodium, which is the clinical and commercial formulation. However, many of the PK/metabolism studies used both sodium and [] formulations, and no finding was shown to be formulation dependent. Therefore, a summary of the results of this study are provided in this section: b(4)

Results: Over the 3 days of the study, only one peak was detected in urine and feces samples, with a signal-to-noise ratio of 80:1 for the first 24 hours. The detection limits are 2% of metabolite. In conclusion no metabolites were detected in urine and feces in monkeys.

Reviewer's comments: The detection limit was 2% in urine, for the first 24 hours, then it fell to 50% in urine and feces in the 24-48 hours. This is consistent with the fact that most of MS-325 is eliminated during the first day.

Report No PTR2003-02: Lack of metabolism of MS-325 by human liver microsomes. Lot 3095p19 (250 mM)

Increasing concentrations of drug were added to microsomes and incubated for 0 to 60 minutes. Phenacetin (100 μ M) and ephedrine (100 μ M) were used respectively as positive and negative controls. The assay buffer was prepared by adding glucose-6-phosphate dehydrogenase to reconstituted NADPH regenerating system (NRS). NRS-containing tubes on ice were added with MS-325 to achieve concentrations of 0.01, 0.1, 1, and 3.6 mM, and reactions were started by adding 20 mg/mL human liver microsomes. The reaction was stopped by addition of ice cold methanol followed by 10 min centrifugation at 10,000 rpm (to pellet precipitate protein). The supernatant was subjected to LC-MS determination of the parent and possible metabolites. The detection limits were 0.05, 0.1, and 0.8 μ M for MS-325, phenacetin, and ephedrine respectively.

Two runs were performed, each with a different lot of microsomes. No metabolism was seen at any time in this assay. At all concentrations of MS-325 essentially 100% of the starting material was found after 60 minutes of incubation. Similarly, no metabolism was detected for ephedrine at any time and phenacetin was metabolized with 74 and 32% remaining compound after the first and the second experiment respectively.

Conclusion: MS-325 is not metabolized by human liver microsomes at concentrations of up to 3.6 mM (~8X the concentration reached in circulation for the intended clinical dose) after 60 min.

Reviewer's comments: Agree with sponsor.

3.3.4 Excretion

Report RTAZ-113: Assessment of breast milk gadolinium content of intravenously administered ^{153}Gd -MS-325 in lactating rats.

Conducted by [] GLP, ^{153}Gd -MS-325 Lot JG392-6, and Lot JG392-7.

b(4)

The objective of this study was to measure levels of ^{153}Gd -MS-325 in rat milk, and corresponding blood levels, after intravenous administration.

Study design: Two groups of lactating Sprague-Dawley rats were used. Group 1 was composed of 5 animals and Group 2 was composed of 4 animals. ^{153}Gd -MS-325 was administered as a single intravenous bolus injection to rat dams on day 12 to day 14 postpartum, after removal of the pups, at 0.03 mmol/kg/~7 μ Ci/kg (0.16X the human dose) in Group 1 and 0.3 mmol/kg/~6.5 μ Ci/kg (1.6X the human dose) in Group 2. Prior

to each scheduled milking time, oxytocin in saline (1 IU/mL) was administered at a dose of 1 IU/mL/kg to the dams by intraperitoneal injection. The scheduled milk and blood sample (250 μ L each) collection times were prior to dose administration (3 animals/group), at 1, 2, and 4 hours post-dose (3 animals/group), and at 24, 48, 72, and 96 hours post-dose (all animals). Triplicates weighed aliquots of milk and blood were analyzed by gamma counting for radioactivity content. The animals were also observed for clinically relevant abnormalities during dosing and sample collection. Whole blood and milk samples were analyzed for ^{153}Gd -activity in a gamma scintillation counter for 2 minutes.

Results

The animals were said to appear healthy during the course of the study. However, no observations were recorded.

In Group 1: Three rats sampled at 1 hour post-injection had detectable levels of radioactivity in the blood (5979 ± 1109 cpm/g). At 2 hours post-injection, only 2 of 3 rats sampled had levels in the blood that were above the 2 times background detection limit (mean 1698 ± 2144 cpm/g). Radioactivity (MS-325) was not detected in the blood for the remainder of the 96 hours study. Radioactivity was detected in neither breast milk sample obtained from 2 rats at 1 hour post-injection. At 2 hrs post-injection in the single sample of breast milk obtained, a detectable level of radioactivity of 11494 cpm/g was observed, which corresponds to approximately 0.2% of the injected dose per gram. Radioactivity was not detected in the breast milk for the remainder of breast milk sampling (48 hours).

In Group 2: Three rats sampled had detectable levels in the blood at 1 hour post-injection (7324 ± 2310 cpm/g). At 2 hours post-injection, all 3 rats sampled had levels in the blood that were below the 2 times background detection limit. Radioactivity (MS-325) was not detected in the blood for the remainder of the study (96 hours). Breast milk samples were obtained from 3 rats at 1 hour post-injection; radioactivity was detected in only 1 sample (3382 cpm/g). At 2 hours post-injection, detectable levels of radioactivity were observed in 2 of 3 rats (3149 ± 3923 cpm/g), which corresponds to approximately $0.07 \pm 0.08\%$ of I.D./gram. All breast milk samples obtained 4-24 hours post-injection did not contain radioactivity that was above the 2 times background cutoff. After 24 hours post-injection, no milk samples were obtained in sufficient volume to permit analysis.

Conclusion: The sponsor concluded that $^{153}\text{Gd-MS-325}$ is transiently found at low levels in the breast milk of rats from 1 to 2 hours post-injection, and that by 4 hours post-injection and for the remainder of breast milk sampling, $^{153}\text{Gd-MS-325}$ was not detected in the breast milk.

Reviewer's comments: Agree with the sponsor's conclusion. A wide interanimal variability was observed, the excreted amount was up to 0.2% of the intravenously administered dose of gadolinium with no apparent correlation between the amount of gadolinium and the injected dose. For instance, there was no excretion at some time

points for some animals. Because the conducting laboratory did not have a standard for the Gadolinium, it was not possible to establish the concentrations of gadolinium found in breast milk. Drugs that are protein bound are generally less likely to be transferred into breast milk. However, MS-325 is excreted in the breast milk at rates, and this finding is common with gadolinium compounds. The results of this study suggest also the possibility of a dissociation or extraction of gadolinium from the drug via physiological mechanisms. Although the amount excreted in the milk is small, the findings of this study

b(4)

3.3.5 Pharmacokinetics

Report 301: Plasma kinetics of unformulated MS-325 in rats

The drug was administered to anesthetized male Sprague-Dawley rats as an IV bolus, n=5/dose for MS-325, and n=2-4/dose for GdDTPA.

Compounds tested

¹⁵³Gd-labeled MS-325 formulated with sodium at 0.025, 0.05, and 0.10 mmol/kg, (respectively 0.14X, 0.27X, and 0.54X the human dose). Lot TJM24-196

¹⁵³Gd-labeled MS-325 formulated with [] at 0.025, 0.05, and 0.10 mmol/kg (respectively 0.14X, 0.27X, and 0.54X the human dose). Lot TJM24-189.

¹⁵³Gd-labeled GdDTPA formulated with [] at 0.05, and 0.10 mmol/kg (respectively 0.27X, and 0.54X the human dose). Lot TJM-B3-62, TJM-B3-5.

b(4)

Study design

Plasma kinetics were determined at different doses in Sprague-Dawley rats.

Different formulations of MS-325 were shown to have equivalent potency in Report 305 (in rabbits), therefore the sponsor decided to combine all MS-325 data. GdDTPA, which does not bind to plasma proteins was used as a standard. Plasma kinetics were determined by gamma counting for ¹⁵³Gd content, on whole blood samples collected for 5 and 10 minute time points, up to 35 minutes for MS-325 and 30 minutes for GdDTPA.

Results

Plasma kinetics were described by the sponsor as a standard bi-exponential model.

Results are summarized in the following table

	MS-325 (all doses)	Gd-DTPA 0.05 mmol/kg	Gd-DTPA 0.10 mmol/kg
Distribution T _{1/2} (min)	~6.8	~0.5	~0.5
Elimination T _{1/2} (min)	~22	~16.5	~12.7
Distribution volume (L/kg)	~0.23-0.25	~0.23-0.25	
Plasma clearance (mL/min/kg)	~7	~8	~12
Dose-corrected AUC			

(mM min)/(mmol/kg)			
0-10 min	~42-46	~35-37	~35-37
0-30 min	~90	~70	~70

(Table prepared by reviewer)

Reviewer's comments

Elimination and distribution half-lives were higher in MS-325 probably due to the binding of MS-325 to albumin. MS-325 and GdDTPA had similar distribution volumes, although the binding of MS-325 to albumin would have predicted a smaller distribution volume. This is probably due to the low lipophilicity of both drugs, which hinders the diffusion of the drugs across the membranes. In addition, the relatively high (~25% of ID in the feces at 0.10 mmol/kg) biliary excretion rate likely increases the plasma clearance, resulting in a larger than expected volume of distribution.

Report 302: Plasma kinetics of unformulated MS-325 in rabbits.

The drug was administered to mildly sedated female New Zealand White rabbits as an IV bolus, n=2-6/dose.

Compounds tested

¹⁵³Gd-labeled MS-325 formulated with sodium, administered as IV bolus at 0.025 and 0.10 mmol/kg (respectively 0.27X, and 1.1X the human dose). Lot TJM83-61

¹⁵³Gd-labeled MS-325 formulated with [] administered as IV bolus at 0.025 and 0.10 mmol/kg (respectively 0.27X, and 1.1X the human dose). Lot TJM24-189.

¹⁵³Gd-labeled GdDTPA formulated with [] administered as IV bolus at 0.10 mmol/kg (1.1X the human dose). Lot TJM24-132.

b(4)

Study design

Plasma kinetics were determined by gamma counting for ¹⁵³Gd content, on whole blood samples collected from the central ear arterial cannula, while injection was performed via marginal ear vein. Some of the rabbits were used in multiple pharmacokinetic studies. These rabbits were never injected more than once with the same compound at the same dose, and were allowed to fully recover for at least a week prior to initiation into a new study. Blood samples were retained for T1 measurements and plasma binding experiments reported in Report 136. The different formulations of MS-325 were shown to have equivalent potency in Report 305 (in rabbits), therefore the sponsor decided to combine all MS-325 data. However, as noted previously, equivalent potency does not necessarily mean equivalent kinetics.

Results

The plasma kinetics followed a bi-exponential model with a distribution half-life of approximately 3 min, and an elimination half-life of 118 to 174 minutes for 0.10 and 0.025 mmol/kg MS-325 respectively. A similar dose-effect was observed for dose-corrected AUC with 221 and 282 (mM min)/(mmol/kg) at 0.10mmol/kg and 0.025 mmol/kg respectively. This effect was attributed to the fact that at higher doses of MS-325, the unbound fraction is increased, thus resulting in a shorter elimination half-life,

and a more rapid plasma clearance. Plasma clearance was ~0.5 and 0.8 mL/min/kg at 0.025 and 0.10 mmol/kg MS-325, respectively, compared to 5.4 for 0.10 mmol/kg GdDTPA. The distribution volume was 0.114 and 0.137 L/kg for 0.025 and 0.10 mmol/kg MS-325, and 0.299 L/kg for GdDTPA.

Reviewer's comments

Rabbit plasma kinetics and rat plasma kinetics exhibit significant differences, the most striking one being the distribution volume. Furthermore, distribution and elimination half lives, and AUC were dose-dependent in rabbit but not in rat, perhaps indicating that the binding sites were not saturated at the highest dose employed in the present study.

Negatively charged hydrophobic compounds do not easily cross the membrane, resulting in a smaller distribution volume. The binding of MS-325 to albumin further decreases its distribution volume making it even smaller than that of GdDTPA, which does not bind to plasma proteins. According to the sponsor, the rabbits' volume distribution is not affected by biliary excretion, unlike for the rats where the distribution volume was affected by the relatively significant biliary excretion. However, there is no report in this NDA that provides evidence for such claim.

Report 309: Plasma kinetics of unformulated MS-325 in monkeys

The drug was administered to anesthetized male Cynomolgus monkeys as an IV bolus, n=2-5/dose. The monkeys in which the blood samples were obtained after MS-325 injection were the ones reported in Report 303.

Compounds tested

¹⁵³Gd-labeled MS-325 formulated with sodium, administered as an IV bolus at 0.025 mmol/kg (0.27X the human dose). Lot TJM24-201.

¹⁵³Gd-labeled MS-325 formulated with [] administered as an IV bolus at 0.10 mmol/kg (1.1X the human dose). Lot TJM24-186A, TJM83-60.

¹⁵³Gd-labeled GdDTPA formulated with [] administered as an IV bolus at 0.10 mmol/kg (1.1X the human dose). Lot TJM24-192.

b(4)

Study design

Similarly to the previous studies, the sponsor decided to combine MS-325 data from different formulations. Plasma kinetics were determined by gamma counting for ¹⁵³Gd content, on whole blood samples collected via femoral venipuncture, or femoral arterial cannula, while injection was performed via femoral vein.

Some monkeys were used in multiple pharmacokinetic studies but allowed to recover. GdDTPA is an extracellular contrast agent used as a reference standard; it does not bind to proteins and it is almost entirely eliminated by the kidneys. Creatinine clearance was not determined for each monkey, and a literature clearance (Øksendal) of ~3.0mL/min/kg was used.

Results

The plasma kinetics of MS-325 followed a bi-exponential model with a distribution half-life of 4.7 and 2.7 min, and an elimination half-life of 212 min and 143 min at 0.025 and

0.10 mmol/kg respectively, compared to 2.36 and 56 min in GdDTPA. The volume of distribution ranged from 0.13 to 0.14 L/kg, and was similar at both doses of MS-325, whereas it was 0.214 for GdDTPA. A similar dose-corrected AUC_{0-30} was obtained at both doses of MS-325, with 291 and 224 (mM min/(mmol/kg)) at 0.025 and 0.10 mmol/kg respectively. GdDTPA had an AUC_{0-30} of 134 (mM min/(mmol/kg)). Plasma clearance was 0.555, 0.765, and 2.57 mL/min/kg for low-dose, high-dose MS-325 and GdDTPA respectively, whereas renal clearance was determined to be 0.508 and 0.728 at the 2 doses of MS-325.

Reviewer's comments

There was no dose-dependent effect for MS-325, and the sponsor attributed this no effect observation to the percentage of MS-325 bound in plasma: at the higher dose this percentage is somewhat lower than that observed for the low dose. The lower percent bound (higher $f_{unbound}$) at the higher dose is consistent with the larger V_d , shorter elimination half-life, and more rapid renal elimination. Both MS-325 and Gd-DTPA had similar distribution kinetics. MS-325 had a longer elimination half-life, a higher dose-corrected AUC, and a smaller volume distribution than GdDTPA. In addition renal clearance was more rapid for GdDTPA. These differences observed with MS-325 compared to GdDTPA are characteristic of drugs that bind to plasma albumin, and that are subject to liver extraction.

Report 400: MS-325 plasma kinetics and relaxation rates in baboons.

The objective of this study was to determine the in vivo plasma kinetics and relaxation rates of MS-325 in anesthetized male Baboons, administered 0.10 or 0.20 mmol/kg as an IV bolus (n=3/dose). Lot KM89-179, YZ104-148.

Study design

Anesthetized animals were injected with 0.10 mmol/kg MS-325. Blood samples were collected via percutaneous femoral venous catheters, at determined time points from 1 to 120 minutes post-injection. After a two week "washout" period, the same baboons were administered the second dose of 0.20 mmol/kg MS-325. Whole blood was obtained approximately 2 weeks prior to study initiation. In vitro plasma protein binding was determined via radiolabeled ^{153}Gd -MS-325 ultra filtration. Relaxivity was determined by titrating various concentrations of MS-325 in baboon plasma, and fitting the $1/T_1$ data versus concentration, and extrapolating the relaxivity from the slope.

