

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-711

PHARMACOLOGY REVIEW(S)

MEMO TO FILE

TO: ADEBAYO LANIYONU, PH.D., SUPERVISORY PHARMACOLOGIST
FROM: SIHAM BIADE, PH.D., PHARMACOLOGIST
SUBJECT: AMMENDED RECOMMENDATIONS FOR VASOVIST® LABELING
NDA #: 21-711
DATE: 12/18/2008
CC: JAMES MOORE, PHARM. D., M.A.

The pharmacology/toxicology recommendations for labeling have been modified from the original NDA review to reflect the maternal health team (MHT) suggestions. The revised recommendations are as follows:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

[REDACTED]

b(4)

[REDACTED]

b(4)

8.3 Nursing Mothers

[REDACTED]

b(4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of gadofosveset. [REDACTED] Gadofosveset was negative in the *in vitro* bacterial reverse mutation assay, *in vitro* CHO chromosome aberration assay, and the *in vivo* mouse micronucleus assay. Administration of up to 1.5 mmol/kg (8.3 times the human dose) to female rats for 2 weeks and to male rats for 4 weeks did not impair fertility. [REDACTED]

b(4)

Appears This Way
On Original

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Siham Biade
12/18/2008 04:03:20 PM
PHARMACOLOGIST

There was no open assignment for nonclinical for this
review cycle. I checked in the Memo using
the original NDA submission

Adebayo Lanionu
12/18/2008 04:06:08 PM
PHARMACOLOGIST



**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-711
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	5/23/05
PRODUCT:	VASOVIST
INTENDED CLINICAL POPULATION:	Adults with suspected or known vascular disease
SPONSOR:	Epix Pharmaceuticals
DOCUMENTS REVIEWED:	Complete response to approvable letter
REVIEW DIVISION:	Division of Medical Imaging Imaging and Radiopharmaceutical Drug Products (HFD-160)
PHARM/TOX REVIEWER:	Siham Biade, Ph.D.
PHARM/TOX SUPERVISOR:	Adebayo Laniyonu, Ph.D.
DIVISION DIRECTOR:	George Mills, MD, MBA
PROJECT MANAGER:	James Moore, Pharm.D.

Date of review submission to Division File System (DFS): 11/16/05

**Appears This Way
On Original**

**Appears This Way
On Original**

TABLE OF CONTENTS

EXECUTIVE SUMMARY	4
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	6
2.6.1 INTRODUCTION AND DRUG HISTORY	6
2.6.2 PHARMACOLOGY.....	8
2.6.2.1 Brief summary.....	8
2.6.2.2 Primary pharmacodynamics.....	8
2.6.2.3 Secondary pharmacodynamics.....	8
2.6.2.4 Safety pharmacology.....	8
2.6.2.5 Pharmacodynamic drug interactions.....	11
2.6.3 PHARMACOLOGY TABULATED SUMMARY	11
2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....	11
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	11
2.6.6 TOXICOLOGY	12
2.6.6.1 Overall toxicology summary.....	12
2.6.6.2 Single-dose toxicity.....	12
2.6.6.3 Repeat-dose toxicity.....	12
2.6.6.4 Genetic toxicology.....	12
2.6.6.5 Carcinogenicity.....	12
2.6.6.6 Reproductive and developmental toxicology.....	12
2.6.6.7 Local tolerance.....	12
2.6.6.8 Special toxicology studies.....	16
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	19
APPENDIX/ATTACHMENTS	24

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The preclinical studies conducted support safety and efficacy (measured by relaxation rates). This reviewer recommends VASOVIST™ be approved.

B. Recommendation for nonclinical studies

No additional studies are required.

C. Recommendations on labeling

Changes in the label would more appropriately reflect findings from preclinical studies. Please see pages 19-23 under "Suggested labeling"

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The sponsor submitted five preclinical study reports. The Division did not request these studies.

1. Effect of MS-325 in cisplatin-induced rat model of renal impairment

Previously, the sponsor submitted the blood and urine analysis results of a study evaluating the effect of MS-325 in a cisplatin-induced rat model of renal impairment. The present report contains histopathological data not submitted in the original report. Based on these new findings, the sponsor provided a re-analysis of serum and urine chemistry data. However, there was no correlation between the severity of histopathological damage and the extent of changes in the clinical chemistry, thus the sponsor did not succeed in establishing a reproducible model of cisplatin-induced renal impairment in rats. Therefore, in spite of the additional histopathological data, the original conclusions made by this reviewer have not changed.

As stated in the original review, availability of clinical data ameliorates the lack of adequate preclinical data in renally impaired animal model.

2. Local tolerance studies in rabbits using the IV, IA, and IM administration routes

The sponsor submitted three reports assessing local tolerance in rabbit using the intravenous, intraarterial, and intramuscular administration routes. These studies include

observation periods of up to 8 days, which allowed for complete recovery in the IV injection study. Single intraarterial administration of MS-325 resulted in marked local reactions with signs of incomplete reversibility. It is noted that the sponsor was not seeking the intraarterial route as a route of administration. Single intramuscular administration of MS-325 caused mild to moderate foetal necrosis associated with proliferative inflammatory reaction and phagocytosis of damaged muscle fibers at the injection site. These effects were slightly but not completely reversible within 7 days of treatment. Therefore, inadvertent administration into the muscle in the proximity of the vein in humans may induce similar reactions.

3. Effects of MS-325 and its Ligand In Vitro on Cytochrome P450 Dependent Metabolism of Model Substrates.

The range of concentrations equal to or below the anticipated clinical plasma concentration did not allow for the determination of Vasovist IC_{50} s. However, in these experimental conditions, IC_{50} s for the CYP enzymes studied were greater than 300 μ M. Thus, Vasovist does not inhibit the studied enzymes activity in vitro at concentrations equivalent to the maximum plasma concentration reached at the proposed clinical dose.

Overall Conclusions and Recommendations:

Overall, the studies submitted in the present complete response do not affect the previous conclusions and recommendations from a pharmacology/toxicology perspective. No additional nonclinical studies are needed. From a nonclinical pharmacology and toxicology perspective, this reviewer recommends approval for VASOVIST™.

B. Pharmacologic activity

No additional data were submitted

C. Nonclinical safety issues relevant to clinical use

This reviewer did not identify new issues relevant to clinical use, based on the five nonclinical study reports submitted by the sponsor in their complete response.