Results

Plasma kinetics were described as a bi-exponential model with a distribution phase of 3.5 to 6.1 min, and an elimination phase ranging from 119 to 174 min for 0.10 and 0.20 mmol/kg respectively. Volume of distribution was approximately 0.13-0.15 at both doses, and plasma $AUC_{0-60\text{min}}$ was 457 and 384 for low and high dose respectively.

The dose corrected $\Delta 1/T_1$ $AUC_{0-60\text{min}}$ was determined and compared to that of human at doses of 0.01, 0.025, and 0.05 mmol/kg, and to that of cynomolgus monkey at doses of 0.025 and 0.10 mmol/kg. In all the species studied, MS-325 relaxivity was lower at higher doses than at lower doses. The compound was found to be most potent in humans.

The baboon plasma protein binding of MS-325 was approximately 91% at 0.10 mmol/kg (87% in cynomolgus), and the R1 was 45 mM⁻¹s⁻¹ (43 mM⁻¹s⁻¹ in cynomolgus).

Table. *In Vitro* Relaxivity and Fraction Bound of 0.10 mM MS-325 in Plasma of Various Species. (By sponsor)

Species	r ₁ (mM ⁻¹ s ⁻¹)	Fraction Bound (bound)
Human	53.5	0.96
Monkey	43.5	0.87
Rabbit	32.5	0.97
Rat	28.0	0.76
Mouse	22.6	0.67
PBS Buffer	6.6	NA (0.0)

Reviewer's comments

Results for in vitro plasma protein binding and relaxivity were similar for baboons and cynomolgus monkeys. Plasma kinetics were also similar for most parameters, however, a dose-dependent effect was observed in baboons but not in cynomolgus monkeys. The higher relaxivity noted in baboons as compared to that observed in cynomolgus may be due to the higher plasma protein binding. However, because these differences were very small, this effect may not be biologically relevant.

Report No. 6754-127: Metabolism and pharmacokinetics of MS-325 following intravenous administration to monkeys. Lab conducting study: []
[] GLP compliance. Lot b3095p39.

b(4)

The objective of this study was to assess the metabolism and pharmacokinetics of 0.03 mmol/kg MS-325, equivalent to 0.3X the intended clinical dose, given to monkeys by an intravenous route of administration.

Study design

Three male and three female cynomolgus monkeys were used in this study. In Group 1, 2 males and 2 females each received a single intravenous bolus injection of 0.03 mmol/kg MS-325 (0.3X the human dose). Blood was collected at 1, 5, 15, and 30 minutes, and at 1, 4, 8, 24, 48, and 96 hours post-dose. Based on the pharmacokinetic parameters calculated from the Group 1 plasma gadolinium concentration data, the blood collection time points for Group 2 were determined. After an appropriate washout period, the Group 1 animals and an additional male and female were assigned to Group 2. Each animal received an IV injection of 0.03 mmol/kg MS-325. Blood was collected from one animal per sex per time point at 1, 4, and 8 hours post-dose. Urine was collected at 0-6, 6-24, 24-48, and 48-72 hours post-dose from both Groups 1 and 2. Plasma and urine samples from Groups 1 and 2 were analyzed for gadolinium concentrations using ICP methodology. Group 2 plasma and urine samples were also analyzed for MS-325 concentrations utilizing liquid chromatography/mass spectrometry (LC/MS/MS) methodology.

Results

No metabolites were detected in the plasma or urine using this methodology.

Results of the hematology, clinical chemistry, and urinalysis tests, conducted prior to and/or after administration of MS-325 showed no abnormalities, and no drug-related adverse effects.

After IV administration, gadolinium plasma levels declined in a bi-exponential manner with a rapid distribution phase followed by a relatively slower elimination phase. The mean $t_{1/2}$ of 0.0575 hours (3.5 min) observed for males was slightly shorter than that of 0.0834 hours (5 min) observed in females. The mean terminal elimination half-life determined by compartmental ($t_{1/2}$) or noncompartmental ($t_{1/2elim}$) analysis was generally similar between males and females.

(Summary tables (3) prepared by sponsor)

Summary of Mean (n=2) Gadolinium Pharmacokinetic Results: Group 1

Gender		AUC _{0-t} ($\mu\text{g}\cdot\text{hr}/\text{mL}$)	AUC _{0-t} ($\mu\text{mol}\cdot\text{hr}/\text{mL}$)	AUC _{0-∞} ($\mu\text{g}\cdot\text{hr}/\text{mL}$)	AUC _{0-∞} ($\mu\text{mol}\cdot\text{hr}/\text{mL}$)	$t_{1/2}$ (Hours)	Cl _T (mL/hr/kg)	V _{DSS} (mL/kg)	MRT (Hours)
<u>Noncompartmental Parameters</u>									
Mean	M	136	0.868	159	1.01	2.89	30.8	116	3.78
Mean	F	128	0.815	150	0.954	2.94	32.8	123	3.77
Combined Gender									
Mean		132	0.841	155	0.984	2.92	31.8	120	3.77
SD		16	0.102	20	0.125	0.06	4.1	12	0.12

Summary of Mean (n=2) Gadolinium Pharmacokinetic Results: Group 1 (Continued)

Gender		A ($\mu\text{g}/\text{mL}$)	A ($\mu\text{mol}/\text{mL}$)	B ($\mu\text{g}/\text{mL}$)	B ($\mu\text{mol}/\text{mL}$)	$t_{1/2\alpha}$ (Hours)	$t_{1/2\beta}$ (Hours)
<u>Compartmental Parameters</u>							
Mean	M	53.3	0.339	36.9	0.235	0.0575	2.85
Mean	F	49.4	0.314	33.7	0.214	0.0834	2.88
Combined Gender							
Mean		51.3	0.326	35.3	0.225	0.0704	2.86
SD		3.9	0.025	3.9	0.025	0.0165	0.11

Urine Gadolinium Concentrations (µg/mL) and Amounts Excreted in Urine (µg)

Group	Animal	Gender	Concentration (µg/mL)												
			0-6 hr	Volume (mL)	Ae	6-24 hr	Volume (mL)	Ae	24-48 hr	Volume (mL)	Ae	48-72 hr	Volume (mL)	Ae	
1	I08605	Male	↑		4312	↑		1940	↑		360	↑		128	
	I08656	Male	↓		4232	↓		1686	↓		627	↓		219	
	Mean			246		4272	39.0		1813	4.92		494	2.46		174
	I08673	Female	↑		3188	↑		2285	↑		732	↑		493	
	I08735	Female	↓		NA	↓		3167	↓		1703	↓		1310	
	Mean			23.1		3188	59.9		2726	16.2		1217	7.30		902
Combined Gender															
	Mean			172		3911	49.4		2269	10.6		855	4.88		538
	SD			143		627	24.2		647	10.6		586	4.24		538

b(4)

b(4)

Ae Amount excreted calculated as concentration (µg/mL) x volume (mL).

NA Not applicable.

NS No sample.

a Observation of urine appears to contain water noted for Animal No. I08673 for the 0-6 hour interval.

b No sample was present for Animal No. I08735 for the 0-6 hour interval.

There were no apparent gender differences in the mean pharmacokinetic parameters. The mean V_{Dss} values in both genders were approximately 13% of the total body weight, indicating that gadolinium was not highly distributed into the tissues.

Reviewer's comments

The findings of this study are concordant with previous studies on different species. The pharmacokinetics of MS-325 follows a bi-exponential model, with a very short distribution time (5-8 min) followed by a slower elimination. This model is close qualitatively to humans. The distribution phase seems to be the same across species, in the order of minutes, whereas the elimination is different from a species to another (see summary). However, in this case, the distribution half-life was determined to be 3-5 hours for monkeys, compared to 16 hours in humans.

Report PTR 2003-40: Pharmacokinetics of MS-325-Gd¹⁵³ in non-naïve cynomolgus monkeys. (This is part of the report RTAZ-0114, following below)

Study design: Cynomolgus monkeys (n=2/sex/dose) received a single IV injection of 0.03 or 0.1 mmol/kg ¹⁵³Gd-MS-325. Blood samples were collected at 1, 5, 15, 30 min, and 1, 4, 8, and 24 hrs post-dose, and plasma concentrations determined.

Results

Table 1. Pharmacokinetic parameters in Cynomolgus monkeys following IV administration of 0.03 or 0.10 mmol/kg MS-325.

	0.03 mmol/kg		0.10 mmol/kg	
	Males	Females	Males	Females
Distribution half-life (min)	21	5	11	23
Elimination half-life (hr) (1)	5.6	3.4	4.1	4.4
(2)	3.2	2.1	2.2	2.2
AUC (µmol/mL/hr)	1.7	0.98	3.4	3.0
Cl (mL/hr/kg)	18	31	28	34
Vss (mL/kg)	136	146	161	195

(1) compartmental parameter (2) non-compartmental parameter
 (Table prepared by reviewer)

Plasma concentrations decreased in a bi-exponential fashion, thus a two-compartment model was appropriate for analysis of the plasma kinetic data. A one compartment model was also used.

-At 0.03 mmol/kg, distribution half-life was 4 times more rapid in females (5 min), compared to that in males (21 min). At 0.1 mmol/kg, distribution half-life was 11 min in males and 23 min in females.

-The elimination phase was similar in males and females at both doses studied, with a half-life ranging from 3.4 to 5.6 hrs.

-AUC appeared to be dose-dependent for both sexes, although a smaller value was found in females at low dose. Volume of distribution was also similar across sexes and doses.

Reviewer's comments:

This study confirmed most of the previous findings in monkeys, with plasma kinetics following a bi-exponential model. However, the very low number of animals used in this study does not allow for proper comparison between males and females, thus differences found may not be interpreted.

3.3.6 Pharmacokinetic drug interactions

Not applicable

Appears This Way
 On Original

3.3.7 Tables and figures to include comparative TK summary

Table: Summary of MS-325 pharmacokinetic results in rats, rabbits, and cynomolgus monkeys. (By sponsor)

Species	Study Number	Dose ($\mu\text{mol/kg}$)	$T_{1/2\alpha}$ (hr)	$T_{1/2\beta}$ (hr)	$AUC_{0-\infty}$ ($\text{hr} \cdot \mu\text{mol/L}$)	V_{dss} (L/kg)	Cl (L/hr/kg)
Rat	301	25	0.014 \pm 0.002	0.38 \pm 0.05	58 \pm 4	0.254 \pm 0.040	0.432 \pm 0.031
Rat	301	100	0.015 \pm 0.003	0.39 \pm 0.05	259 \pm 50	0.236 \pm 0.034	0.397 \pm 0.069
Rabbit	302	100	0.05 \pm 0.01	1.97 \pm 0.38	2198 \pm 665	0.137 \pm 0.016	0.049 \pm 0.013
Monkey	6754-127	30	0.07 \pm 0.02	2.86 \pm 0.11	984 \pm 125	0.120 \pm 0.012	0.032 \pm 0.004
Monkey	PTR2003-40	30	0.18 \pm 0.06	4.07 \pm 0.10	1190 \pm 40	0.144 \pm 0.006	0.025 \pm 0.001
Monkey	PTR2003-40	100	0.21 \pm 0.04	3.88 \pm 0.06	3194 \pm 70	0.167 \pm 0.004	0.031 \pm 0.001

b(4)

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

General toxicology:

1. Single-dose toxicity

Acute toxicity was evaluated in cynomolgus monkeys administered single doses of MS-325 at 0.2, 1, and 3 mmol/kg (2.2X, 11X, and 32X the human dose respectively based on BSA) followed by a 14 day observation period. Salivation, focal accumulation of vacuolated and foamy macrophages was observed in the lung, and cytoplasmic vacuolation of the reticuloendothelial cells was noted in animals at 11X and 32X. NOEL was established at 2.2 times the human dose.

In rats given a single IV dose of 0.5, 1.0, 2.0, 3.0, and 5.0 mmol/kg (2.7, 5.4, 11, 16, and 27 times the human dose respectively), followed by a 14-day observation period, the incidence and severity of kidney alterations, from acute vacuolation to chronic renal tubular degeneration/regeneration and single cell/brush border necrosis of the proximal convoluted tubules were dose-dependent and more severe in males. Most of the effects appeared to be reversible. Some of these lesions persisted at 2.7X and 11X the clinical dose. Since renal vacuolation was the only finding in the low and the high intermediate dose groups (0.12 and 0.50 mmol/kg MS-325) without accompanying functional signs of nephrotoxicity, the no observable adverse effect level (NOAEL) was determined to be 0.50 mmol/kg (2.7 times the human dose).

2. Repeat-dose toxicity

(i) Repeat dose toxicity was evaluated in monkey intravenously administered doses of 0.1, 0.5, and 2.0 mmol/kg/day (1, 5.4, and 22X) for 14 days followed by a 28 day recovery period. Salivation, which was noted in a monkey acute toxicity study, was observed during and immediately following high-dose (2.0 mmol/kg) on the first day of dosing.

Liver and kidney weights were increased at all doses. Although a significant degree of recovery was observed, full recovery in kidney weights was not attained at 22X after a 28 day period. AST and ALT were increased at 2 mmol/kg, however no histopathological change was noted. AST, ALT and absolute liver weights returned to normal values after the 28 day recovery period. There was no statistically significant change in the serum chemistry, the urinalysis, and the hematological parameters. However, hemoglobin and hematocrit were slightly lower at the intermediate sacrifice interval with increase at recovery. Histopathological changes included cytoplasmic vacuolation of the kidney, and the reticuloendothelial cells of lymph nodes, and increased number of foamy vacuolated alveolar macrophages. Renal vacuolation and foamy macrophages appearance were reversible at 5.4X whereas the recovery of lymph nodes vacuolation was complete at ~1X the clinical dose. NOEL was established at 0.1 mmol/kg/day (1X the human dose).

(ii) Repeat dose toxicity was evaluated in rats intravenously administered doses of 0.05, 0.1, and 2.0 mmol/kg/day (respectively 0.3, 0.54, and 11X the human dose) for 14 days followed by a 28 day recovery period. Vacuolation was the most significant finding in kidney, lungs and reticuloendothelial systems mainly occurring at 11 times the human dose. Effects were dose-dependent and more pronounced in males. In most low and mid dose group animals, toxicity was reversible after a 28-day recovery period. However, many alterations persisted after the end of the recovery period. Therefore, no NOEL could be established. Overall, the types of toxicity revealed in these studies have been described for marketed gadolinium agents.

In general, rats were more sensitive to MS-325 than monkeys, perhaps because of their smaller size; the hyperosmolality of MS-325 may affect more significantly smaller volumes of blood.

Genetic toxicology:

The mutagenicity potential of 2 formulations of MS-325 was evaluated using 1) the *in vitro* reverse mutation assay on *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2uvrA, 2) the *in vitro* chromosomal aberrations in Chinese hamster ovary cells (CHO), and 3) the *in vivo* mouse bone marrow micronucleus assay. In all studies, MS-325 was not mutagenic when compared to the negative control in either the activated or the non-activated assay under the conditions of these studies. Overall genetic toxicology studies were acceptable.

Carcinogenicity:

Not conducted

Reproductive toxicology:**4.1. Fertility and early embryonic development:**

Vasovist was shown to reduce by 22% the number of spermatids per gram testicular tissue and to increase the epididymides and testicle weights in rats treated, daily for 4 weeks, with 1.5 mmol/kg/d (8X HD). Furthermore, the number of foamy vacuolated macrophages in the epididymides, and the number of foamy macrophages in the testicular interstitium was increased in rats treated with 1.5 mmol/kg/d. Most of the effects were observed at 1.5 mmol/kg/day (8X the human dose). At 0.21 mmol/kg/day the only finding was the increase in kidney weights, therefore NOEL could be established at 0.21 mmol/kg/day, 1X the human dose.

4.2. Embryofetal development:

MS-325 at 0.1, 0.45, and 2 mmol/kg (0.5, 2.5, and 11 times the human dose) was injected in rats between the 6th and 17th day of pregnancy. There was a significant increase in the number of skeletal variations and in the number of skeletal retardations at 11 times the human dose. MS-325 was not teratogenic in rats at all doses tested. NOEL was established at 2.5 times the human dose.