Appears This Way
On Original

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-711
Review number: 2

Document Type	Sequence number	Date	Mod Type
N	000	5/23/05	AZ
N	216	4/26/05	IT
N	000	10/11/05	BP

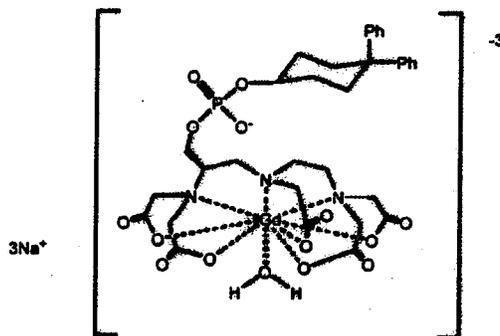
Information to sponsor: Yes (X) No ()
Sponsor and/or agent: Epix Medical
Manufacturer for drug substance: Mallinckrodt

Reviewer name: Siham Biade, Ph.D.
Division name: Medical Imaging and Radiopharmaceutical
Drug Products
HFD #: 160
Review completion date: 08/01/2005

Drug:

Trade name: VASOVIST™
Generic name: Gadofosveset Trisodium
Code name: MS-32520-R
Chemical name: Trisodium-{(2-(R)-[(4,4-diphenylcyclohexyl)phosphono]oxymethyl]diethylenetriamine pentaacetato)(aquo)gadolinium(III)}
CAS registry number: 211570-55-7, 193901-90-5 (anhydrous form)
Molecular formula/molecular weight: C₃₃H₄₀GdN₃Na₃O₁₅P/ 975.88

Structure:



Gadofosveset Trisodium

Relevant INDs/NDAs/DMFs: IND# 51,172

Drug class: Diagnostic contrast agent with MRA

Indication: Use with magnetic resonance angiography
 [] in adults with suspected or known vascular disease

b(4)

Clinical formulation: Clear, colorless to slightly yellow solution, pH 6.5 to 8.0.

Parameter	Condition	Value
Osmolality (mOsmol/kg water)	@ 37°C	825
Viscosity (cP)	@ 20°C	3.0
Density (g/mL)	@ 25°C	1.1224
pH		6.5 to 8.0

The osmolality of the solution (825 mOsmol/kg) is 2.89 times that of plasma (285 mOsmol/kg).
 Marketed as 10 mL vials containing 10 mL of solution and 20 mL vials containing 15mL [] of solution.

b(4)

Route of administration: Intravenous bolus injection, manually or by power injection, at a dose of 0.12 mL/kg (0.03 mmol/kg) over a period up to 30 seconds followed by a 25-30 mL normal saline flush.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

REPORT PTR2004-019	Assessment of MS-325 in Cisplatin-Induced Rat Model of Renal Impairment.
REPORT A20491	Local Tolerance Test in the Rabbit (M+F) after a Single Injection into the Congested and Uncongested Vein of the Ear
REPORT A20492	Local tolerance study in rabbits after single i.a. administration
REPORT A20493	Local Tolerance Test in the Rabbit (M+F) after Single Intramuscular Administration
REPORT A25601	Inhibitory Effects of ZK 236018 (MS-325) and its Ligand ZK 233284 In Vitro on Cytochrome P450 Dependent Metabolism of Model Substrates

Studies not reviewed within this submission:
None

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

N/A

2.6.2.2 Primary pharmacodynamics

N/A

2.6.2.3 Secondary pharmacodynamics

N/A

2.6.2.4 Safety pharmacology

Renal effects:

REPORT PTR2004-019: Assessment of MS-325 in Cisplatin-Induced Rat Model of Renal Impairment (Lot B0002PR, March 2004)

Introduction/Background

Previously, the sponsor submitted the blood and urine analysis results in the original NDA submission. The present report contains the blood and urine analysis along with the results of the histopathological examination of the kidneys. In light of both the clinical and pathology reports, the sponsor noted that several animals did not exhibit tubular nephrosis in spite of cisplatin treatment. This observation affected 3 animals in the vehicle+cisplatin treated Group 2, 1 animal in the cisplatin+0.1mmol/kg MS-325 treated Group 4, 1 animal in the cisplatin+0.3mmol/kg MS-325 treated group 5, and 2 animals in the cisplatin+1mmol/kg MS-325 treated Group 6. The same animals also had serum and urine parameters similar to the vehicle control group (non-cisplatin treated) suggesting that in these animals, cisplatin was not effective at inducing renal damage. Based on these observations, the sponsor performed a re-analysis of the serum and urine data excluding these animals' data.

The in-life portion of this study along with the blood and urine analysis were conducted by [] (Appendix I) while the histopathological analysis was performed by [] (Appendix II).

b(4)

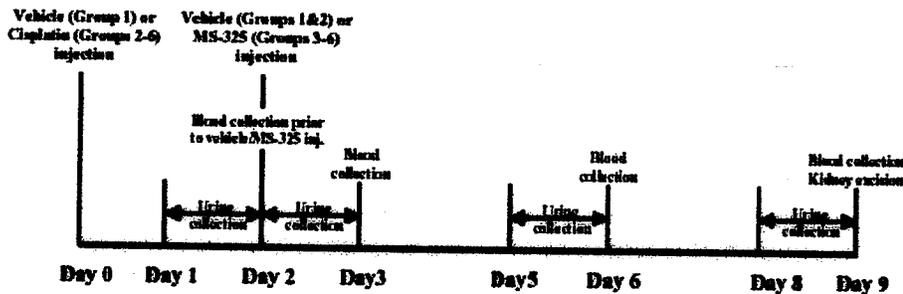
Study objective:

The objective of this study was to evaluate the effect of 0.03, 0.1, 0.3, and 1 mmol/kg (0.16, 0.54, 1.6, and 5.4X the human dose based on BSA) of MS-325 on renal function in a cisplatin-induced model of renal impairment in the rat.

Study design:

MS-325 was administered intravenously at 0.03, 0.1, 0.3, and 1 mmol/kg at 48 hours (day 2) after 7.5 mg/kg intraperitoneally cisplatin (day 0). Urine samples were collected over 24 hours on day 1, 2, 5, and 8 for measurements of urine volume, electrolytes (Na⁺, K⁺, Cl⁻), creatinine and proteins. Blood samples were collected on day 2, 3, 6, and 9. Concentrations of serum albumin, enzymes, creatinine, BUN and electrolytes (Na⁺, K⁺, Cl⁻) were measured. The mean ± SEM value for each treatment group was calculated and Dunnett's test was applied for comparison between vehicle and treatment groups at various time points. Results were compared between all cisplatin-treated groups and naïve rats. Differences were considered significant at P<0.05 level. The rats were sacrificed on day 9, and their kidney was weighed and sent for histopathological analysis.

Figure 1 illustrates the protocol timeline for this study*.