Embryofetal development was studied in rabbits dosed once daily with 0.3, 0.8, and 2.0 mmol/kg/day (respectively 3.3, 9, and 22 times the intended clinical dose). Embryotoxicity was associated with an increase of post-implantation loss and total resorptions at 22X HD. Total resorptions increased from 3 in the control group to 20, 17, and 35 at 3X, 9X, and 22X the human dose, whereas the number of live fetuses decreased from 127 in the control group to 109, 108, and 92 in the same groups. At 22 times the human dose, MS-325 produced teratogenic effects (hydrocephalus with malrotated fore paws and hind limbs, cleft palates, nasal region adhesion and vacuoles in cerebral hemispheres). The teratogenic effects were not observed at 9 times the human dose.

4.3. Prenatal and postnatal development in rat:

Prenatal and postnatal development was studied in rats treated once daily with 0.03, 0.21, and 1.5 mmol/kg. F0, F1, and F2 generations development, including reproduction indices (birth index, live birth index, viability index, lactation index, and overall survival index) was not affected by MS-325 treatment. No malformation was observed in the pups of either generation. A decrease in the mean entire litter and in body weights was observed in F1 and F2 pups. This effect was marginal and not significant.

In view of the fact that MS-3235 treatment was found to be embryotoxic in rat and rabbit, and teratogenic in rabbit, we recommend the pregnancy label proposed by the sponsor be Category C.

b(4)

Special toxicology:

Perivascular injection of 0.5 mL of MS-325 at 0.25 mmol/mL (0.5X clinical dose), in rabbits produced mild irritation of the skin and subcutaneous tissue. This effect persisted through 96 hours (end of observation). This study suggests that local reaction is likely to occur after accidental extravasation of Vasovist. The study was not conducted until resolution of local irritation, however, the decrease in the sign severity indicates that the effect was likely reversible. Clinical equivalent dose was not evaluated.

Intravenous injection of 1 mL of MS-325 (1X clinical dose) produced no significant irritation.

MS-325 did not demonstrate in-vitro hemolytic potential following 45 min incubation with human whole blood at ratios ranging between 1:64 and 1:1 (MS-325: whole blood) nor did it cause precipitation or coagulation following a 20' incubation with plasma, at 1:1 to 1:8 ratios (MS-325: plasma)

At doses of MS-325 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 an IgE mediated allergic reaction. In addition, MS-325 did not exhibit a potential to produce dermal sensitization in guinea pigs. In a rat study, the T cell-dependent antibody response was not affected by the treatment with MS-325. In the same study, microscopic observation of severe vacuolation in alveolar lumina of the lungs at 10X the human anticipated dose, and not 0.16X and 2.7X the human dose, suggests that macrophage function and morphology will be affected at the high dose level.

3.4.2 Single-dose toxicity

Study report no6622-102: Single dose intravenous toxicity study of MS-32516 in rats.

The purpose of this study was the evaluation of the effects of the ligand (MS-32516) following single intravenous administration in rats.

Key study findings: A single dose injection of MS-32516 (ligand) did not induce significant toxicity at doses of 0.01 mmol/kg (at least 30 times the clinical intended dose).

Study no.: 6622-102
Volume #, and page #: 5, 3902
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: 11/20/1997
GLP compliance: Yes
QA report: yes () no (X)
Drug, lot #, and % purity: MS-32516, 2749P018, no reported purity.

Methods

Doses: 0.01, 0.05, 0.1, and 0.125 mmol/kg, (respectively 32, 162, 324, and 405 times the maximum human dose, based on body surface area) followed by 14 day observation.

Species/strain: Rat/Sprague-Dawley — CD®(SD)BR

Number/sex/group or time point (main study): 5/sex/dose

Route, formulation, volume, and infusion rate: Single bolus intravenous via tail vein, aqueous solution, dose volumes of 0.1, 0.5, 1.0, and 1.25 mL/kg, injection over ~ 1min. Physiological saline at a dose volume of 1.25 mL/kg as control.

Satellite groups used for toxicokinetics or recovery: none

Age: approximately 7 weeks old.

Weight: Males: 246g-282g; Females: 160g-187g.

Unique study design or methodology (if any): None

b(4)

Doses (mmol/kg)	0.01	0.05	0.1	0.125	NOEL
					0.01
Dose multiple (based on BSA)	32	162	324	405	32

Observation times and results

Mortality:

Observation was performed twice daily for mortality and moribundity, on Day 1 observation immediately post injection, 15, 30 minutes, and 1, 2, and 4 hours post-injection.

Results: one male animal treated with 1.25 mL/kg (400X HD) was found dead after dosing on day 1. Clinical signs noted in this animal immediately after dosing included labored respiration, pallor of the entire body and dorsal recumbency.

Clinical signs:

Observation performed once daily.

Results: Immediately following dosing on Day 1, slight to moderate hypoactivity was noted in respectively 1 male and 2 females of Group 3 (162X HD). All animals appeared normal within 15 minutes of dosing.

Body weights:

Observation: twice prior to treatment and prior to initiation of dosing on Day 1, then weekly during the study, and at termination.

Results: A gain in body weight was observed in all animals. However, there was no statistical difference between treated and control group animals.

Food consumption:

Recorded once during pretest, then weekly during the study.
No significant mean food consumption differences were noted.

Ophthalmoscopy:

Not conducted

EKG:

Not conducted

Hematology and clinical chemistry:

Observation: At termination of the study, blood samples were collected via orbital plexus. Animals were fasted overnight prior to clinical sampling. Prothrombin time and activated partial thromboplastin time were also measured.

Results: A significant increase of 66% and 54% in mean alkaline phosphatase values was noted in females of Group 4 and Group 5 respectively (325 and 400X HD respectively). A slight decrease, 28% and 35%, of mean total cholesterol was observed in females of Groups 2 (32X) and 4 (325X). No other hepatic enzyme seems to have been affected by treatment. Neither do the coagulation parameters.

Urinalysis:

Not conducted.

Gross pathology:

Observation: After 18 days of observation, all surviving animals were fasted overnight, weighed, anesthetized, exsanguinated, and necropsied. At necropsy, macroscopic observations were performed.

Results: one dilatated pelvis, one dark lung, one enlarged liver, kidney, and adrenal were observed in females at 1.25 mL/kg.

Organ weights:

Adrenal, brain, liver, kidney, testis and epididymis were weighed at necropsy.

Results: Mean kidney weight was slightly elevated in males of Groups 2 and 5 while a 39%, 37%, and 29% increase was noted in females of Group 5, in, respectively the mean absolute weight, in the organ relative to body weight, and in the organ relative to brain weight. There were no significant treatment-related changes in the mean of other organs weight.

Histopathology: Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Observation: Adrenal, brain, heart, injection site, kidney, lesions, lung, liver, lymph node (mesenteric, mandibular, and axillary), testis with epididymis, urinary bladder were examined microscopically.

Results: Liver vacuolation was present in males and females of all groups, however, the incidence was greater in treated animals, predominantly in females, compared to control animals. Liver congestion was noted in 1 male and 1 female of the highest dose level

group, and liver necrosis observed in 1 female in groups 1, 2 and 4, whereas it was noted in 2 males of Group 4. Adrenal congestion and necrosis, lung hemorrhage and pigmentation were sporadically noted in animals of Group 5. Heart degeneration was observed in 1 male of group 2 and in 1 female of group 3 and group 5. There was one case of pyelonephritis, one case of lymphoid necrosis, and one case of urinary bladder hyperplasia and cystitis in the group 5 females. Dilatation of the uterus was observed in 1 female of group 2 and 5.

Table. Distribution of Toxic effects

Dose (mmol/kg)	Control (n=10)	0.01 (n=10)	0.05 (n=10)	0.1 (n=10)	0.125 (n=10)
Liver vacuolation	+	++	++	++	++
Liver congestion					1M +1F
Liver necrosis	1F	1F		2M + 1F	
Adrenal congestion, necrosis, lung hemorrhage & pigmentation					Sporadic
Heart degeneration		1M	1F		1F
Pyelonephritis					1F
Lymphoid necrosis					1F
Urinary bladder hyperplasia, cystitis					1F
Uterus dilatation		1F			1F

M, male; F, female

+ present

++ present at higher incidence

(Table prepared by reviewer)

Toxicokinetics:

Not performed.

Conclusions: Sponsor concluded that microscopic evaluation did not reveal any evidence of compound-related histopathologic changes associated with the administration of MS-32516-R. All microscopic changes present in the treated rat were considered incidental and treatment unrelated.

Reviewer's comments:

↓ Each mL of VASOVIST Injection contains 244 mg of gadofosveset trisodium (0.25 mmol), 0.27 mg of fosveset, and water for injection.

↓ With a clinical dose of 0.03 mmol/kg of Gadofosveset drug substance and a maximum of — Fosveset allowed in the clinical formulation, the maximum clinical dose of

Most of these observations were reported for the highest dose level, which is about 400 times the clinical intended dose. Moreover, there was no clear dose-dependency except perhaps for the liver vacuolation where it was evident that the incidence was greater in treated animals compared to the control animals. There was no microscopic finding in the kidney, which is one of the target organs. This observation suggests that the liver

b(4)

vacuolation may not be clinically relevant. Overall, a single dose injection of MS-32516 did not induce significant toxicity at doses at least 30 times the intended clinical dose.

Study title No 211: A single-dose toxicity study of MS-325 in the cynomolgus monkey via intravenous administration.

Key study findings: No effect was observed in monkey administered a single dose of 0.2 mmol/kg (2.2 X human dose), which the sponsor established as the NOEL.

Study no.: 95-3288
Volume #, and page #: 5, 2931
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: 02/27/1996
GLP compliance: Yes
QA report: yes () no (X)
Drug, lot #, and % purity: MS-325-DPI (abbreviates as MS-325) (0.251 mmol/mL (244 mg/mL), 730 mOsM/Kg, MS-325-DP1), 512016, no purity % provided (analysis was the sponsor's responsibility)

Methods

Doses: 0.2, 1.0, and 3 mmol/kg (2.2, 11, and 32 times the intended clinical dose based on body surface area).
 Species/strain: Monkey/Cynomolgus (macaca fascicularis)
 Number/sex/group or time point (main study): 2/sex/dose
 Route, formulation, volume, and infusion rate: Intravenous, aqueous solution, 12, 0.8, 4.0, and 12.0 mL/kg (for 0.9% saline and 0.2, 1.0, and 3.0 mmol/kg, respectively), 6 mL/min.
 Satellite groups used for toxicokinetics or recovery: No
 Age: Young adults (sic)
 Weight: Males: 1.8-2.3 kg, Females: 1.8-2.2 kg
 Unique study design or methodology: None

Dose (mmol/kg)	0.2	1.0	3	NOEL 0.2
Human dose multiple (based on BSA)	2.2	11	32	2.2

Observation times and results

A single dose of MS-325 was injected followed by a 14 day observation period. Animals were examined twice daily, once in the morning and once in the afternoon.

Mortality:

No mortality occurred during the course of the study.

Clinical signs:

Post-dose observations were performed immediately following dosing, and 15, 30, and 45 min, and 1, 2, and 4 hrs after dosing.

Immediately post dosing, salivation was noted in animals from the 1 and 3 mmol/kg groups, and retching was observed in one female given 3 mmol/kg. These signs lasted up to 15 minutes post dose. Unformed stool was observed in the females at 1 mmol/kg on Days 5, 6, 7, and 14. One male animal from the 1 mmol/kg group had an opacity in the right eye that was not present pretest. One female in the 1 mmol/kg group had an ovary cyst.

Body weights:

Twice pre-test and weekly thereafter, and terminally after fasting.

No treatment-related changes were observed.

Food consumption:

Estimation was made 7 times weekly beginning 1 week pretest.

No difference was noted between treated and control animals.

EKG:

Not performed

Hematology:

Blood was collected on unanesthetized monkeys fasted overnight, via femoral venipuncture.

There was no significant treatment related change.

Clinical chemistry:

(Performed at the same times as hematology)

No treatment related change

Urinalysis:

Urine was collected

No treatment-related change

Gross pathology:

A few macroscopic changes were observed at necropsy. However, these changes occurred in controls and treated animals and were therefore deemed incidental findings.

Organ weights:

Adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, spleen, testes with epididymides, and thyroid/parathyroid glands.

No treatment related changes.

Histopathology:

Adequate Battery: yes. However, the brain was not examined, should have been examined as well. There are no data providing direct demonstration that the drug does not cross the BBB. This information would have been valuable, in view that although the sponsor claims that MS-325 dose not reach the brain, several clinical signs, detected in different animal species, seem to point to central stimulation.

Peer review: no

Microscopic examination was performed on heart, injection site, kidneys, liver, lungs, lymph nodes (axillary, mediastinal, mesenteric, submandibular, inguinal)

Results

Macroscopic examination revealed minimal or slight focal accumulation of vacuolated macrophages, including foamy macrophages, in the lung of 2 females at 1 mmol/kg and one male at 3 mmol/kg. Foamy macrophages were present as a loose cluster of cells, free within the individual alveoli.

Cytoplasmic vacuolation of the reticuloendothelial cells was observed in lymph nodes of 1 male and 2 females within each group of 1 mmol/kg and 3 mmol/kg.

No effect was observed at the lowest dose of 0.2 mmol/kg (2.2 X human dose), which the sponsor established as the NOEL in this study.

Toxicokinetics:

Not conducted

Other:

Not conducted

Reviewer's comments: Previous rat studies showed drug-related vacuolation of the kidney, which did not occur in monkeys of this study. On the other hand, the lung is clearly identified as a significant target for MS-325. Foamy macrophages may result from the specific binding to albumin, which may stimulate a macrophage-mediated response.

Study title-Report 210: A single-dose toxicity study of MS-325 in the rat via intravenous administration

Study objective: To assess the potential toxicity of MS-325 when administered as a single dose via intravenous injection to rats followed by a 14-day observation period.

Key study findings: LD50 of MS-325 was 3.9 mmol/kg for both sexes (~21 times the human dose). Histological findings show that the target tissue is the renal proximal convoluted tubules. Microscopic changes seen in the kidneys were less severe in females. NOAEL was established at 0.5 mmol/kg (2.7X HD)

Study no.: 96-1403

Volume #, and page #: 5, 2624

Conducting laboratory and location: T

b(4)

b(4)

Date of study initiation: 02/06/1996
GLP compliance: Yes
QA report: yes () no (X)
Drug, lot #, and % purity: MS-325-DPI (abbreviates as MS-325) (0.251 mmol/mL (244 mg/mL), 730 mOsM/Kg, MS-325-DP1), 512016, no purity % provided (analysis was the sponsor's responsibility)

Methods

Doses: 0.5, 1.0, 2.0, 3.0, and 5.0 mmol/kg (2.7, 5.4, 11, 16, and 27 times the intended clinical dose). The 2 mmol/kg group was added due to the mortality at 3.0 mmol/kg. Control animals received 0.9% saline at 19.9 ml/kg.
 Species/strain: Albino rats (Outbred) VAF/Plus/Sprague-Dawley derived (CD®) — CD (SD) BR]
 Number/sex/group or time point (main study): 4-5/sex/dose
 Route, formulation, volume, and infusion rate: Tail vein intravenous, 0.251 mmol/mL (244 mg/mL), volume of 2, 4, 8, 12, and 19.9 ml respectively at a rate of 4mL/min, bolus as a single dose followed by a 14-day observation period.
 Satellite groups used for toxicokinetics or recovery: No
 Age: 45 or 48 days.
 Weight: not provided.
 Unique study design or methodology: None

b(4)

Dose (mmol/kg)	0.5 grp	1.0 grp	2.0 grp	3.0 grp	5.0 grp	NOAEL 0.5
Human dose multiple (based on BSA)	2.7	5.6	11	16	27	2.7

Observation times and results

Control animals were evaluated on a different day (for hematology).

Mortality:

Observation: Twice daily, once in the morning, and once in the afternoon.
 Re: LD₅₀ was 4.2 and 3.8 mmol/kg for males and females respectively.
 The 2 mmol/kg group comprises 4 males and 4 females, and there was no death in this group. In the 3 mmol/kg, 1 out of 5 females died, and no male died.
 In the 5 mmol/kg group, 4/5 males and 4/5 females died. The cause of death was considered to be cardiovascular collapse based on lethargy and rapid breathing observed immediately post dose in the 3 and 5 mmol/kg dose animals. All survivors were free of systemic signs of toxicity within 2 hrs.

Clinical signs:

Observation: Detailed physical examination for signs of local, or systemic toxicity, pharmacologic effects, and palpation for tissue masses, immediately post-dosing, 15 min, 30 min, 1 hr, 2 hrs, and 4 hrs post-dose, and daily thereafter for 14 days.

Results: Immediately post-dosing, most of the animals in the 3 and 5 mmol/kg groups exhibited lethargy and rapid breathing. Moreover, labored breathing and prostration was observed in 1 animal of Group 5. Two rats from the highest dose group were pale. All survivors were free of systemic signs of toxicity within 2 hours post-dose. Rapid breathing which resolved within 1 hour was observed in one animal of the 1 mmol/kg group, and discoloration of the tail was noted in most animals for up to 4 hours post-dose. The remaining animals were free of pharmacological and toxicological signs throughout the study.

Body weights:

Observation: Twice pretest, weekly during the treatment and terminally (after fasting).

Results: The mean body weights at the 2 highest dose levels showed a slight decrease.

Food consumption:

Observation: Weekly, beginning one week prior to treatment.

Results: The mean food consumption values were generally comparable to the control animals' values, in spite of a slight decrease in the 2, 3, and 5 mmol/kg male groups during week 1.

Ophthalmoscopy:

Not conducted.

EKG:

Not conducted.

Hematology:

Blood was collected at Day 23 or 27, by puncture of the orbital sinus under CO₂O₂ anesthesia. Rats were fasted overnight prior to collection.

There were no treatment-related changes.

Clinical chemistry:

Observation and results: same as hematology.

Urinalysis:

Not conducted.

Gross pathology:

Animals were fasted prior to sacrifice at Day 23 or 27 and examination was performed post-mortem.

Results: Kidney dilatation was observed in 1 male control, and 1 male treated with the highest dose level. The effect was also seen in 1 female of Group 2 and Group 5. Two

males and 3 females of Group 5 that died on Day 1 showed fluid filled lungs, with one discolored lung in each of these groups. Several isolated findings were reported but they were not dose-dependent: In the female Group 4, one animal had an ovary cyst, and one discolored focus spleen; 3 males of Group 5 and 1 female of group 4 had discolored thymus. In both groups 5, fluid was observed in the trachea. There was on scab sore in tail of 1 male and 1 female of Group 6 and one discolored tail in 1 male and 1 female of Group 6.

Organ weights:

Brain, adrenals, kidneys, liver, and testes/epididymis were weighed.

Results: No changes were observed in brain, adrenals, liver and testis. In males treated with 2, 3, and 5 mmol/kg, the mean absolute and relative kidney weights were significantly increased. No changes were noted for females.

Histopathology:

Adequate Battery: no. Most of the organs were preserved. Microscopic evaluation was performed on heart, injection site, kidney, liver, lungs (with mainstem bronchi), lymph node (axillary, mesenteric, mediastinal, submandibular, and inguinal). Brain and reproductive organs should have been examined as well. Information on the potential toxicity on the brain is not well documented throughout this NDA.

Peer review: no.

Results

Kidneys: Proximal convoluted tubules nephrosis occurred in 1 female of Group 4 and in 3 males and 4 females of Group 5. Proximal tubules vacuolation was observed in nearly all animals of Groups 3, 6, and 4, and in 3/10 Group 2 rats. A dose-response urothelium vacuolation was noted in males with a lower incidence in females. Only males showed a clear dose-dependent nephropathy. This effect was not observed in any of the female rats. Mineral deposit was observed in several males treated at doses higher than 2 mmol/kg, and in females of all groups including one female control. Pelvis dilatation, interstitial fibrosis, and proteinaceous casts were observed in some animals but these events were not dose-dependent.

Lungs: Edema was observed in 2 control males, and 8 out 10 rats in group 5. Lung congestion was observed in 8 animals of the highest dose groups but it was also found in 3 of the male controls.

Two males from Group 5 had submandibular/max lymph nodes hemorrhage. Some alterations were observed at the injection site in Groups 6 and 5, with a higher incidence of tissue hemorrhage at the highest dose level. However, these effects were not observed in other dose groups. Moderately severe tail hemorrhage, inflammation, and acanthosis were observed in 1 male and 1 female of dose Group 6. Hemorrhage of the thymus was observed in all males of the highest dose level. Likewise, trachea lymphoid cell infiltrate and hemorrhage were only observed in high dosed males.

Toxicokinetics:

Not conducted.

Conclusion: A single dose of MS-325 in rats resulted in a dose-dependent increase in the incidence and/or severity of tubular alterations in the kidneys, with effects being more severe in males compared to females. These changes were not associated with concomitant changes in chemistry and hematological parameters. Therefore, they were not considered to be clinically relevant by the sponsor. The NOAEL was established at 0.5 mmol/kg, a dose level at which cytoplasmic vacuolation was noted in 3/10 animals. This effect is common to gadolinium agents.

Reviewer's comments: The NOAEL is established at 0.5 mmol/kg, 2.7 times the intended human dose. Vacuolation of the kidney is a common occurrence observed with gadolinium agents. The clinical chemistry did not reveal dramatic concomitant changes in blood urea, and creatinine, however, and because the kidney is a target organ for MS-325, the renal function needed to be more thoroughly investigated, including urinalysis, in normal animals and in an established model of renal impairment.

Study title N A06874: Systemic tolerance study in rats after single intravenous administration of ZK236018 (SH L03588A; Epix Medical, MS-325) over a period of 4 and 15 days

Key study findings: After a single intravenous administration of SH L03588A (ZK236018) the no observed effect level (NOEL) was determined to be 0.03 mmol/kg. Since renal vacuolation was the only finding in the low and the high intermediate dose groups (0.12 and 0.50 mmol/kg ZK 236018) without accompanying signs of nephrotoxicity the no observable adverse effect level (NOAEL) was determined to be 0.50 mmol/kg (2.7 times the human dose).

Study no.:	TXST20010093
Volume #, and page #:	5, 3444
Conducting laboratory and location:	Schering AG, Experimental Toxicology, D-13342 Berlin, Germany
Date of study initiation:	06/06/2001
GLP compliance:	Yes
QA report:	Yes () No () Information not provided.
Drug, lot #, and % purity:	Gadofosveset trisodium (ZK236018), 3095p19, 99.3%. 0.9% NaCl (w/v) for dilution.

Methods

Doses: 0.03, 0.12, 0.50, and 2.0 mmol/kg (respectively 0.16, 0.6, 2.7, and 10 X the clinical dose based on body surface area).
Species/strain: rat, Wistar (Shoe:WIST)

Number/sex/group or time point (main study): 10/sex/dose (5/sex/group were sacrificed on Day 4 and the rest on Day 15 to determine reversibility of potential effects).

Route, formulation, volume, and infusion rate: single intravenous, aqueous solution, 8mL/kg, 6.0 mL/min.

Satellite groups used for toxicokinetics or recovery: 3 males and 3 females added to the 4 treatment groups. Blood samples were analyzed for Gd content in a research laboratory that is not part of Schering's GLP program.

Age: Not provided.

Weight: Males: 204-247 g. Females: 174-218 g.

Unique study design or methodology: None

Dose (mmol/kg)	0.03	0.12	0.50	2.0	NOAEL 0.5
Human dose multiple (based on BSA)	0.16	0.6	2.7	10	2.7

Observation times and results

Mortality:

There was no drug-related death. One female in Group 5 died reportedly due to accidental puncture of the carotid artery during blood sampling on Day 2.

Clinical signs:

Observation: twice daily, once in the morning and once in the afternoon.

There were no test article-related changes in the general condition compared to baseline observations.

Body weights:

Recorded at Day -10 (pre-value), Day 1 and Day 4 in group sacrificed on Day 4, and at Day -10 (pre-value), Day 1, Day 4, Day 8, and Day 13 in group sacrificed on Day 15. A weight loss was observed in all groups including control group, between Day 1 and Day 4.

Food consumption:

Recorded from day 1 to Day 4 in the group sacrificed on Day 4 and from Day 1 to Day 4, Day 4 to Day 8, and Day 8 to Day 13 in the group sacrificed on Day 15. No drug-related change was noted. Water consumption was increased in 2/5 males of Group 5. This effect was not seen in the same group animal sacrificed at Day 4; therefore, it was considered to be accidental by the sponsor

Ophthalmoscopy:

Examinations were carried out in week -1, at Day 3 in collective 1 and at Day 12 in collective 2 in 5 males and 5 females per group. Visible sutures in the lens seen in animal occurred in 1 animal of group 4 on day 3. However, this event was not dose-dependent and the finding was considered to be a chance finding.

EKG:

Not performed.

Hematology:

Performed in EDTA-blood samples of 7 to 10 males and 9 to 10 females per group on day 2, and 5 males and 4 to 5 females per group on day 9.

Parameters evaluated: red and white blood cell count, hemoglobin, packed cell volume (PCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), platelet count and differential count (neutrophils, lymphocytes, eosinophils, basophils, monocytes, large unstained cells and lobularity index).

Results:

Females of Group 5 had slightly increased neutrophil counts on Day 2, and a decrease in MCHC on Day 9-10.

Citrated plasma samples of 2 to 4 males and 3 to 5 females per group on days 14-15 was evaluated for the following parameters: thrombin time, thromboplastin time, activated partial thromboplastin time and fibrinogen.

A slight prolongation in activated partial thromboplastin time was observed in male animals at 0.50 mmol/kg. This effect was not dose-dependent.

Clinical chemistry:

The following parameters were evaluated in serum samples of 9 to 10 males and 10 females per group on day 2, and from 5 males and 4 to 5 females per group on day 9: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, glucose, urea nitrogen, creatinine, sodium, potassium, calcium, chloride, total protein and protein electrophoresis.

Results: Increases in serum urea nitrogen, serum creatinine and serum chloride were observed in male animals of the high dose group on day 2. Significantly increased chloride levels though to a smaller extent than in males were also seen in female animals of the high intermediate (0.5 mmol/kg) and the highest dose group (2 mmol/kg).

Urinalysis:

The following parameters were measured in urine samples collected over a period of about 18 hours from 5 males and 4 to 5 females per group on days 4 and 11: pH value, specific gravity, urinary volume, protein, glucose, ketones, urobilinogen, bilirubin and blood.

Results: None of the findings in urine of treated animals were considered to be compound-related either because similar findings were observed in urine of control animals or because of lack of dose dependence or because they occurred sporadically only.

Gross pathology:

On completion of treatment, all animals were sacrificed under general chloroform and post mortem examination was performed in each animal.

Organ weights:

Weight was determined for liver, kidney, heart, lung, salivary glands, pancreas, pituitary gland, thyroid glands with parathyroid glands, adrenal glands, ovaries, uterus, testes, prostate, seminal vesicle, thymus, spleen, iliac lymph node, brain.

Results: There was a slight increase in kidney weights of males of the high dose group at Day 4 and Day 15. Changes found in the absolute weights of other organs were not reflected when compared to total body weights.

Histopathology: Adequate Battery: yes

Peer review: no

All rats of all dose groups were necropsied. Complete histopathologic examination was performed on all protocol designated tissues of all rats of groups 1 and 5, and all gross lesions (see histopathology inventory table below). The kidneys of all rats of groups 2, 3, and 4, were also examined.

Results:

On Day 4: There were no findings in Group 2. In the kidneys, cytoplasmic vacuolation of proximal cortical tubular epithelium was observed in 4 females of Group 3 and nearly all animals of Groups 4 and 5. Tubular degeneration/regeneration, granular and hyaline casts as well as single cell necrosis in the pars recta of proximal tubules, associated with the acute tubular lesions occurred with various degrees of severity in all males of group 5.

On Day 15: There was no finding in Group 2 and 3. Cytoplasmic vacuolation was found in 1 male and 3 females of Group 4. Only 1 female in the Group 4 had slight hyaline casts in the pars recta associated with cytoplasmic vacuolation of tubular epithelium. The findings in nearly all the male animals of Group 5 were similar in nature to those made on Day 4, however they were less severe and occurred in a slightly lower incidence, suggesting that the findings were reversed to some extent. However, the microscopic examination revealed a higher rate of lung alveolar histiocytosis in Group 5 compared to the control group: this alteration was observed in 8/10 treated animals on Day 3 and 9/10 on Day 15, compared to 2/10 and 4/10 control animals, respectively.

Microscopic observation of other organs did not show significant differences between control and treated animals.

Toxicokinetics:

PK parameters were calculated from mean values of male and female rats. The mean maximum Gd-concentration in serum (C_{max}) and its point in time (T_{max}) were determined directly from the data by plotting the mean serum concentration against the different points in time. The mean area under the serum concentration-time curve from the first (2 min) to the last (60 min) observation time point (AUC_(2-60 min)) was calculated according to the linear trapezoidal rule. The mean AUC values are presented in

$\mu\text{mol}\cdot\text{min}/\text{L}$. The average serum concentration per min, C_{av} , was calculated for the same time interval by dividing the AUC value by the time period of 58 min.

Results: Mean maximum Gd-concentrations in serum increased almost proportionally with the dose and were observed at the first sampling time at 2 min after i.v. administration. A 67-fold increase of the dose from 0.03-2.0 mmol/kg resulted in a 45-fold increase of the C_{max} -value from 0.28 mmol/L to 12.8 mmol/L. The mean $\text{AUC}_{(2-60 \text{ min})}$ values for Gd indicated also an almost dose-dependent linear increase between the dose level of 0.03 mmol/kg up to 2 mmol/kg in male and female rats. A 67-fold increase of the dose resulted in a 49-fold increase of the $\text{AUC}_{(2-60 \text{ min})}$ -value from 5.26 mmol \cdot min/L to 257 mmol \cdot min/L.

Other:

Bone marrow was collected from femur at necropsy on days 4 and 15. However, myelograms were not examined due to the absence of serious histological or hematological deviations.

Histopathology inventory

Study	A0687 4			
Species	Rats wistar			
Adrenals	*X			
Aorta				
Bone Marrow smear				
Bone (femur)	X			
Brain				
Cecum	X			
Cervix				
Colon	X			
Duodenum	X			
Epididymis				
Esophagus	X			
Eye	X			
Fallopian tube				
Gall bladder				
Gross lesions				
Harderian gland	X			
Heart	*X			
Ileum	X			
Injection site				
Jejunum	X			
Kidneys	*P			
Lachrymal gland				
Larynx				
Liver	*X			
Lungs	*X			

Lymph nodes, iliac	*			
Lymph nodes mandibular	X			
Lymph nodes, mesenteric				
Mammary Gland	X			
Nasal cavity				
Optic nerves				
Ovaries	*X			
Pancreas	*X			
Parathyroid	*X			
Peripheral nerve				
Pharynx				
Pituitary	*X			
Prostate	*X			
Rectum	X			
Salivary gland	*			
Sciatic nerve				
Seminal vesicles	*X			
Skeletal muscle				
Skin	X			
Spinal cord	X			
Spleen	*X			
Sternum	X			
Stomach	X			
Testes	*X			
Thymus	*X			
Thyroid	*X			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	*X			
Vagina	X			
Zymbal gland				

X, histopathology performed
 *, organ weight obtained

Statistical analysis:

The Dunnett test was used for parametric values to assess the statistical significance of differences between the control and treatment groups.

Reviewer's comments:

The slight increase in neutrophil count in female animals at the high dose group of 2.0 mmol/kg may result from a phagocytosis mechanism as suggested by the sponsor. The lung alveolar histiocytosis was significantly higher in treated animals than in the control group, indicating again that lung is a target of gadolinium toxicity. In general, males displayed a consistent pattern of renal impairment at high dose level with increases in serum creatinine, urea nitrogen and chloride associated with observed

increased kidney weights and the histological alterations in kidneys. Males of the high dose group were most affected. However, the biochemical changes were absent at day 15 post treatment and are therefore considered to be of transient nature.