* Figure provided by sponsor

Table 1. MS-325 rat doses and corresponding human dose multiples

Group (8 rats/group)	Vehicle 0.9% NaCl	Cisplatin 7.5 mg/kg	MS-325 mmol/kg	MS-325 mmol/m ²	MS-325 Human dose multiples
1	+	-	-	-	-
2	+	+	-	-	-
3	-	+	0.03	0.18	0.16X
4	-	+	0.1	0.6	0.54X
5	-	+	0.3	1.8	1.6X
6	-	+	1	6	5.4X

Results:

Best Possible Copy

Histopathological analysis:

[Appendix II) performed histopathological analysis. Histological evaluation was limited to the kidneys. [received tissue samples to be processed into microscopic slides and prepared the report. A staff veterinary pathologist examined the slides.

b(4)

Microscopic findings included characteristic cisplatin-induced tubular nephrosis of the pars recta of the proximal tubules within the outer strip of the medulla in some but not all treated animals. Tubular nephrosis is typically characterized by several changes including tubular epithelial vacuolization/degeneration, necrosis/apoptosis and/or loss with resultant tubular epithelial regeneration, karyomegaly/megalocytosis, tubular lumen dilatation, intraluminal accumulations of sloughed/necrotic cellular debris and/or protein casts. Other findings may include variable amounts of interstitial fibrosis, edema, or hemorrhage, tubular and/or interstitial mineralization, tubular and/or interstitial cellular infiltrates composed of either neutrophils, histiocytes/macrophages and/or lymphocytes/plasma cells.

Table 2. Summary tables of number of animals with microscopic findings by organ/group. (Table provided by sponsor)

 TEST ARTICLE : MS-325
 TEST SYSTEM : RAT (Wistar), 8 Days, IV
 SPONSOR : EPIX Medical, Inc.
 PATHOL. NO.: 59313 JRS
 DATE : 15-JUL-04
 PathData® System V5.1b

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
 STATUS AT NECROPSY: K0
 Day 8 - Kidney

Best Possible Copy

SEX :	MALE					
DOSE GROUP:	01	02	03	04	05	06
NO. ANIMALS:	8	8	8	8	8	8
KIDNEYS :	8	8	8	8	8	8
- Tubular epi. swell. :	-	1	-	-	1	-
- Mineralization :	-	-	1	1	-	-
- PT rarification :	-	5	6	5	6	6
- Urothelial hyperpla. :	-	-	1	1	-	-
- Bacteria :	-	1	-	-	-	-
- Tubular nephrosis :	-	5	8	7	7	6
- Proteinosis :	-	1	6	2	-	-
- Hydronephrosis :	3	2	4	-	-	1
- Chronic inflammation:	5	2	3	5	4	6

Three animals treated with cisplatin alone did not develop tubular nephrosis. The cause for the absence of tubular lesions in these three Group 2 animals is unknown. Except for Group 3 (MS-325, 0.03 mmol/kg), all other groups had one (Groups 4 and 5) or two animals (Group 6) with no evidence of tubular damage. All groups treated with MS-325, regardless of dose, had more animals with tubular nephrosis than cisplatin/vehicle control Group 2. The nephrosis in the MS-325-treated animals was more severe compared to the cisplatin/vehicle control Group 2. The histopathological findings were not dose-dependent.

Sponsor's conclusions:

Regardless of MS-325 doses, MS-325+cisplatin treated animals typically had an increase in incidence and severity of cisplatin-induced nephrosis, compared to cisplatin/vehicle

controls, as well as a slight delay in the resolution of the nephrosis. The number of unaffected animals was higher in the cisplatin/vehicle group than in any MS-325+cisplatin-treated group, hence the sponsor ascribed the increase in incidence and severity in the MS-325-treated animals to the cisplatin's inability to induce nephrosis in some animals. The sponsor concluded that there were no dose-dependent, adverse test article effects or trends.

Reviewer's comments:

The cisplatin-induced rat model of renal impairment report contained histopathological data not submitted in the original report. Some animals treated with cisplatin alone or cisplatin+MS-325 did not develop tubular damage as determined by histopathological examination. The sponsor removed these animals from the present analysis and provided a re-analysis of serum and urine chemistry data. However, because of the discrepancy between histopathological and clinical chemistry and urinalysis findings, the data are not acceptable. There was no correlation between the severity of histopathological damage and the extent of changes in the clinical chemistry. For instance, many animals with grade 4 tubular damage had lower BUN than animals with milder tubular damage. Moreover, the study did not include a group of animals treated with MS-325 alone. Thus, in animals showing histopathological or clinical chemistry signs of renal impairment, it was impossible to ascribe the tubular damage to cisplatin or MS-325 or to the combination of both. These observations are evidence of major deficiencies in this study; they indicate that the sponsor failed to establish a reliable animal model of renal impairment prior to conducting the study. Therefore, in spite of the additional histopathological data, the original conclusions made by this reviewer have not changed.

As stated in the original review, the goal of this study was to evaluate the effect of MS in an animal model of renal impairment; however, the sponsor did not unequivocally develop such a model thus making result interpretation difficult. Human PK data showed that Vasovist elimination half-life was extended up to 70 hrs (normal: 16 hrs) in subjects with severe renal impairment. The availability of clinical data ameliorates the lack of adequate preclinical data in renally impaired animal model.

2.6.2.5 Pharmacodynamic drug interactions

N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

N/A

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

N/A

2.6.6.2 Single-dose toxicity

N/A

2.6.6.3 Repeat-dose toxicity

N/A

2.6.6.4 Genetic toxicology

N/A

2.6.6.5 Carcinogenicity

N/A

2.6.6.6 Reproductive and developmental toxicology

N/A

2.6.6.7 Local tolerance

The sponsor submitted three local tolerance studies evaluating the effects of MS-325 following single intravenous, intraarterial, and intramuscular administration in rabbits. The previous local tolerance studies submitted by the sponsor included observation periods of up to 96 hours, and most of the effects, although showing signs of reversibility did not completely disappear. In contrast, the local tolerance studies submitted within the present complete response include observation periods of up to 8 days, which in some cases allowed for observation of complete recovery (intravenous injection study).

In the original NDA submission, the sponsor submitted an acute intravenous and perivascular study in rabbits (Report 2006). This reviewer had concluded the following:

1. Intravenous injection:

Intravenous injection produced subcutis hemorrhage, heterophil/lymphoid cell infiltrate, edema, vein and soft tissue necrosis, acute necrotizing inflammation at the injection site in both treated and control groups. These effects were observed at 24 hours and were slightly more marked in the treated group. At 96 hours, persistent subcutis heterophil/lymphoid cell infiltrate and edema, and epidermis surface cell debris/inflammatory cells were minimal (grade 1) in 2 treated animals and slight (grade 2) in 1 control animal. One animal in the treated group presented minimal (grade 1) soft tissue necrosis indicating a reversibility of the effects. The effects observed proximal to the injection site at 24 hours post intravenous administration were absent at 96 hours.

2. Perivascular injection:

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

This reviewer recommended the following be included under "Precautions" of the label:

"Precautions



b(4)

REPORT A20491: Local Tolerance Test in the Rabbit (M+F) after a Single Injection into the Congested and Uncongested Vein of the Ear.

(Shering AG, Experimental Toxicology, D-13342 Berlin, Germany, Study TXST20040004, January 2004, Batch 3095p19, 250mM)

This study evaluates the local effect of intravenous injection of MS-325 in the congested and uncongested vein in rabbits.