These results are in accordance with the results obtained with gadolinium compounds, which are known to cause renal vacuolation in rats treated with clinically relevant doses. The incidence and the severity of kidney alterations, which ranged from acute vacuolation to chronic renal tubular degeneration/regeneration and single cell/brush border necrosis of the proximal convoluted tubules were dose-dependent. Most of the effects appeared to be reversible at doses equal or lower than 0.12 mmol/kg as observed on Day 15 post-treatment. However, some lesions persisted at doses of 0.5 and 2.0 mmol/kg, which are 2.7, and 11 times the clinical dose. In addition, the observed collagen deposition may result in irreversible scarring at these dose levels. Because biochemical changes such as increased serum chloride levels, underlying renal impairment were reversible at doses of 0.5 mmol/kg, the sponsor established the NOAEL at this dose level.

Pharmacokinetics data showed that the mean $AUC_{(2-60min)}$ value, systemic exposure increased almost dose-linearly and that a single intravenous administration of 0.03-2 mmol/kg, resulted in a gadolinium exposure similar in both genders. These data suggest that the higher susceptibility of males compared to females observed in this study is not due to different pharmacokinetic profiles.

3.4.3 Repeat-dose toxicity

Report 215: Study title: A 2-week toxicity study of MS-325 in the Cynomolgus Monkey via intravenous administration with a 28-day recovery period.

Key study findings:

-MS-325 induced an increase in liver and kidney weights at all doses. Changes in absolute liver weights were reversible at 5.4 and 22X, after a 28 day recovery period. Although signs of recovery were noted, changes in kidneys persisted at 2 mmol/kg/day.
-MS-325 induced histological changes in several systems including cytoplasmic vacuolation of the kidney, and the reticuloendothelial cells of lymph nodes, and increased the number of foamy vacuolated alveolar macrophages. Most of the effects were reversible after the 28 day recovery period.

NOEL for MS-325 administered by intravenous injection for 2 weeks to cynomolgus monkeys was established at 0.1 mmol/kg/day (1X clinical dose)

Study no.: 95-3289
Volume #, and page #: 5, 5713
Conducting laboratory and location:

Date of study initiation: 01/22/1996
GLP compliance: Yes

b(4)

QA report:

yes () no (X)

Drug, lot #, and % purity:

MS-325-DPI (abbreviates as MS-325)
 (0.251 mmol/mL (244 mg/mL), 730
 mOsM/Kg, MS-325-DP1), 512016, no
 purity % provided (analysis was the
 sponsor's responsibility)

Dose (mmol/kg)	0.1	0.5	2.0	NOEL 0.1
Human dose multiple (based on BSA)	1	5.4	22	1

Methods

Doses: 0.1, 0.5, and 2.0 mmol/kg/day (respectively 1, 5.4, and 22 times the clinical dose) for 14 days followed by a 28 day recovery period

Species/strain: Monkey/Cynomolgus (*macaca fascicularis*)

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: Bolus intravenous, aqueous solution, 8 mL for controls, and 0.4, 2.0, and 8.0 mL (respectively for 0.1, 0.5, and 2.0 mmol/kg), 6mL/min.

Satellite groups used for recovery: 2/sex/group

Age: Young adults (sic)

Weight: Males: 1.8-2.2 kg; Females: 1.7-2.3 kg

Unique study design or methodology: None

Observation times and results

Mortality:

Observation twice daily during pretest and test periods.

No death occurred during the study.

Clinical signs:

Observations performed at 15, 30, and 45 minutes and 1, 2, 3, and 4 hours after dosing on day 1, then daily prior and after dosing during the treatment period, and once during the recovery period.

Results: Salivation was observed during and immediately following dosing in all males and 5/6 at the high-dose (2.0 mmol/kg) on the first day of dosing. The effect decreased over the course of the study until Day 14, when only 2 females salivated.

One male of the 2 mmol/kg group had swelling of its lower legs during week 1 and 2.

Body weights:

Recorded twice pretest, weekly during the treatment, and at termination.

No treatment-related effects were observed.

Food consumption:

Estimated 4 times weekly prior to treatment and 7 times weekly during the study.

No treatment-related effects were observed.

Ophthalmoscopy:

Evaluated pretest and at study termination.
No treatment-related effects were observed.

EKG:

Not conducted.

Hematology:

Blood collection was performed pretest, at day 15 and at termination.

Results: Hemoglobin, hematocrit, and red blood cell count were slightly lower at the terminal sacrifice interval.

AST increased by approximately 2 fold compared to pre-test values in males and females of the high dose group. Similarly increase in ALT was observed in the 2 high dose groups. At recovery, AST and ALT values were similar to control and pre-test values. No histological alterations were detected in the liver at this dose level.

Clinical chemistry:

Blood collection was performed pretest, at day 15 and at termination.

Urinalysis:

Performed pretest and at termination, included microscopic examination of the urine of presence of renal cells and epithelial cells. No treatment related effect was observed.

Gross pathology:

Complete macroscopic post mortem on all animals, at termination or at termination following recovery period.

Organ weights:

Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, and thyroid/parathyroid were weighed at termination.

Results: At the end of the treatment period, kidney and liver weights were increased in both males and females at the 2 mmol/kg dose level (kidney: ~40% absolute and relative to the body weight. In addition, a significant dose-dependent increase was observed in both liver and kidney weights of males. In females, the increase was observed at 0.5 and 2.0 mmol/kg. At the end of the recovery period, weights were still elevated in the 2.0 mmol/kg in kidneys, although to a lesser extent than seen at the end of the treatment period, suggesting that recovery has partially occurred. The recovery seemed complete for the liver, since there was no difference between weights in control and high dose treated animals at termination of the recovery period.

Histopathology: Adequate Battery: yes

Peer review: yes for ophthalmoscopic examination

Observation: All organs removed and preserved were analyzed histologically including blood smears following hematology analysis.

Results: Proximal convoluted tubules of the kidney showed cytoplasmic vacuolation at 0.5 and 2.0 mmol/kg in both sexes. Full recovery was achieved after 28 days for the 0.5 mmol/kg/day group, and vacuolation persisted although to a lesser degree in the higher dose group, indicating that recovery has taken place. These histological modifications were not associated with perturbation of the renal function as determined by serum chemistry and urinalysis values.

Foamy vacuolated alveolar macrophages were found in treated animals at higher incidence and severity, and were not detected in the recovery animals suggesting full recovery had taken place.

Reticuloendothelial cells including mediastinal, submandibular, mesenteric, and inguinal lymph nodes, were affected by all dose levels, as determined by the presence of cytoplasmic vacuolation: 3 males and 1 female in the lowest dose group had vacuolation in the axillary, mesenteric, and inguinal lymph nodes, whereas most of the lymph nodes were affected in males and females in the 0.5 and 2.0 mmol/kg/day groups. Full recovery was noted in the 0.1 mmol/kg, and partial in the higher dose groups.

Histopathological changes noted in the injection sites were fully reversed following the 28 day recovery period.

There was no noticeable histological finding in the lymphocytes of blood smears.

Toxicokinetics:

None

Conclusion: Based on the fact that the only finding at the low-dose was reticuloendothelial cell vacuolation in lymph nodes that was resolved by the end of the recovery period, the NOEL for MS-325 administered by intravenous injection for 2 weeks to cynomolgus monkeys was 0.1 mmol/kg/day (1 time the human dose).

Reviewer's comments:

The only remarkable clinical sign caused by MS-325 was salivation during and immediately following administration of the drug at the highest dose level, which persisted in 2 females only, until Day 14. This may be due the drug's hypertonicity. However, there were no controls for the hypertonicity in this study where animal controls received 0.9% saline.

MS-325 induced an increase in liver and kidney weights at all doses. The changes in liver weights were reversible at 0.5 and 2.0 mmol/kg, 5.4 and 22 times the clinical dose respectively, after a 28 day recovery period. Although reduced, changes in kidneys persisted at 2 mmol/kg/day. AST and ALT increase was parallel to an increase in liver weights at high dose, and both AST/ALT and absolute liver weights returned to normal values after the 28 day recovery period.

MS-325 induced histological changes in several systems including cytoplasmic vacuolation of the kidney, and the reticuloendothelial cells of lymph nodes, and increased the number of foamy vacuolated alveolar macrophages. Most of the effects were reversible after the 28 day recovery period. In addition, proximal convoluted tubules vacuolation did not result in renal function impairment, with the vacuolation being reversed for at least 5.4 times the clinical dose. Recovery was also complete in the lung with the disappearance of foamy macrophages after the recovery period even at the highest dose. The recovery of lymph nodes vacuolation was complete only at the low dose, which is ~1X the clinical dose. Therefore, this reviewer is in agreement with the sponsor establishing a NOEL at 0.1 mmol/kg/day.

Report 214: Study title: A 2-week toxicity study of MS-325 in the rat via intravenous administration with a 28-day recovery period.

Key study findings: Toxicity was described in kidney, lung and reticuloendothelial system, with a higher degree of severity and incidence in rat compared to monkey. Effects were dose-dependent and more pronounced in males. In most animals doses with 0.05 and 0.1 mmol/kg (0.27 and 0.54 times the human dose) toxicity was reversible after a 28-day recovery period. However, many alterations persisted until the end of the recovery period. No NOAEL was established.

Study no.: 95-2435
Volume #, and page #: 5, 5173
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: 02/27/1996
GLP compliance: Yes
QA report: yes () no (X)
Drug, lot #, and % purity: MS-325-DPI (abbreviates as MS-325) (0.251 mmol/mL (244 mg/mL), 730 mOsM/Kg, MS-325-DPI), 512016, no purity % provided (analysis was the sponsor's responsibility)

Dose (mmol/kg)	0.05	0.1	2.0	NOEL <0.05
Human dose multiple (based on BSA)	0.27	0.54	11	<0.27

Methods

Doses: 0.05, 0.1, and 2.0 mmol/kg/day (respectively 0.27, 0.54, and 11 times the clinical dose) for 14 days followed by a 28 day recovery period
 Species/strain: Albino Rats/Sprague-Dawley

Number/sex/group or time point (main study): 10/sex/dose
Route, formulation, volume, and infusion rate: Bolus intravenous, aqueous solution, 8 mL/kg for controls, and 0.4, 2.0, and 8.0 mL/kg (respectively for 0.05, 0.1, and 2.0 mmol/kg), 6mL/min.
Satellite groups used for recovery: 5/sex/dose
Age: 43 days
Weight: Males: 201-231g; Females: 137-172g.
Unique study design or methodology: None

Observation times and results

Mortality:

Examination twice daily: once in the morning and once in the afternoon.

Results: There were no deaths related to treatment.

Clinical signs:

Examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses.

Results: There were no remarkable clinical signs during the course of the study.

Body weights:

Twice pre-test, weekly during treatment and terminally (after fasting)

Weight increased in males at 0.05 and 0.1 mmol/kg compared to the control group, ranging from 1.2% to 6% on week 2 to week 6, whereas a decrease was observed in males at 2 mmol/kg (~8% in week 1 to 1.6% in week 6). The weight loss was considered to be drug-related by the sponsor. There were no weight changes in females.

Food consumption:

Weekly beginning one week prior to treatment.

Very slight variations were noted: not relevant toxicologically .

Ophthalmoscopy:

Pretest, at termination of treatment and at termination of the recovery period.

Focal retinopathy was observed in 2 males and 1 male of group 2 and 3 respectively.

Iritis was observed in 2 males in group 3, and one female of group 3 had intravitreal hemorrhage. All these signs were observed on week 2.

EKG:

Not conducted

Hematology:

Blood collection performed via venopuncture at Day 15 (end of treatment) and Day 43 (end of the recovery period).

Results: Hemoglobin, hematocrit, and red blood cell counts were slightly (~8%) decreased in the high-dose males at termination of the treatment, but were similar to control values at the end of the recovery period. White blood cells were increased by 25%

in the 2 high-dose groups at the end of treatment, and by 42%, 15%, and 30% in the treated groups at the end of the recovery period. Lymphocyte counts were concomitantly increased. A 42%, 38%, and 55 % increase in platelets was observed in males treated with respectively 0.05, 0.1, and 2 mmol/kg/day at the end of the treatment. At the end of the recovery period, there was still a 23% increase at the highest dose compared to the control group. Hematological changes did not occur in females. Total iron-binding capacity elevated in mid and high dose males and in high dose females at termination, and remained elevated at the end of recovery period. However, these changes were not considered toxicologically significant.

Comments: Drug-related changes (within 50 %) were mainly observed in the 2 highest doses (0.54X and 11X the clinical dose); although they may be seen as mild changes, they did not fully disappear at the end of the recovery period.

Clinical chemistry:

Performed on plasma samples collected at Day 15 (end of treatment) and Day 43 (end of the recovery period).

Results: Up to 35% decrease in mean ALT was observed in males and females at 2 mmol/kg, at termination. The decrease persisted in the females at the end of recovery period, which was then accompanied with a decrease in the AST.

BUN was slightly increased at termination in both males and females given 2 mmol/kg/day, and in females of the 0.1 mmol/kg group. At the end of recovery, bun values were similar throughout control and treated groups. Only in the high-dose group males at termination, was the creatinine slightly increased.

Urinalysis:

Gross pathology:

Pale kidneys were observed in high dose animals (10 males and 4 females) at Day 14, and in 3/5 males at the end of the 28-day recovery period. Kidney dilatation was noted in 1 and 2 males given respectively 0.1 and 2 mmol/kg/day, at termination; it was also found in one male control. Uterus was distended in one high dose female and scabs/sores were observed in the tail of 2 high dose females. After the 28 day recovery period one female had tail necrosis.

Organ weights:

Kidneys, testes with epididymides, liver, ovaries weights were determined at termination and recovery.

Results: Kidney weights were elevated in both high-dose males and females at termination, and remained elevated, although to a lesser degree, in males at the end of recovery. Weight recovery was complete in females.

Histopathology: Adequate Battery: yes

Peer review: no

Renal findings: In general, males were more affected than females, and the severity of the effect on renal histopathology was dose-dependent. Cytoplasmic vacuolation of the

proximal convoluted was observed at all doses with a dose-dependent severity. In the distal/collecting tubules, the cytoplasmic vacuolation was limited to high dose groups with males being more severely affected than females. Tubular dilatation/cell debris with peritubular fibrosis was detected in high kidneys of all high-dose males, whereas the fibrosis was found at all doses and in a dose-dependent manner. Ten out of 10 males of the 2 mmol/kg group, 3/10 control group, and 2/10 low-dose group animals had tubular proteinaceous casts. Females were less affected (1 female in low-dose and 1 female in high-dose). Tubular degeneration/atrophy, which occurred at all doses, was found to be dose-dependent, and was also noted in the control group at both termination and recovery. These changes persisted to a lesser degree in mid- and high dose group animals after the recovery period. Although observed in most cases, the recovery at 28 days was generally incomplete. Overall, recovery was more pronounced in females, where a lower incidence of milder histopathological alterations compared to males, persisted after the recovery period.

Alveolar macrophages: foamy vacuolated alveolar macrophage accumulation was mild to moderately severe in both males and females treated with 2 mmol/kg/day. Females fully recovered after the 28-day period. This was not the case for all animals in the high dose group, and for one animal in the mid-dose group where a slight cytoplasmic vacuolation of foamy alveolar macrophages was still visible at the end of the recovery period.

Reticuloendothelial system (RES): RES of the spleen, the lymph nodes, the liver, the interstitial tissue of testis, epididymis, prostate, pituitary, and adrenal medulla exhibited cytoplasmic vacuolation at doses of 2 mmol/kg. The low and mid dose tissues will be examined and results will be submitted by the sponsor in a report amendment. The severity of this histopathological finding depended on the tissue and on the dose.

Urinary bladder: vacuolation was also observed in the urinary bladder of all high dose animals. No effect was noted at the low dose and mid dose. After the recovery period all animals had larger vesicle present in the surface epithelium, with the small vacuoles that had seemingly coalesced into a single large vesicle.

Injection site: there was an increase in the incidence and severity of hemorrhage and chronic inflammation at the injection sites of high dose animals at termination. At the end of the recovery period, the injection site was normal in all animals.