Study design:

Two male and two female New Zealand white rabbits received intravenous injection of 0.5 mL (125 µM) of MS-325 in the congested marginal vein of the right ear within 30 seconds. Another group of rabbits (2M+2F) received the same treatment into the uncongested vein of their right ear. All animals were used as self-controls, and were injected 0.5 mL saline respectively into the congested or uncongested vein of their left ear. Congestion of the marginal vein was performed using a clamp in the proximal third part of the ear. The clamp was removed immediately at the end of injection. The reactions at the injection sites were observed immediately, 2 and 4 hours after injection and then daily until day 8 after administration. Ear tips and injection sites were examined histologically.

Results:

Slight reddening around the puncture track or around the injection point was noted as well as vessel injection and formation of hematoma in both control and treated groups. The incidence of these findings was similar in the uncongested vein for both groups, with a slightly faster recovery in the treated animals compared to controls. In the congested

vein, hematoma was more frequent in the MS-325 treated group. However, none of these signs persisted beyond day 6, except for a slight reddening and blue colored vessel in the control group.

The macroscopic and histological examination at necropsy of the injection sites of the test article treated congested and uncongested ear vein did not reveal any signs of compound-related morphological alterations.

Reviewer's comments:

This study showed that intravenous administration of MS-325 drug product does not induce morphological changes at the injection site in rabbits whether the vein is not congested or artificially congested with a clamp. The gross examination revealed that slight reddening, blue coloration, and hematoma incidence was overall similar in drug treated animals compared to controls. On day 8, none of the treated animals presented signs of local irritation.

This study provided additional information to the findings of the previous acute vascular administration study submitted in the original NDA. In that study, observations were performed up to 3 days post administration, thus reversibility of the local effects was not complete. In contrast, the present study showed that IV injection of 0.5 mL (125 µM) MS-325 induces slight local and completely reversible effects.

Study No. A20492: Local tolerance study in rabbits after single i.a. administration (Shering AG, Experimental Toxicology, D-13342 Berlin, Germany, Study TXST20040004, January 2004, Batch 3095p19, 250mM)

This study, conducted in rabbits, evaluates the local effect of intraarterial injection of MS-325 into the *Arteria centralis* of the ear.

Study design:

Two male and two female New Zealand white rabbits each received intraarterial injection of 0.5 mL (125 µM) of MS-325 into the *Arteria centralis* of the right ear within 30 seconds. The same rabbits received 0.5 mL of NaCl solution into the *Arteria centralis* of their left ear as control. The injection sites were observed during and immediately, 2 and 4 hours after injection and then daily until day 8 after administration. The animals were sacrificed on day 8 and tissue samples of the ear tips and at approximately 1 cm distal to the marked injection sites, were histopathologically examined.

Results:

1. *Gross examination:* Administration of 0.5 mL of MS-325 into the central artery of the ear induced transient light to severe reddening distal, proximal and around the puncture track and slight swelling proximal of the puncture track. A marked dark-red area around

the puncture track, as well as a bluish vessel injection in the proximal half of the ear, were observed post administration. Most of these findings, with the exception of dark red area and slight swelling, were also observed in the control group although at a much lower incidence. In both treated and control groups, the findings showed a tendency to reversibility during the course of the observation period. However, reddening persisted through the end of the study.

2. Histological examinations: Examinations revealed that intraarterial administration of 0.5 mL (125 µM) MS-325 did not result in pathomorphological findings.

Sponsor's conclusions and recommendations

Similar clinical effects have to be reckoned with when SH L03588A (Vasovist) is inadvertently applied intraarterially to humans.

Reviewer's comments:

Although histopathological examination of the injection sites did not reveal MS-325 related effects following intra arterial administration of 0.5 mL (125 µM), this study showed that this route of administration may result in marked local reactions such as reddening, hematoma, and clear delimited dark red area. On Day 8, there were signs of reversibility, but the process was not complete. The sponsor did not seek the intraarterial route of administration in the NDA. Based on the results of this study, intraarterial administration of Vasovist is not recommended in humans.

Study No. A20493: Local tolerance test in the rabbit (M+F) after single intramuscular administration

(Shering AG, Experimental Toxicology, D-13342 Berlin, Germany, Study TXST20040004, January 2004, Batch 3095p19, 250mM)

This study, conducted in rabbits, evaluates the local effect of intramuscular injection of MS-325 into the sacrospinal muscle.

Study design:

New Zealand White rabbits (4M+4F) were each injected with 1 mL of SH L03588A into one side of the sacrospinal muscle, and the control muscle was injected with 1 mL of a 0.9% (w/v) NaCl-solution under the same conditions, on the opposite side.

Injection sites were observed immediately after injection, as well as 2, 4 and 24 hours after administration and thereafter once daily until day 3 or day 7. At day 3 or day 7, 2 male and 2 female animals each were sacrificed for macroscopic inspection of all injection sites and histological examination of altered injection sites only. When size permitted, portions of muscle that were macroscopically altered were also weighed.

Results:

The weights of altered muscle tissue of the treated animals were markedly increased compared to control injection sites in the samples taken on day 3. The size of the muscle was too small on day 7 and was therefore not weighed.

On day 3:

-Macroscopic examination revealed mild and slight hemorrhage at the administration sites in 1 and 2 control animals respectively, and moderate hemorrhage in all 4 animals injected MS-325.

-Histological examination of the *altered* injection sites, following saline injection, revealed signs of local irritation in form of mild and slight focal necrosis (puncture track) in 1 and 2 control animals respectively, whereas in the four animals administered MS-325, focal necrosis was moderate (more severe grading 3) and was associated with proliferative inflammatory reaction and phagocytosis of damaged muscle fibers. The sponsor attributed the morphological alterations at the control injection sites to mechanical trauma during insertion of the injection needle (puncture track).

On day 7:

-Macroscopic examination revealed no findings at the administration sites in any of the control group animals. One animal in the MS-325 group exhibited slight reddening, and one animal slight hemorrhage.

-Histological examination was performed only on those altered injection sites. It revealed mild focal necrosis with proliferative inflammatory reaction and phagocytosis of damaged muscle fibers associated with mild muscle fiber regeneration. According to the sponsor, these observations indicate a sign of reversibility in the local irritation process.

Reviewer's comments:

A single intramuscular administration of 1.0 mL of MS-325 into the sacrospinal muscle of rabbits caused mild to moderate focal necrosis associated with proliferative inflammatory reaction and phagocytosis of damaged muscle fibers at the injection site. These effects were slightly but not completely reversible within 7 days of treatment. Therefore, inadvertent administration into the muscle in the proximity of the vein in humans may induce similar reactions.