Toxicokinetics:

None conducted

Other:

None

Conclusions: Based on the fact that the only finding at the low-dose was mild reversible vacuolation of renal proximal tubule epithelial cells, the NOEL for MS-325 administered by intravenous injection for 2 weeks to rats was established by the sponsor at 0.05 mmol/kg/day, 0.3X the intended clinical dose.

Reviewer's comments: The dose selection was not adequate: 0.05, 0.1, and 2.0 mmol/kg/day (respectively 0.27, 0.54, and 11 times the clinical dose), and did not include

the human dose equivalent. The interval between dose multiples, 0.54 times to 11 times the clinical dose, does not provide an appropriate dose range.

Vacuolation was the most significant finding of this study. It was dose-dependent and more pronounced in males compared to females. In most low and mid dose group animals, toxicity was reversible after a 28-day recovery period. The type of toxicity detected is somewhat expected with gadolinium compounds. Lung and reticuloendothelial cell system were affected by the treatment at all dose levels. Because many of the histopathological, hematological, and chemical alterations were not completely reversible even at low doses equivalent to 0.27 times the clinical dose, it was not possible to determine a NOEL. Toxicity to the kidney, which is a signature of these compounds, needs to be further characterized at the functional level.

3.4.4. Genetic toxicology

Study 17419-0-409R: Mutagenicity test on MS-325-DP1 in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay.

Study objective: The purpose of this study is to evaluate MS-325-DP1 for its ability to induce reverse mutations at the histidine locus in the genome of specific *Salmonella typhimurium* tester strains, and at the tryptophan locus in an *Escherichia coli* tester strain both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes.

Key findings: Under the conditions of this study, MS-325-DP1, did not show a significant increase or a dose related response in the number of histidine revertant and tryptophan revertant colonies when compared to the negative control in either the activated or the non-activated assay.

Study no.:	17419-0-409R	
Volume #, and page #:	5, 7924	
Study type:	Genotoxicity study	
Conducting laboratory and location:	<input type="checkbox"/>	b(4)
Date of study initiation:	January 29, 1996	
GLP compliance:	Yes	
QA reports:	Yes (X) no ()	
Drug, lot #, Radiolabel, and % purity:	MS-325-DP1 (250 mM, 730 mOsM/kg), 512016	
Formulation/vehicle:	Normal saline used as vehicle and diluent for test article.	

Methods:

Plate incorporation and pre-incubation methods.

Strains/species/cell line: *Salmonella typhimurium* tester strains, TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2uvrA.

Metabolic activation: microsomal enzymes prepared from AroclorTM-induced male Sprague Dawley rat liver (S9).

Doses used in definitive study: The mutagenicity test was performed using 5 concentrations of test article, 5000, 3330, 1000, 333, 100 µg per plate, in presence and in absence of S9mix fraction, using three plates per concentration.

Basis of dose selection:

The initial mutagenicity assay was performed on TA100 and WP2uvrA, using 5000, 3330, 1000, 667, 333, 100, 66.7, 33.3, 10, and 6.67 µg per plate, in presence and in absence of S9mix fraction, using three plates per concentration. The maximum dose of 5000 µg is equivalent to approximately 0.19 mM, about 0.5 times the peak plasma concentration of 0.43 mM anticipated for a clinical dose of 0.03 mmol/kg. Cytotoxicity was assessed by examining bacterial lawn density and number of spontaneous revertants per plate. Since no cytotoxicity was observed the highest dose used in the confirmatory assay was the same as that used in the initial assay.

Negative controls: Vehicle control consisted of deionized water.

Positive controls: Positive controls are listed in the table below:

Table Positive controls

Tester strain	S9 mix	Positive control	Conc/plate (µg)
TA98	+	Benzo(a)pyrene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	-	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	-	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	-	ICR-191	2.0
WP2uvrA	+	2-aminoanthracene	25.0
WP2uvrA	-	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: The tester strains were exposed to the test article, via the plate incorporation methodology, for 48 ± 8 hr at 37 ± 2°C. Following this incubation, revertant colonies were counted. For the article, the vehicle control, and the positive controls, 3 plates per dose level were tested in the presence or absence of metabolic activation.

Results:

Study validity: Selection of bacterial tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). Positive controls produced expected responses. Dose selection for the plate incorporation method was adequate based upon use of the limit dose level (i.e., 5000 µg/plate). A minimum of 3 non-toxic concentrations was required to evaluate assay data.

Study outcome: No cytotoxicity was observed in the dose range finding assay in either the presence or the absence of S9mix. In addition, a confirmatory assay was performed for the other strains. In both assays, no positive increases in the number of revertants were observed with any of the tester strains with or without activation, except for the strain TA1535, where there was a 2 fold increase in the mean revertants per plate at a dose of 3330 µg. This was an isolated event.

It was concluded that under the conditions of this study, test article MS-325-DP1 did not cause a positive increase in the number of revertants per plate with any of the tester strains either in the presence or in the absence of microsomal enzymes prepared from AroclorTM-induced rat liver (S9).

Reviewer's comments: Agree with the conclusion. However, vehicle control consisted of a saline solution, providing an isotonic solution, when the test article had an osmolality of approximately 720 mOsm/kg. Study #19423-0-409OECD and 194430-0-409OECD addressed this issue.

Study 19423-0-409OECD, and 194430-0-409OECD: Mutagenicity test with MS-325-DP1 and MS-325-DP2 in the salmonella-escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay.

Study objective: This assay evaluated the test articles and/or their metabolites for their ability to induce reverse mutations at the histidine locus in the genome of specific *Salmonella typhimurium* tester strains and at the tryptophan locus in an *Escherichia coli* tester strain both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from AroclorTM-induced rat liver (S9).

Key findings: Under the conditions of this study, MS-325-DP1 and MS-325-DP2, did not cause a positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from AroclorTM-induced rat liver (S9).

Volume #, and page #: 5, 8027

Conducting laboratory and location: J

Date of study initiation: 04/17/1998

b(4)

GLP compliance: Yes
QA reports: yes (X) no ()
Drug, lot #, and % purity: MS-325-DP1, Epix 610007, Not provided
 MS-325-DP2, Mallinckrodt 2749P028, Not provided
 Vehicle control, E800139 (410 mM NaCl, 240 mg/ml MS-32516 provided by Epix)

Methods

Plate incorporation and pre-incubation methods.

Strains/species/cell line: *Salmonella typhimurium* tester strains, TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2uvrA.

Doses used in definitive study: The mutagenicity test was performed using six concentrations of each test article 5000, 3330, 1000, 333, 100 and 33.3 µg per plate, in presence and in absence of S9mix fraction, using three plates per concentration.

Basis of dose selection:

The initial mutagenicity assay was performed using five concentrations of each test article: 5000, 3330, 1000, 333, and 100 µg per plate, in presence and in absence of S9mix fraction, using three plates per concentration. The maximum dose of 5000 µg is equivalent to approximately 0.19 mM, about 0.5 times the peak plasma concentration of 0.43 mM anticipated for a clinical dose of 0.03 mmol/kg. Cytotoxicity was assessed by examining bacterial lawn density and numbers of spontaneous revertants per plate. Due to the cytotoxicity observed at initial doses for tester strain TA100 strains the confirmatory assay included a lower dose of 33.3 µg per plate.

Negative controls: Vehicle control consisted of a mixture of 410 mM NaCl and 240 µg/mL MS-32516, providing a hypertonic solution of 720 mOsm/kg.

Positive controls: Positive controls are listed in the table below:

Table Positive controls

Tester strain	S9 mix	Positive control	Conc/plate (µg)
TA98	+	Benzo(a)pyrene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	-	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	-	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	-	ICR-191	2.0
WP2uvrA	+	2-aminoanthracene	25.0
WP2uvrA	-	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: The tester strains were exposed to the test article, via the plate incorporation methodology, for 52 ± 4 hr at $37 \pm 2^\circ\text{C}$. Following this incubation, revertant colonies were counted. For each article, vehicle controls, and positive controls, 3 plates per dose level were tested in the presence or absence of metabolic activation.

Results

Study validity: Selection of bacterial tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). Positive controls produced expected responses. Dose selection for the plate incorporation method was adequate based upon use of the limit dose level (i.e., 5000 $\mu\text{g}/\text{plate}$).

Study outcome: Cytotoxicity in the absence of S9mix was detectable for the 3 highest concentrations tested for TA 100 tester strain as a decrease in the number of revertant colonies per plate compared to the vehicle control. The assay was repeated for this strain and included an additional low dose of 33.3 μg per plate. In the second assay, there were at least 3 non-toxic concentrations. Hence, the criteria for the test validity were met. No positive increases in the number of revertants were observed with any of the tester strains with or without activation.

It was concluded that under the conditions of this study, test article MS-325-DP1 and MS-325-DP2 did not cause a positive increase in the number of revertants per plate with any of the tester strains either in the presence or in the absence of microsomal enzymes prepared from AroclorTM-induced rat liver (S9)

Reviewer's comments: Agree with the conclusion.

This study is somewhat a bridging study between MS-325-DP1 and MS-325-DP2, both of which have been used in clinical studies. Initial genotoxicity tests had been conducted for DP1 compound. A new method of synthesis was later used to produce MS-325-DP2, which was used in the clinical trials. Vehicle control contains the ligand as well, indicating that the ligand is not mutagenic.

Study 19423, 19443-0-437OECD: Mutagenicity test on MS-325-DP1 and MS-325-DP2 measuring chromosomal aberrations in Chinese hamster ovary cells.

Study objective: The objective of this in vitro assay was to evaluate the ability of MS-325-DP1 and MS-325-DP2 to induce chromosomal aberrations in Chinese hamster ovary cells with and without metabolic activation.

Key findings: Under the conditions of this study, MS-325-DP1 and MS-325-DP2 were considered negative for inducing chromosome aberrations in CHO cells with and without metabolic activation

Volume #, and page #: 5, 8079

Conducting laboratory and location: b(4)

Date of study initiation: 04/15/1998

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: MS-325-DP1, Epix 610007, Not provided
MS-325-DP2, Mallinckrodt 2749P028, Not provided
Vehicle control, E800139 (410 mM NaCl, 240 mg/ml MS-32516 provided by Epix)

Method: Cell cultures were exposed to both test substances, with and without metabolic activation. At 3 and 17.8 hours after exposure of cell cultures to the test substance, they were treated with a metaphase-arresting substance, Colcemid, harvested, stained and metaphase cells are analyzed microscopically for the presence of chromosomal aberrations (polyploidy or endoreduplication).

Experimental design: CHO cell cultures, 1.2×10^6 / 10mL, were treated in duplicate at each concentration level, including the appropriate controls for 3 hrs and 17.8 hrs, in the presence or absence of S9 fraction, for the initial and the confirmatory assay, respectively. Colcemid at 0.1 μ g/ml was added 2 hours before cell harvest. Toxicity was visually evaluated by the confluence of the cell monolayers, the presence of mitotic cells, and the dead cells floating in the medium. After Giemsa staining, cells were evaluated for mitotic index and chromosomal aberrations. Chromosomal aberrations (polyploidy or endoreduplication) were assessed. Numerical aberrations, i.e. change in the number of chromosomes, were not determined.

Doses selection: Concentrations of 500, 998, 1990, 2990, 4000, and 5000 μ g/ml were tested for MS-325-DP1. Concentrations of 500, 998, 1990, 2990, 3980, and 4960 μ g/ml were tested for MS-325-DP2.

Controls: Negative controls contained only cells and culture medium, and solvent controls contained the solvent for the test article, at the highest dilution volume of 20.5 μ l/mL. For the non activation series, mitomycin C (MMC) was used as a positive control at 0.75 and 1.5 μ g/mL in the initial assay, and at 0.08 and 0.10 μ g/mL in the confirmatory assay. In the metabolic activation series, cyclophosphamide (CP) was used as positive control at 5.0 and 10.0 μ g/mL. One of the concentrations was used in each of the aberration assays.

Metabolic activation: S-9 fraction was prepared from livers obtained from Aroclor 1254 pretreated male Sprague-Dawley rats. The S-9 percentage was 1.5%, which is within the acceptable range. According to the sponsor, this concentration caused CP to be highly clastogenic for many different lots.

Validity: A test article was considered positive if a significant increase in the number of cells with chromosomal aberrations was observed at one or more concentrations. In addition, a concentration-related response should be observed. A test article was considered negative if no significant increase was observed at any of the concentrations. Equivocal evaluation was left to the consideration of the study director.

Results: Only cells with 21 ± 2 centromeres were analyzed. Whenever possible, 100 cells from each replicate at 4 concentrations, and at least 25 cells were analyzed for cultures that had greater than 25% of cells with one or more aberrations. Mitotic index and percentage of polyploidy and endoreduplication were determined by evaluating 100 metaphases when available. Chromatid and isochromatid breaks were not considered true aberrations and were not included in the calculations.

In the 3 hr incubation series:

- For MS-325-DP1, without metabolic activation, reductions of 17%, 27%, 20%, and 13% in the mitotic index were observed for MS-325-DP1 at doses of 998, 1990, 2990, and 5000 $\mu\text{g/mL}$ respectively. With metabolic activation reductions of 22%, 27%, and 11% were observed at 1990, 2990, and 4000 $\mu\text{g/mL}$ respectively.
- For MS-325-DP2 incubated without metabolic activation, reductions of 7%, 35%, 14%, and 25% were observed at doses of 998, 1990, 3980, and 4960 $\mu\text{g/mL}$ respectively. With metabolic activation, a 15% reduction in confluence and a slight decrease in the mitotic index were observed at 4960 $\mu\text{g/mL}$. Also, confluence was slightly reduced in cultures treated with 2990 and 3980 $\mu\text{g/mL}$, with a 14% reduction in the MI for 2990 $\mu\text{g/mL}$.
- No significant increase in the chromosomal aberrations was observed at the concentrations analyzed with or without activation.

In the 17 hr incubation series:

- For MS-325-DP1, in the absence of metabolic activation, reductions of 9%, 2%, and 25% were observed at doses of 1990, 2990, and 5000 $\mu\text{g/mL}$ respectively. In the presence of metabolic activation, the mitotic indices were reduced by 21%, 17%, and 13% at doses of 1990, 4000, and 5000 $\mu\text{g/mL}$ respectively.
- For MS-325-DP2, without activation, reductions of 11%, 5%, 11%, and 9% were observed at doses of 1990, 2990, 3980, and 4960 $\mu\text{g/mL}$ respectively. In the presence of metabolic activation, reductions in the mitotic index of 8, 17, and 31% were observed at doses of 1990, 2990, 3980 $\mu\text{g/mL}$ respectively.
- No significant increase in the chromosomal aberrations was observed at the concentrations analyzed with or without activation.

The sponsor concluded that MS-325-DP1 and MS-325-DP2 were considered negative for inducing chromosome aberrations in CHO cells with and without activation.

No cytotoxicity was observed for either MS-325-DP1 or MS-325-DP2, with and without metabolic activation. A higher percent of dicentric was observed following 3hr treatment with MS-325-DP1 in the absence of metabolic activation. This effect was observed for all

concentration levels and did not seem to be dependent on the slight toxicity that was observed at all doses.

Reviewer's comments: Negative and vehicle controls contained less than 5% cells with aberrations. The positive controls were significantly higher (~40% in average) than the vehicle controls. The assays included at least 3 analyzable concentrations. Thus, the study was overall valid. However, cyclophosphamide was not tested in the absence of S9 to demonstrate its S9-dependence in the cell line being used in that laboratory.

A slight increase in the % cells with aberrations, which was not concentration-dependent, was observed with MS-325-DP1. The increase was found mostly in the dicentric aberrations. However, this effect was not observed in the 17hr incubation series.

The maximum dose tested was 5000 µg/mL, equivalent to approximately 5.12 mM, which is more than 10 times the anticipated peak plasma concentration of 0.43 mM achievable at the intended clinical dose of 0.03 mmol/kg.

In conclusion, the slight increase was not dose-dependent, and was only seen at 3 hr incubation. Moreover, the dose at which the effect is observed could not theoretically be achieved at intended clinical dose. Therefore, MS-325-DP1, and MS-325-DP2 do not seem to induce chromosomal aberrations in the conditions of the assay.