2.6.6.8 Special toxicology studies

Study title: Inhibitory Effects of ZK 236018 (MS-325) and its Ligand ZK 233284 In Vitro on Cytochrome P450 Dependent Metabolism of Model Substrates

Study no.:

Report A25681

Volume #, and page #:

Conducting laboratory and location:

**Schering AG, Clinical Pharmacokinetics,
Metabolism and Bioanalysis, 13342 Berlin,
Germany**

Date of study initiation: 15 Oct 2004
GLP compliance: No
QA reports: yes () no (x)
Drug, lot #, and % purity: ZK 236018 (MS-325) & ZK 233284 (Ligand), K14301 & MGK-D15387, 99.83% & 99.25 % respectively
Formulation/vehicle: 10 mL vial containing 250 mM Solution /Sodium hydroxide, hydrochloric acid, water for injection
 Ligand: 1 mM in Potassium phosphate

Study design:

ZK 236018 and ZK 233284 were dissolved in potassium phosphate, 100 mM, pH 7.4, and diluted to yield final concentrations ranging between 0.37 μ M and 300 μ M with a constant solvent concentration; 300 μ M is approximately the plasma concentration in human at 30 min post administration of a dose of 0.03 mmol/kg. The isoenzyme specific model substrates were dissolved in methanol with exception of chlorzoxazone, which was dissolved in buffer. The final solvent concentration was 1 % methanol (v/v) for testosterone and paclitaxel. The final solvent concentration for the other model substrates was 0.5 % methanol (v/v).

The microsomes were incubated with isoenzyme specific model substrates at various concentrations and their metabolism was studied for different concentrations of ZK 236018 and for one concentration of ZK 233284 (ligand). Model substrate metabolism was monitored by the quantitative determination of the produced metabolite. The metabolite was quantified by HPLC separation with UV absorbance, fluorescence detection or radiometric detection. Each data point was tested in duplicate incubations.

Assay conditions for determination of MS-325 ZK 236018 IC50 values

Relevant isoenzyme	CYP2C9	CYP2C19	CYP2D6	CYP3A4
Model substrate	Diclofenac ZK 701010	S-mephenytoin ZK 180082	Bufuralol ZK 305707	Testosterone ZK 5040
Substrate concentration	10 μ M	30 μ M	15 μ M	50 μ M
Reaction	Diclofenac 4'-hydroxylation	S-mephenytoin 4'-hydroxylation	Bufuralol 1'-hydroxylation	Testosterone 6 β -hydroxylation
Test system	HLM	Supersomes	HLM	HLM
Concentration of protein [mg/mL] or CYP [pmol/mL]	0.2 mg/mL	80 pmol/mL	0.6 mg/mL	0.4 mg/mL
Preincubation	20 min	20 min	20 min	20 min
Reaction time	30 min	60 min	60 min	30 min

Concentration of MS-325 [μM]	0, 0.37, 1.1, 3.3, 10, 30, 100, 300	0, 0.37, 1.1, 3.3, 10, 30, 100, 300	0, 0.37, 1.1, 3.3, 10, 30, 100, 300	0, 0.37, 1.1, 3.3, 10, 30, 100, 300
Model inhibitor (MI)	Sulfaphenazole ZK 3520	Omeprazole ZK 305708	Quinidine ZK 800931	Ketoconazole ZK 111216
Concentration (MI)	5 μ M	20 μ M	1 μ M	1 μ M

Relevant isoenzyme	CYP1A2	CYP2A6	CYP2C8	CYP2E1
Model substrate	Phenacetin ZK 376	Coumarin ZK 43577	Paclitaxel ZK 133917	Chlorzoxazone ZK 26937
Substrate concentration	50 μ M	5 μ M	20 μ M	50 μ M
Reaction	Phenacetin O-deethylation	Coumarin 7-hydroxylation	Paclitaxel 6 α -hydroxylation	Chlorzoxazone 6-hydroxylation
Test system	HLM	HLM	HLM	HLM
Concentration of protein [mg/mL] or CYP [pmol/mL]	0.6 mg/mL	0.2 mg/mL	0.6 mg/mL	0.4 mg/mL
Preincubation	20 min	20 min	20 min	20 min
Reaction time	60 min	20 min	60 min	30 min
Concentration of MS-325 [μM]	0, 0.37, 1.1, 3.3, 10, 30, 100, 300	0, 0.37, 1.1, 3.3, 10, 30, 100, 300	0, 0.37, 1.1, 3.3, 10, 30, 100, 300	0, 0.37, 1.1, 3.3, 10, 30, 100, 300
Model inhibitor	Furafylline ZK 169611	Pilocarpine ZK 889323	Quercetin ZK44634	DDC ZK 4353
Concentration of model inhibitor	2.5 μ M	50 μ M	5 μ M	5 μ M

IC₅₀ estimation

Isoenzyme specific inhibitors were used as positive controls (data not shown). The reference values (samples without inhibitor) were determined in triplicate. The inhibitory profiles of ZK 236018 towards the various human microsomal CYP activities were presented as semilogarithmic plots with the remaining activity (y-axis) versus concentration of ZK 236018 (log scale, x-axis). IC₅₀ values were estimated from these plots.

Results:

The sponsor showed that 50% inhibition could not be achieved for any of the substrates, and up to the highest concentration of 300 μ M of MS-325. The ligand ZK 233284 had no inhibitory effect on the same substrates at a final concentration of 1 μ M.

The sponsor concludes that the data clearly show that ZK 236018 (MS-325) exerts no drug-drug interaction potential when co-administered at diagnostic doses with commonly

prescribed drugs known to be cleared predominantly by cytochrome P450s. The sponsor draws the same conclusion for ZK 233284 (ligand).

Reviewer's comments:

The selection of the concentrations of MS-325 was inadequate. The sponsor tested seven concentrations of MS-325 with the highest one equivalent to the plasma level reached at the proposed clinical dose. Thus, the number of low doses tested seems excessive. The sponsor should have evaluated the effect of the drug at concentrations of at least 2 to 3 fold the anticipated clinical concentration.

The mean turnover for some enzymes (CYP2C8, CYP2C9, CYP3A4) was greater than that of negative control, which resulted in negative inhibition percents reaching up to 80% for CYP3A4. According to the sponsor, the negative values in the mean inhibition reported for these enzymes are due to assay variability.

The range of concentrations equal to or below the anticipated clinical plasma concentration did not allow for the determination of IC₅₀s. However, in these experimental conditions, IC₅₀s for the CYP enzymes studied were greater than 300 μM,

Thus, Vasovist does not inhibit the studied enzymes activity in vitro at concentrations equivalent to the maximum plasma concentration reached at the proposed clinical dose.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Overall Conclusions and Recommendations:

Upon review of the five preclinical study reports submitted within the sponsor's complete response, this reviewer concludes that no additional nonclinical studies are needed. From a pharmacology/toxicology perspective, an approval of Vasovist is recommended.

Suggested labeling:

[

b(4)

4 Page(s) Withheld

 Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

Reviewer: Siham Biade, Ph.D.

NDA No.21-711

Reviewer Signature Siham Biade, Ph.D.

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

NONE

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Siham Biade
11/16/2005 04:04:31 PM
PHARMACOLOGIST

Adebayo Lanionu
11/16/2005 04:11:09 PM
PHARMACOLOGIST

MEMORANDUM

Jan. 12, 2005

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-711

I concur with the determination by Dr. Siham Biade and Dr, Adebayo Laniyonu that the marketing application for Vasovist[®] (gadofosveset trisodium) is approvable from the pharmacology/toxicology perspective. The product label should be amended as recommended by Drs. Biade and Laniyonu. In particular, I concur that Pregnancy Category C is appropriate for this drug.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.

Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Kenneth Hastings
1/12/05 01:59:45 PM
PHARMACOLOGIST

Note:

This will be the Standard CDER Coversheet

TABLE OF CONTENTS

EXECUTIVE SUMMARY	1
PHARMACOLOGY/TOXICOLOGY REVIEW	13
3.1 INTRODUCTION AND DRUG HISTORY	13
3.2 PHARMACOLOGY.....	18
3.2.1 Brief summary.....	18
3.2.2 Primary pharmacodynamics.....	18
3.2.3 Secondary pharmacodynamics.....	28
3.2.4 Safety pharmacology.....	28
3.2.5 Pharmacodynamic drug interactions.....	51
3.3 PHARMACOKINETICS/TOXICOKINETICS.....	55
3.3.1 Brief summary.....	55
3.3.2 Distribution.....	56
3.3.3 Metabolism.....	60
3.3.4 Excretion.....	62
3.3.5 Pharmacokinetics.....	64
3.3.6 Pharmacokinetic drug interactions.....	71
3.3.7 Tables and figures to include comparative TK summary.....	72
3.4 TOXICOLOGY.....	72
3.4.1 Overall toxicology summary.....	72
3.4.2 Single-dose toxicity.....	75
3.4.3 Repeat-dose toxicity.....	91
3.4.4 Genetic toxicology.....	99
3.4.5 Carcinogenicity.....	111
3.4.6 Reproductive and developmental toxicology.....	111
3.4.7 Local tolerance.....	128
3.4.8 Special toxicology studies.....	130
3.5 OVERALL CONCLUSIONS AND RECOMMENDATIONS	134
3.6 APPENDIX/ATTACHMENTS.....	134

EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability

The preclinical studies conducted support safety and efficacy (measured by relaxation rates). No additional studies are required. This reviewer recommends VASOVIST™ be approved.

1.2 Recommendation for nonclinical studies

None

1.3 Recommendations on labeling

The following changes in the label would more appropriately reflect findings from preclinical studies.

[REDACTED]

b(4)

[REDACTED]

b(4)

2 Page(s) Withheld

 Trade Secret / Confidential (b4)

 ✓ Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

PHARMACOLOGY

Magnetic Resonance Angiography (MRA) is a more recent development in Magnetic Resonance Imaging (MRI). Enhancement of MR images with exogenous contrast agents such as chelates of gadolinium (Gd) has become standard clinical practice in many settings. This NDA is for the use of Vasovist at a dose of 0.03 mmol/kg, with MRA [] in adults with suspected or known vascular disease. b(4)

Gadofosveset trisodium, Vasovist™, is a trisodium salt of a gadolinium (III) complex of a substituted diethylenetriaminepentaacetate (DTPA) ligand. The basic coordination environment of the gadolinium ion is identical to that observed for the unsubstituted gadolinium diethylenetriaminepentaacetate (Gd-DTPA). In Gadofosveset trisodium, the DTPA ligand is substituted by a phosphodiester moiety, which confers the albumin binding properties to the drug. This binding is supposed to prolong plasma half-life, retain the agent in the blood pool, and thus is expected to increase the relaxation rate of water protons in plasma, and imaging for up to at least one hour after administration into humans. In vitro and in vivo mechanistic studies demonstrated that the drug binds in a specific and reversible fashion to albumin in different species, and increases water proton relaxation time, used as a surrogate for MRA imaging. Imaging was studied in 2 animal models, where Vasovist was shown to improve the images compared to pre-contrast measurement.

1. Primary pharmacodynamics

The potency of a MRI contrast agent is related to its magnetic efficiency, or relaxivity. A high relaxivity contrast agent alters the water proton relaxation time (T1) more efficiently than a low relaxivity agent does. This results in a greater proton relaxation rate ($1/T1$, in units of s^{-1}) which is detected as a brighter MR image (higher signal intensity). The relaxation rate, or $1/T1$, is used in describing contrast agent effects because, unlike T1 relaxation times, relaxation rates can be added arithmetically to assess their cumulative effects.

Vasovist (MS-325) consists of 2 isomers, A and B with an equilibrium ratio of [] Both isomers were shown to be potent, in terms of enhancement of relaxation rates, isomer B being [] more potent than isomer A. Two formulations of MS-325, sodium and [] were used in several binding and relaxivity studies. A study in rabbits (Report 105) demonstrated that relaxation rates were similar for [] and sodium formulations. b(4)

1.1. In vitro studies

In vitro protein binding of unformulated MS-325 and enhancement of relaxation rates in rat, mouse, rabbit, monkey, and human plasma, and in 4.5% (Human Serum Albumin) HSA, 4.5% dog albumin, and 4.5% pig albumin solutions were assessed. At a

concentration of 0.10 mM MS-325, which is 4 times lower than the anticipated clinical plasma level, the binding was greatest in rabbit and lowest in mouse, with the following ranking: rabbit > human > pig > 4.5% HSA > monkey > dog > rat > mouse. Differences between species were not quantitatively large and may reflect differences in plasma composition (for example, fatty acid composition was shown to affect the binding to albumin), and/or differences in the abundance of specific binding sites on albumin across species. The sponsor showed that MS-325 is bound at the same rate by human plasma and by 4.5% HSA prepared in PBS. Moreover when α_1 -acid glycoprotein and γ -globulins were present in the human plasma, chromatograms showed that MS-325 co-eluted only with albumin and not with the two other plasma proteins, suggesting that the binding in vivo occurs almost exclusively through albumin. Using fluorescent probes specific for albumin binding sites I and II, MS-325 was shown to displace dansylsarcosine with an inhibition constant K_i of $\sim 85 \mu\text{M}$ and dansyl-L-asparagine with a K_i of $1500 \pm 300 \mu\text{M}$, indicating that site II is the main binding site for MS-325. Warfarin known to bind to site I was not significantly displaced by MS-325 at concentrations of up to $400 \mu\text{M}$.

At the same concentration of 0.10 mM of MS-325, the relaxivity (relaxation rate) was greatest in humans and lowest in mouse, and was as follows: human > pig > monkey > 4.5%HAS > rabbit > dog > rat > mouse.