Study 202: Mutagenicity test on MS-325-DP1 in an in vivo mouse micronucleus assay

Objectives: To evaluate the clastogenic potential of the MS-325-DP1 as measured by its ability to induce micronucleated polychromatic erythrocytes in mouse bone marrow.

Key findings: MS-325-DP1 did not induce a significant increase in micronucleated polychromatic in either male or female mice.

Study no: 17419-0-455CO
Study type: Genotoxicity study
Conducting laboratory and location: T

b(4)

Date of study initiation: January 29, 1996
GLP compliance: Yes
QA reports: Yes (X) no ()
Drug, lot #, Radiolabel, and % purity: MS-325-DP1 (250 mM, 730 mOsM/kg), 512016
Formulation/vehicle: Normal saline used as vehicle and diluent for test article.

Methods:

Strains/species/cell line: ICR/Mice

Dose selection criteria:

Basis of dose selection: a range finding study was performed.

Range finding studies: Test article was administered by intravenous injection at 1200, 1500, 1800, 2100, 2400, 2880, 3600, and 5040 mg/kg, and in a repeat assay at 4000 and 4500 mg/kg. Six animals (3 males and 3 females) were assigned to each group dose, except the 5040 mg/kg group, which had 2 males and 2 females. Animals were observed for 3 days after dosing for toxic signs and/or mortality. The high dose was selected at 4000 mg/kg, as 80% of the MTD.

Controls:

Vehicle: 0.9% saline

Negative controls: 0.9% saline, IV bolus at equal volume of high dose (16.67 ml/kg).

Positive controls: Cyclophosphamide, 80 mg/kg, in a 10ml/kg ad administered as an oral gavage.

Comments: valid controls for the study.

Exposure conditions:

Incubation and sampling times: Bone marrow cells were collected at 24, 48, and 72 hr for all doses, and for vehicle control group. Positive control groups, euthanized approximately 24 hrs after dosing, were included in the assay.

Doses used in definitive study: 1000, 2000, and 4000 mg/kg.

Study design: mice were administered a single intravenous bolus injection of the test article at volumes of 4.17, 8.33, and 16.67 ml/kg respectively. Body weights were noted before treatment to determine the dose volume. Animals were observed for clinical signs following injection.

Analysis:

No. of replicates: two slides were prepared from each mouse.

Counting method: One thousand polychromatic erythrocytes were scored for the presence of micronuclei, which are defined as round, darkly staining nuclear fragments with sharp contour and diameters usually from 1/20 to 1/5 of the erythrocyte. Almond and ring-shaped micronuclei occasionally occurred. The proportion of polychromatic erythrocytes to total erythrocytes was also recorded per 1000 erythrocytes.

Criteria for positive results: a response was considered positive if a statistically significant dose-related increase in micronucleated PCEs was detected, or a reproducible and statistically significant positive response was detected for at least one dose level. A test article that induced neither a statistically dose-response, nor a statistically significant and reproducible increase at one dose level was considered negative. In either case, the final decision was based on scientific judgment. Data analysis was performed using analysis of variance (Winer, 1971). If the analysis of variance was significant ($p < 0.05$), a Dunnett's t-test was used to determine which dose groups were significantly different from the negative control.

Summary of individual study findings:

Study validity: Adequate dosing, species and number of animal/sex/group acceptable, analysis adequate, positive controls exhibited expected response.

There was no hypertonic control, and the injected volume was not the same for all doses.

Study outcome: All animals in the 1000 and 2000 mg/kg dose groups, and in the vehicle control group appeared normal immediately after dosing and remained healthy until harvest time.

In the 4000mg/kg group, immediately after dosing, one female and 3 males were found dead, whereas all other animals were hypoactive but recovered within 5 minutes of treatment; one male died at 19 hrs, 1 female at 47hrs and one female at 66 hrs post-dosing. At 43 hrs, 1 female appeared hypoactive, lethargic, with lacrymation and squinting.

The ratio of polychromatic erythrocytes to all red blood cells was significantly but slightly reduced in the male mice from the 1000 and 2000 mg/kg and 48 hour harvest groups. This reduction was ~35% of the control value at 1000 mg/kg and at 2000 mg/kg. According to CFSAN Redbook 2000, OECD Guideline, the proportion of immature erythrocytes among total erythrocytes should not be less than 20% of the control value. However, the relevance of this non-dependent reduction may be indicative of direct bone marrow toxicity.

The number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in test article treated groups was not statistically increased relative to the vehicle controls in either male or female mice, regardless of dose level or bone marrow collection time whereas cyclophosphamide induced a significant increase.

Genetic toxicology summary:

The sponsor concluded that MS-325-DP1 did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative.

Reviewer's comments:

A few deficiencies in the study design were identified and are listed under paragraph "study validity". At 48 hrs, there was a 1.8 fold increase in the number of micronuclei at 1000 mg/kg, while the increase was 2.3 and 2 fold for 1000 and 2000 mg/kg respectively at 72 hrs. Although the sponsor found them to be statistically insignificant, some changes occurred outside the range of historical controls. However, in the absence of adequate controls mimicking hypertonic conditions of the test article, these data are overall equivocal. This issue is addressed in Study # 19423, 19443-0-455OECD.

Study 19423, 19443-0-455OECD: Mutagenicity test on MS-325-DP1 and MS-325-DP2 in the in vivo mouse micronucleus assay.

Objectives: To evaluate the clastogenic potential of the test article as measured by its

ability to induce micronucleated polychromatic erythrocytes (PCE) in mouse bone marrow .

Key findings: The test articles MS-325-DP1 and MS-325-DP2 induced signs of clinical toxicity in the treated animals but were not cytotoxic to the bone marrow. They did not induce a statistically significant increase in micronuclei in bone marrow PCEs and were considered negative in the mouse bone marrow micronucleus test under the conditions of exposure of this assay.

Study no:	19423, 19443-0-455OECD
Study type:	Genotoxicity study
Volume #, and page #:	5, 8121
Conducting laboratory and location:	✓
Date of study initiation:	04/20/1998
GLP compliance:	Yes
QA reports:	yes (X) no ()
Drug, lot #, and % purity:	MS-325-DP1, Epix 610007, Not provided MS-325-DP2, Mallinckrodt 2749P028, Not provided Vehicle control, E800139 (410 mM NaCl, 240 µg/ml MS-32516 provided by Epix)

b(4)

Methods:

Strains/species/cell line: CD-1[®](ICR) BR/Male mice

Dose selection criteria:

Mice (n=47) were assigned to groups of 6 animals/dose level/harvest point, with one additional animal for the dose/harvest point of 4001.6 mg/kg/48hr and one for the vehicle control at 48 hr harvest point, to replace animals that died during or right after dosing. The animals received an IV injection of 1000.4, 2000.8, and 4001.6 mg/kg of MS-325-DP1 and 992.2, 1984.4, and 3968.8 mg/kg for MS-325-DP2. The top dose is 80 times greater than the clinical dose.

All animals in the vehicle and the positive control groups appeared normal after dosing and remained healthy until the appropriate harvest time points, except for the animal that died during dosing. At low and mid-dose levels, no abnormalities were noted in the animals treated with either test article.

All animals treated with highest doses of either test article were slightly hypoactive immediately after test article injection, and one of each group remains so at 24 hrs. Three mice died immediately after administration of 4001.6 mg/kg of MS-325-DP1. One animal in each group exhibited pale body 1 day after dosing. In the MS-325-DP1 group, convulsions were observed in one mouse immediately following dosing.

Controls:

Vehicle: MS-325 containing 410 mM NaCl, and 240 µg/ml MS-32516, to mimic osmolality of test article.

b(4)

Negative controls: none

Positive controls: Cyclophosphamide (CAS # 6055-19-2), 80 mg/kg. Solubilized in sterile deionized water.

Comments: valid controls for the study; vehicle controls and positive controls were the same for both test articles.

Exposure conditions:

Incubation and sampling times: Bone marrow cells were collected at 24 hr for all doses. An additional collection was performed at 48 hr for the vehicle and the highest dose.

Doses used in definitive study: 1000.4, 2000.8, and 4001.6 mg/kg for MS-325-DP1, and 992.2, 1984.4, and 3968.8 mg/kg for MS-325-DP2.

Study design: mice were administered a single intravenous injection of the test article at a volume of 4.1, 8.2, and 16.4 ml/kg for low, mid and high doses respectively. Body weights were noted before treatment to determine the dose volume. Animals were observed for clinical signs following injection.

Analysis:

No. of replicates: 1 slide was prepared from each mouse.

Counting method: Two thousand polychromatic erythrocytes were scored for the presence of micronuclei, which are defined as round, darkly staining nuclear fragments with sharp contour and diameters usually from 1/20 to 1/5 of the erythrocyte. Almond and ring-shaped micronuclei occasionally occurred. The number of micronucleated normochromatic erythrocytes in the field of 2000 polychromatic erythrocytes was enumerated for each animal. The proportion of polychromatic erythrocytes to normochromatic erythrocytes was also recorded.

Criteria for positive results: a response was considered positive if a statistically significant increase in micronucleated polychromatic erythrocytes was observed for at least one dose level, with a statistically significant dose-related response. Additionally, the biological relevance of the results was evaluated by the study director.

Summary of individual study findings:

Study validity: Adequate dosing, species and number of animal/group acceptable; no female was included in the assay. Only one slide was prepared per animal, although replicates are usually prepared. Positive controls exhibited expected response.

Study outcome: Clinical signs including hypoactivity and convulsions were observed at high doses only, and 3 animals died in the MS-325-DP1 highest dose group. The number of micronucleated polychromatic erythrocytes per 10000 polychromatic erythrocytes in test article treated groups was not statistically increased relative to the vehicle controls with either test articles, regardless of dose level or bone marrow collection time whereas cyclophosphamide induced a significant increase.

Genetic toxicology summary:

Under the conditions described, the sponsor concluded that neither MS-325-DP1 nor MS-325-DP2 exhibited a clastogenic activity as measured by the micronucleus test.

Reviewer's comments: Agree with sponsor's conclusion.

The number of deaths and signs of toxicity were significantly more marked with test article DP1. The injection volume was not the same for all dose levels, thus, the osmolality reached in the test article was not the same. The sponsor did not provide evidence that the difference in osmolality did not affect the outcome of the assay. Slides were not prepared in replicates and no female was studied.

In conclusion, the objective of this study was to evaluate the clastogenic potential of MS-325-DP1 and MS-325-DP2, which did not exhibit clastogenic potential at doses tested under the conditions of the assay. It is of note that the results of the toxicity evaluation suggest different toxicity profiles of 2 compounds.

Reviewer's additional comments:

The commercial formulation contained [redacted] with specifications set at [redacted]. However, the formulation used in mutagenicity studies did not contain [redacted]. For a clinical proposed dose of 0.03 mmol/kg, the anticipated plasma level is 0.43 mM, equivalent to 0.420 mg/mL of Vasovist. The specification of [redacted] means that a maximum concentration of [redacted] could be reached, following Vasovist administration. This plasma concentration is approximately [redacted] fold higher than normal [redacted]. In terms of mutagenicity, it was shown that, at concentrations ranging from [redacted] [redacted] was not mutagenic in the Ames test (IARC 1987), and did not induce sister chromatid exchange (SCE) in Chinese hamster V79 cells at a range of concentration [redacted]. Moreover, in vivo bone marrow cytogenetic tests did not produce significant increases in the proportion of cells with chromosome damage, hence, did not demonstrate clastogenic potential [redacted]. Therefore, the results of genetic toxicology are acceptable even if the test article did not contain [redacted].

b(4)

3.4.5. Carcinogenicity

Not conducted

3.4.6. Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Study of fertility and early embryonic development to implantation of Sprague-Dawley rats with ZK236018 (SH L03588A; Epix Medical, MS-325) after daily intravenous administration to the animals of the F0-generation.

Study no.: A10565
Volume #, and page #: 5,8155
Conducting laboratory and location: T

Date of study initiation: 09/13/2001
GLP compliance: Yes and ICH guideline 4.1.1 compliance.
QA reports: yes (X) no ()
Drug, lot #, and % purity: Gadofosveset trisodium, b3095p39, 99.3%.

b(4)

Background:

The dose levels were selected based on a previous 28-day subchronic toxicity study in Wistar rats (Report A10568). In that study, no effects were observed on clinical signs, clinical chemistry, and food consumption, at 0.03, 0.21 and 1.5 mmol/kg/day (0.16, 1, and 8 times the human dose based on BSA). Body weight was decreased, and kidney weights increased in the high dose group. In the latter group, one animal died. Renal tubular vacuolation was observed in all groups, in a dose-dependent fashion.

Key study findings:

- Two males and two females treated with 1.5 mmol/kg/day died prematurely during mating or the gestation period. The deaths were considered to be test substance-related.
- Pale kidneys were observed in all males and 9 out of 20 females of 1.5 mmol/kg/day group with enlarged kidneys found in one dam.
- Increased kidney weights were noted at 0.21 and 1.5 mmol/kg/d. Increased absolute and relative epididymis weights were noted at 1.5 mmol/kg/d, with a 22% decrease in the number of spermatids per gram of testicular tissue, and a relative testicle weight increase.
- Minimal to moderate number of foamy, vacuolated macrophage cells were present in the epididymal and testicular interstitial connective tissue in all males at 1.5 mmol/kg/d.
- Fertility indices and embryonic development were not affected by doses of up to 1.5 mmol/kg.
- At 0.21mmol/kg/day the only finding was the increase in kidney weights, therefore NOEL could be established at 0.21 mmol/kg/day, 1X the human dose.

Methods

Doses: 0.03, 0.21, and 1.5 mmol/kg/d, respectively 0.16X, 1X, and 8X the clinical dose based on body surface area. A control group received 0.9% saline.

Species/strain: Rat/Sprague-Dawley

Number/sex/group: 20/sex/dose

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

Satellite groups used for toxicokinetics: None

Study design: Male rats were treated daily for 4 weeks prior to mating and during the mating period. Female rats were treated for 2 weeks prior to mating, during mating through gestation day 7. Pairings were on a 1 male to 1 female basis for each group. Dams were laparotomized on the 13th day of gestation and signs of fertility were assessed.

Parameters and endpoints evaluated: mortality, clinical signs, body weight, food consumption, gross pathology of adults, reproductive organs and females kidney weights and histopathology. Evaluation included fertility, the number of corpora lutea, and implantation sites, live and dead conceptuses, sperm count in the epididymis and sperm viability as well as the organ weights of the reproductive organs. Dunnett's and the Student t-test as well as the Fisher test were used, if applicable, for statistical analysis of intergroup differences of mean values.

Dose (mmol/kg)	0.03	0.21	1.5	NOEL 0.21
Human dose multiple (based on BSA)	0.16	1	8	1

Results

Mortality: Five animals died. One female of the control group died on day 3 of gestation. Two males and two females of the 1.5 mmol/kg/day group died, the males on day 6 and day 9 of mating, and the females on day 3 of gestation, and day 6 of mating. The macroscopic evaluation revealed pale kidneys in one male, enlarged kidney in the other male, and no pathological findings in the deceased female animals.

Clinical signs: No remarkable effects.

Body weight: There were a few statistically significant differences from the control animals, observed for the males at mid-dose, and for the females at low-dose. Determination of carcass weight revealed an increase at the low dose only. However, no dose-dependency could be established.

Food consumption: A few statistically significant differences, within ~10% ranges, were observed in treated animals when compared to the control animals. However, these changes were transient, and no clear dose-dependence could be established.

Toxicokinetics: Not performed.

Necropsy: No effect was observed at 0.03 and 0.21 mmol/kg/day. At 1.5 mmol/kg/day, necropsy revealed pale kidneys in all surviving males, and in 9/20 females.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

1. Spermatid number and sperm viability.

The number of spermatids per gram testicular tissue was decreased by 22% in the males treated with 1.5 mmol/kg/d. Data were outside the background range of controls, therefore, the effect was attributed to the drug substance. No changes were noted in the percentage of motile spermatozoa in the epididymal cauda at any dose level.

b(4)

2. Organ weights

-Absolute and relative increase in the left and right epididymides weights, and in the left and right testicle weights, were noted in males treated with 1.5 mmol/kg/d. A slight

increase of the right absolute but not relative epididymis weight was observed at 0.21 mmol/kg/d.