Using a standard dialysis system, MS-325 was removed from a 4.5% human serum albumin solution, in a bi-exponential manner. The time to remove 97% of MS-325 was projected to be 10 to 14 hours, compared to 1.42 hours for urea. According to the clinical reviewer, $73 \pm 5\%$ of the dose was recovered in the dialyzate of 6 patients following three dialysis sessions that occurred at 30 minutes, 48 hours and 96 hours after injection. After 14 days, the plasma concentration declined to $5 \pm 2\%$ of the C_{max} .

1.2. In vivo studies

In vivo binding and relaxivity studies in rat, rabbit, and monkey provided similar result. Overall, highest binding capacity was found in rabbits, and relaxivity was not dose-dependent in monkey, suggesting that the latter may not be an appropriate species for efficacy studies. Thus, rabbit would be the best species for albumin binding studies and drug interaction studies.

In a rabbit model, MS-325 produced high contrast MRA images. A dose of 0.05 mmol/kg was found optimal in terms of vessel enhancement. Doses of 0.015 mmol/kg improved visualization of vascular structures compared to pre-contrast MRA, and visualization of small vessel was brighter and lasted longer compared to Gd-DTPA. In a model of pig renal artery stenosis, vessel lumen measurements obtained with 3D MRA were not statistically different from those obtained from contrast X-ray angiography, although artifacts were observed with the MRA technique. In dynamic MRA acquisition, stenosis appearance agreed well with contrast X-ray images in the majority of cases. Steady-state contrast 3D MRA images showed venous structures, with the presence of artifacts.

2. Safety pharmacology

2.1. Cardiovascular system

The effects of a single intravenous administration of MS-325 on cardiohemodynamics and ECG were assessed in anesthetized Beagle dogs (n=5/dose), at doses of 0.1, 0.3 or 1 mmol/kg (1.8, 5.4, and 18 times the human dose based on body surface area). Signs of cardiac arrhythmia were observed in all treated groups. Unifocal PVC (bigeminy) was noted in one dog given 1.8X, 100 min post-dose. Abnormally inverted downsloping T-waves followed by multifocal PVCs were observed in another dog of same group, 30 to 120 minutes post-dose. At 0.3 mmol/kg (5.4X), one animal showed ECG unifocal PVCs and premature atrial complexes 20 and 25 min post-treatment. At 1 mmol/kg (18X), 1 dog exhibited unifocal PVCs (Bigeminy), and 1 dog showed abnormally inverted downsloping T-waves, 2.5 min post-dose. The effect observed is likely drug-related.

In the 0.1 mmol/kg group, Bazzett's QTc intervals did not vary significantly. When compared to control values, QTc intervals were increased by up to 20 ms in 1/5 dogs at 0.3 mmol/kg (5.4X the HD), and by up to 27 ms in 1/5 dogs at 1 mmol/kg (18X the HD).

b(4)

Dose-dependent and transient changes were observed for various hemodynamic parameters. A statistically significant increase was observed in central venous pressure at all doses. Cardiac output and stroke volume increase of 20-35% was observed at 2.5 to 5 min post-dose at 5.4X and 18X, but not at the 1.8X dose. Although not statistically significant, increase in left ventricular end-diastolic pressure was observed at 40 and 120 min post-dose for 1.8X and 5.4X the human dose. At 18 times the HD, mean aortic pressure and total peripheral resistance were reduced by 20-40%. Overall, cardiohemodynamic parameters changes were dose-dependent and occurred immediately after injection. The only drug-related effect observed at 1.8 times the human dose was the increase in mean central venous pressure.

In another study, 4 telemetered monkeys were each intravenously administered doses of 0.1, 0.3, 1.0, 2.0, and 3.0 mmol/kg (1, 3.2, 11, 22, and 32X the human dose based on body surface area) with a washing period of approximately 7 days between dosing. Effects were usually seen immediately after injection. Increases of 26-31% in mean heart rate were detected within 5 min at doses of 1 and 2 mmol/kg. Taken individually, the response to treatment varied between animals. In one monkey the percent changes were more marked than in the 3 other monkeys. At 0.1 mmol/kg, a 43% increase in heart rate was observed in one monkey at 10 and 15'. The value returned to baseline within 1 hour. For the 2 other monkeys, a 20% increase in HR was observed immediately post-dose and the return to baseline value was observed within 15'. The response was dose-dependent up to 1 mmol/kg. At highest doses of 2 and 3 mmol/kg, the effects on heart rate, and on pressure were less severe than those observed with low doses, perhaps because the monkeys were adapting to increasing doses of the drug.

Variations observed in systolic, diastolic and mean arterial pressure were similar, with a maximum decrease of ~35-50%. In general, the MAP, SAP, and DAS returned to normal

base values within 1 to 2 hours post treatment. More pronounced effects were observed at 0.3 mmol/kg, the treatment affecting one monkey more severely with a MAP down to ~60% at 10-15'.

All monkeys receiving 32X the human dose vomited during or immediately following dosing. Changes in hematology parameters were slight and transient.

Because the effects on cardiovascular system were all transient in this monkey study, and the dose-dependent pattern could not be demonstrated for doses higher than 1 mmol/kg, the NOAEL was established by the sponsor at >3 mmol/kg (32X the human dose based on body surface area). However, this may not reflect the toxicity, although reversible, of MS-325, therefore the NOEL was not established in this study. On the other hand, the NOEL for clinical effects can be established at 2 mmol/kg (22X the human dose).

At 23 times the human dose, MS-325 did not affect the hERG channels. MS-325 blocked hERG potassium channels by 70 and 80% at 113 and 236 times the human plasma concentration anticipated for a clinical dose of 0.03 mmol/kg. The positive control, Terfenadine data were obtained in a previous experiment.

MS-325 did not affect cardiac action potential in isolated guinea pig papillary muscle at doses of up to 2.3 times the human plasma concentration at 0.3, 1, and 3Hz. The only finding that can be attributed to MS-325 is the increase of upstroke velocity (up to 20%), which tended to decrease marginally over time in the control group (~10%).

2.2. Central nervous system

Neurotropic effects of MS-325 were assessed in the Irwin test in mouse (n=3/dose) at doses of 20, 39, 78, 156, 313, 625, 1250, and 2500 $\mu\text{mol/kg}$ (0.05, 0.11, 0.21, 0.42, 0.85, 1.7, 3.4, and 6.8 times the human dose based on body surface area). Transient (less than 2 min) signs of hyperexcitability were observed immediately following administration of doses $\geq 3.4\text{X}$ the clinical dose in all animals. At 6.8X the human dose, running fits as manifestation of a convulsive action with preceding pallor and subsequent tremor, loss of righting reflex and dyspnea was observed in 1/3 animal. NOEL for the Irwin's test was established at 1.7 times the human dose.

A single dose toxicity study of MS-325 administered via injection into the lateral ventricle of the brain in rats suggested the possible brain toxicity of the test article if given IV to patients with a compromised brain blood barrier (BBB). This toxicity consisted, amongst others, of vocalization, tremors, eye squint, and salivation.