-A statistically significant increase was observed in the absolute and relative kidney weights of females treated with 0.21 and 1.5 mmol/kg/d.

3. Evaluation of the estrous cycle.

No treatment related findings at any dose.

4. Histopathology

There was an increase in the number of foamy vacuolated macrophages, minimal to moderate in degree, in the epididymides, and an increase in the number of foamy macrophages, minimal to slight in degree, in the testicular interstitium of rats treated with 1.5 mmol/kg/d.

5. Reproduction data of the dams at laparotomy

-The fertility index was 100% in all groups for males. In females, the fertility index was 94.7, 100, 95, and 88.9%, in controls and in animals treated with 0.03, 0.21, and 1.5 mmol/kg/d respectively. In the high dose group, 2 dams had died prematurely and were excluded from the calculation. Hence, the fertility indices were not affected by the treatment.

-The number of corpora lutea and implantation sites was not affected by treatment.

-Reproduction parameters evaluation: there was a significant increase in the pre-implantation loss (mean %) in the 0.21 mmol/kg group, but this effect was not dose-dependent.

Summary of individual study findings:

-One control animal, and 2 males and 2 females treated with 1.5 mmol/kg/d died.

-Treatment related increase of kidney weights starting at 0.21 mmol/kg, accompanied by macroscopic effects at 1.5 mmol/kg/d, in females.

-Treatment related increase of epididymides absolute and relative weights, and of testicle relative weights, and decrease of spermatid number per gram testicular tissue at 1.5 mmol/kg.

-Treatment related presence of foamy macrophages in the epididymal and testicular interstitial connective tissue of all males treated with 1.5 mmol/kg/day.

-No effect on fertility indices and embryonic development at any of the tested dose levels.

Conclusion: Most of the effects were observed at 1.5 mmol/kg/day (8X the human dose). At 0.21mmol/kg/day the only finding was the increase in kidney weights, therefore NOEL for fertility and embryonic development could be established at 0.21mmol/kg/day, 1 time the human dose.

Reviewer's comments:

Increase of epididymides absolute and relative weights, and of testicle relative weights, and decrease of spermatid number per gram testicular tissue were observed at 1.5 mmol/kg. No effect was noted on fertility indices and reproduction parameters at any dose, therefore the NOAEL could be established at 0.21 mmol/kg/day. This dose is

approximately 1X the clinical dose. The next higher dose tested was 1.5 mmol/kg/day. The sponsor should have tested dose multiples comprised between 0.21 and 1.5 mmol/kg/day. Moreover, this study could have been included in the repeat dose toxicity study conducted in rats, thus reducing the number of animals used.

Embryofetal development

Study title: Study of embryo-fetal development in rats with ZK 236018 (SH L03588A; Epix Medical MS-325) by intravenous administration.

Key study findings:

NOEL was established at 2.5X the human dose. There was a significant increase in the number of skeletal variations and in the number of skeletal retardations at 11 times the human dose. MS-325 was not teratogenic at doses up to 11 times the human dose.

Study no.: A10557
Volume #, and page #: 5,8731
Conducting laboratory and location: T

b(4)

Date of study initiation: 11/09/2001
GLP compliance: Yes and ICH guideline 4.1.3 compliance.
QA reports: yes (X) no ()
Drug, lot #, and % purity: Gadofosveset trisodium, 3095p19, 99.4%.

Methods

Doses: 0.1, 0.45, and 2.0 mmol/kg/d, respectively 0.5X, 2.5X, and 11X 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 the 6th to 17th day of pregnancy. The animals were laparotomized on day 20 of pregnancy and embryo-fetal development was assessed.
 Parameters and endpoints evaluated: mortality, clinical signs, body weight, food consumption, macroscopic evaluation of the internal organs and placentae of the dams, kidney and uterus weights for all dams, number of fetuses (alive and dead) and placentae, number and size of resorptions, of corpora lutea, number of live and dead implantations, individual fetal body weight, fetal abnormalities (external, skeletal, and soft tissue examinations), gross evaluation of placenta as well as local and systemic clinical parameters of the dams including post-mortem investigations such as necropsy and kidney/body weight ratio.

Gadolinium concentration was evaluated: on gestation days 6/7 and 17/18, blood samples were collected at 3 min, 0.5 and 24 hours post administration in 5 dams/group, as well as 10 and 60 min post-administration in further 5 dams/group).

Statistical evaluations: Dunnett's and the Student t-test as well as the Fisher test were used, if applicable, for statistical analysis of intergroup differences of mean values.

Dose (mmol/kg)	0.1	0.45	2.0	NOEL 0.45
Human dose multiple (based on BSA)	0.5	2.5	11	2.5

Results

Overall, no treatment related changes were noted in females treated with 0.1 mmol/kg/day. Most of the effects were observed at 2 mmol/kg/day, 10 times the human dose.

Mortality (dams):

No animal died in this study.

Clinical signs (dams):

No remarkable signs were noted.

Body weight (dams):

No remarkable signs were noted.

A slight reduction of approximately 5% of the mean maternal body weight was noted at 2 mmol/kg/day on day 13 to 19. This reduction, although insignificant, corresponded to the reduced food intake, net weight and carcass weight at 2 mmol/kg/day.

In the low dose group, an increase in the mean body weight was noted on day 18 and 20. This effect was not dose-dependent.

Food consumption (dams):

Reduction in the absolute (20%) and relative (16%) food consumption was noted on several days (8, 11, 18, and 20) during the treatment period at 2 mmol/kg/day.

Toxicokinetics:

Table (provided by sponsor): Mean pharmacokinetic parameters of gadolinium (Gd) obtained in pregnant female Sprague-Dawley rats daily intravenous administration of 0.1, 0.45, and 2.0 mmol ZK 236018 per kg body weight from day 6 to day 17 of gestation $C_{3\text{min}}$, $AUC_{3-60\text{min}}$, and C_{24}

Appears This Way
On Original

Best Possible Copy

Parameters For Gd [unit]	Dose (mmol ZK 236018/kg/Day)					
	0.1/d 6	0.1/d 17	0.45/d 6	0.45/d 17	2.0/d 6	2.0/d 17
C ₀ [mmol/L]	0.74	0.83	2.77	2.99	8.58	12.7
C _{3min} [mmol/L]	0.65 ± 0.05	0.73 ± 0.02	2.46 ± 0.32	2.62 ± 0.07	7.86 ± 2.4 [#]	11.0 ± 0.41
DF (C _{3min})	1.00	1.00	0.84	0.79	0.60	0.75
t _{1/2} [min]	21.8	24.5	24.8	25.3	23.4	28.9
AUC _{3-60min} [mmol*min/L]	15.2	17.4	61.6	63.2	204	254
DF(AUC _{3-60min})	1.00	1.00	0.90	0.81	0.67	0.73
C _{av, 3-60min} [mmol/L]	0.27	0.31	1.08	1.11	3.58	4.46
AUC _{0-∞} [mmol*min/L]	20.1	23.9	84.5	87.5	273	372
%AUC _{0-∞} [%]	13.9	17.2	17.8	18.1	16.3	22.0 ^{##}
R	N/A	1.14		1.03		1.25
MRT [min]	28.5	32.1	33.0	33.3	31.2	37.7
Clearance [mL/min/kg]	4.98	4.19	5.32	5.15	7.32	5.38
V _z [L/kg]	0.16	0.15	0.19	0.19	0.25	0.22

The mean of two measurements was used.

The area extrapolated to calculate the AUC_{0-∞} was more than the statistically acceptable 20%.

- C₀: extrapolated serum concentration at time 0
- C_{3min}: maximum Gd-concentration in serum observed 3 minutes after drug administration
- DF (C_{3min}): Dose normalised dose proportionality factor
 $[(C_{3min, x \text{ mmol/kg}}/C_{3min, 0.1 \text{ mmol/kg}}) / (x \text{ mmol/kg}/0.1 \text{ mmol/kg})]$ for the same day
- t_{1/2}: terminal half life
- AUC_{3-60min}: area under the concentration versus time curve from the first to the last point of time
- DF(AUC_{3-60min}): Dose normalised dose proportionality factor
 $[(AUC_{x \text{ mmol/kg}}/AUC_{0.1 \text{ mmol/kg}}) / (x \text{ mmol/kg}/0.1 \text{ mmol/kg})]$ for the same day
- C_{av, 3-60min}: average serum concentration from the first to the last point of time
- AUC_{0-∞}: extrapolated total area under the curve
- %AUC_{0-∞}: area extrapolated to calculate the AUC_{0-∞}
- R: Accumulation factor $[AUC_{3-60min, \text{ day 17}}/ AUC_{3-60min, \text{ day 6}}]$
- MRT: mean residence time (of the unchanged drug in the systemic circulation)
- V_z: volume of distribution during the apparent terminal elimination phase

Mean maximum drug concentrations were observed in serum at 3 min at all dose levels, in a dose-dependent manner. Findings were similar for the AUC. Elimination half-life ranged from 21.8-28.9 minutes. The drug was rapidly distributed within 10 min, with a slight increase of the volume distribution and clearance between 0.1 and 2 mmol/kg/d. Clearance and distribution were similar on day 6 and day 17 of treatment.

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

- No effect was observed following 0.1 and 0.45 mmol/kg/day. An increase of absolute and relative weights of kidney was noted at 2.0 mmol/kg/ with pale kidneys in 7/20 dams.
- Gravid uterus, carcass, and net weight change were not affected with 0.1 and 0.45 mmol/kg/day. At 2 mmol/kg, the carcass weight was decreased (6%), net weight change varied accordingly.
- Kidney absolute and relative weights increased by 19% and 26% respectively. No effect was detected at the 2 lower dose levels.

Offspring (malformations, variations, etc.):

-The number of corpora lutea, implantation sites, total resorptions, live fetuses was statistically unchanged at all doses. Interestingly, and although the global number of resorptions was not remarkably different across doses, the resorptions in the highest dose seemed to consist mostly of early resorptions (<2mm) compared to other doses. Early resorption/late resorption ratio was 3:4, 3:2, 1:6, and 6:2 for control, 0.1, 0.45, and 2 mmol/kg respectively. Although not statistically significantly, the total mean % pre- and post-implantation was increased by 1.2, 1.5, and 1.4 fold for 0.1, 0.45, and 2 mmol/kg, respectively, and a 2.2, 2.8, and 1.7 fold increase was noted in pre-implantation loss at the same doses, whereas post-implantation loss was less severely affected by treatment.

-Mean weight of total and female fetuses per litter was slightly but significantly reduced at 2 mmol/kg/day. However, the values were within the normal range of background data.

- No increase in the incidence of malformations and no macroscopically visible variations were noted at any of the doses tested. Twin sisters and twin brothers were noted at 0.1 and 0.45 mmol/kg/day respectively. One runt was observed at 0.1 mmol/kg and 2 runts each at 0.45 and 2 mmol/kg/day. Although there was no effect in the control group, this incidence is within the background range.

-At 2 mmol/kg/day, there was a 52% increase in the number of skeletal variations such as wavy ribs (not ossified or shortened), and a 4% in retardations, such as not ossified sternebrae and reduced size of pelvic vertebral arches. A 2.5 fold increase in fetal and litter incidence of soft tissue variations was observed at doses of 0.45 and 2 mmol/kg. However, the increase in the number of skeletal variations and in the number of skeletal retardations was statistically significant only at 2 mmol/kg.

Conclusion: Most of the effects were observed at the highest dose of 2 mmol/kg/day. At 0.1 and 0.45 mmol/kg/day, there was a slight but significant increase in the missing ossification of the sternebrae, in 75/137 fetuses and 66/125 fetuses respectively. These values, however, were within the normal range of background data, with a total incidence of retardations on sternebrae similar to that of controls.

Reviewer's comments: The selection of the doses would have been more adequate, had the sponsor included intermediate and lower doses. However, the maternal NOAEL was

2.5X the intended clinical dose. Developmental NOAEL was established at 0.45 mmol/kg (2.5 times the human dose).

Study title (No A10556): Study of embryo-fetal development in rabbits with ZK 236018 (SH L03588A; Epix Medical, MS-325) by intravenous administration.

Key study findings:

In the dams: No treatment-related effect was noted at 0.3 mmol/kg/day. Dose-dependent local tolerance reactions were noted at the injection site at 0.8 mmol/kg. A decrease in the mean gravid uterus weight, and an increase of absolute and relative kidney weights were noted at 2 mmol/kg.

In the fetuses: Malformations were noted in fetuses at the highest dose, equivalent to 22X the human dose. NOEL for teratogenic effects is 0.8 mmol/kg (9X the human dose based on BSA).

Embryotoxicity was associated with an increase of post-implantation loss and total resorption at 22X HD.

Pharmacokinetics: The AUC increased non-linearly and under-dose proportionally. Systemic exposure was similar between all dose levels (the increase in AUC was found to be smaller than the increase in dose)

Study no.: A10556
Volume #, and page #: 5,9825
Conducting laboratory and location:

b(4)

Date of study initiation: 01/11/2002
GLP compliance: Yes and ICH guideline 4.1.3 compliance.
QA reports: yes (X) no ()
Drug, lot #, and % purity: Gadofosveset trisodium, b3095p39, 99.7%.

Methods

Doses: 0.3, 0.8, and 2.0 mmol/kg/d, respectively 3X, 9X, and 22X the clinical dose based on body surface area. A control group received 0.9% saline.

Species/strain: Female rabbits/Himalayan

Number/sex/group: 20/dose

Route, formulation, volume, and infusion rate: Intravenous, aqueous solution, 8mL/kg, bolus 10 mL/min.

Satellite groups used for toxicokinetics: 5/dose

Study design: animals were treated once daily from the 6th to 18th day of pregnancy. The animals were laparotomized on day 29 of pregnancy and embryo-fetal development was assessed.

Parameters and endpoints evaluated: mortality, clinical signs, body weight, food consumption, macroscopic evaluation of the internal organs and placentae of the dams, kidney and uterus weights for all dams, number of fetuses (alive and dead) and placentae, number and size of resorptions, of corpora lutea, number of live

and dead implantations, individual fetal body weight, fetal abnormalities (external, skeletal, and soft tissue examinations), gross evaluation of placenta as well as local and systemic clinical parameters of the dams.

Gadolinium concentration was evaluated: on gestation days 6/7 and 18/19, blood samples were collected at 5 min, 15 min, and 1, 4, and 24 hours post administration.

Statistical evaluations: Bartlett chi-square test, Dunnett's and the Student t-test as well as the Fisher's exact test, were used, for statistical analysis for the comparison of classification measurements.

Dose (mmol/kg)	0.3	0.8	2.0	Embryotoxicity NOEL <0.3	Teratogenicity NOEL 0.8
Human dose multiple (based on BSA)	3	9	22	<3	9

Results

No treatment related local intolerance reactions were noted at 0.3 mmol/kg/day at and around the injection sites during daily macroscopic evaluation. At 0.8 mmol/kg/d, slight to moderate swellings were noted in 8/21 pregnant dams, from gestation day 10 onwards until gestation day 17. These effects were slight to severe in 16 dams treated with 2 mmol/kg/day, accompanied by slight to moderate erythemas in 4 dams, and eschar formation in 14 dams, from gestation day 7 or 11 onwards. Swelling and eschar formation often persisted until laparotomy, whereas erythemas were only notes until end of treatment.

Mortality (dams):

No animal died prematurely in this study. One dam treated with 0.8 mmol/kg/d was sacrificed after abortion on gestation day 25.

Clinical signs (dams):

No remarkable signs were noted.

Body weight (dams):

No remarkable effect was noted. The mean maternal body weight showed a statistically significant increase on gestation days 3-6 in the high dose group, however, this finding had been noted before start of substance treatment, hence it was considered incidental.

Food consumption (dams):

No drug related effect was noted on absolute and relative food consumption in treated animals as compared to the control. A slight reduction was observed in the low-dose group on gestation day 7, and a slight increase in the high-dose group on gestation day 26. These findings were considered incidental.