2.3. Renal system

Three studies were conducted to evaluate the effects of MS-325 on renal function: one in normal monkey, and 2 in a renally impaired rat model. These studies were considered inadequate in part because of failure to establish a stable model (rat study), and

methodological inadequacies (monkey study). None of these studies focused on the effect of an impaired kidney on the fate of Vasovist following its administration to renally impaired animals. Such a study would have provided information on the effects of renal impairment on the drug pharmacokinetic parameters.

Overall, the renal system was inadequately investigated. According to the clinical pharmacology reviewer, human PK data showed that Vasovist elimination half-life was extended up to 70 hrs (normal: 16 hrs) in subjects with severe renal impairment, thus exposing this population to increased risk compared to the non-renally impaired subjects. Availability of clinical data ameliorates the lack of adequate preclinical data in renally impaired animal model.

3. Pharmacodynamic drug interactions

The effect of MS-325 on protein binding of digitoxin, propranolol, verapamil, and warfarin was studied in vitro in human plasma. The percent of unbound digitoxin, propranolol, and verapamil was not affected. According to the clinical pharmacology reviewer, no interaction was found between Warfarin and Vasovist in human trials, whether Vasovist was administered before or after Warfarin.

Clinically relevant concentrations (1X to 10X the maximum therapeutic dose) of Phenprocoumon did not change significantly the 1/T1 effect of MS 325. Ibuprofen was shown to decrease MS-325 percent 1/T1 by 10% whereas a reduction of ~50% was observed with naproxen. The clinical relevance of the in vitro changes in 1/T1 is yet to be demonstrated since according to the clinical pharmacology reviewer, the changes in relaxation rates measured in human subjects did not accurately predict efficacy as measured by imaging.

PHARMACOKINETICS/TOXICOKINETICS

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 $^{153}\text{Gd-MS-325}$ 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)	$\text{AUC}_{0-\text{inf}}$ ($\text{hr} \cdot \mu\text{mol/L}$)	V_{dss} (L/kg)	CI (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	6754-127	30	0.07 ± 0.02	2.86 ± 0.11	984 ± 125	0.120 ± 0.012	0.032 ± 0.004
Monkey	PTR2003-40	30	0.18 ± 0.06	4.07 ± 0.10	1190 ± 40	0.144 ± 0.006	0.025 ± 0.001
Monkey	PTR2003-40	100	0.21 ± 0.04	3.88 ± 0.06	3194 ± 70	0.167 ± 0.004	0.031 ± 0.001

b(4)

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.

TOXICOLOGY

1. Acute toxicity summary

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 body surface area) 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. The NOEL was established at 2.2 times the human dose.

In rats given a single IV dose of 0.12, 0.5, 1.0, 2.0, 3.0, and 5.0 mmol/kg (1, 2.7, 5.6, 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 (2.5 times the human dose).

2. Repeat toxicity summary

(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. 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. The 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, 0.5, 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.

3. Genetic toxicology summary

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.

4. Reproductive toxicology summary

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 the NOEL for fertility 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

respectively 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 (0.16, 1.1, and 8 times the human dose). 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 however 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 be Category C.

b(4)

5. Special toxicology summary

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 a 45' 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.

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-711

Review number: 1

Sequence number	Date	Type of submission
N000	12/12/03	NDA
N000	4/8/04	BP
N000	7/22/04	BP
N000	10/27/04	BP

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Epix Medical

Manufacturer for drug substance: Mallinckrodt

Reviewer name: Siham Biade, Ph.D.

Division name: Medical Imaging and Radiopharmaceutical
Drug Products

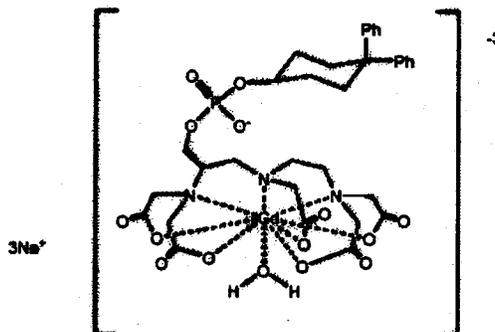
HFD #: 160

Review completion date: 07/01/2004

Drug:

Trade name: VASOVIST™
 Generic name: Gadofosveset Trisodium
 Code name: MS-32520-R
 Chemical name: Trisodium-{(2-(R)-[(4,4-diphenylcyclohexyl)phosphonooxymethyl]diethylenetriamine pentaacetato)(aquo)gadolinium(III)}
 CAS registry number: 211570-55-7, 193901-90-5 (anhydrous form)
 Molecular formula/molecular weight: C₃₃H₄₀GdN₃Na₃O₁₅P/ 975.88

Structure:



Gadofosveset Trisodium

Relevant INDs/NDAs/DMFs: IND# 51,172

Drug class: Diagnostic contrast agent with MRA

Indication: Use with magnetic resonance angiography
] in adults with
 suspected or known vascular disease

Clinical formulation: Clear, colorless to slightly yellow solution, pH 6.5 to 8.0.

b(4)

Parameter	Condition	Value
Osmolality (mOsmol/kg water)	@ 37°C	825
Viscosity (cP)	@ 20°C	3.0
Density (g/mL)	@ 25°C	1.1224

Marketed as 10 mL vials containing 10 mL of solution and 20 mL vials containing 15mL] of solution.

b(4)

Route of administration: Intravenous bolus injection, manually or by power injection, at a dose of 0.12 mL/kg (0.03 mmol/kg) over a period of time up to 30 seconds followed by a 25-30 mL normal saline flush.

Proposed use:

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Pharmacology

Report No. 104: A Safety Study to Monitor the Cardiovascular Effects of Unformulated MS-325 Given Intravenously to Cynomolgus Monkeys

Report No. 105: Effect of different formulations of unformulated MS-325 on enhancement of relaxation rates in rabbit plasma

Report No. 106: Assessment of unformulated MS-325 isomers A & B: effects on enhancement of relaxation rates in rabbit plasma

Report No. 110: Magnetic Resonance Angiography Dose Response with Unformulated MS-325 in Rabbits

Report No. 113: Comparison of Pig Renal Artery Stenosis Grading Using MS-325 Magnetic Resonance Angiography, Non-Contrast Magnetic Resonance Angiography, and Contrast X-ray Angiography

- Report No. 114:** In vitro Magnetic Resonance Imaging of MS-325 and Some Commercially Available Contrast Agents in Human Plasma
- Report No. 130:** In vitro Protein Binding and Enhancement of Relaxation Rates of Unformulated MS-325 in Human Plasma
- Report No. 131:** Specificity of Binding of Unformulated MS-325 to Plasma Proteins
- Report No. 132:** Qualitative Analysis of the In Vitro Binding Affinity Of Unformulated MS-325 To Human Serum Albumin
- Report No. 133:** In vitro Protein Binding of Unformulated MS-325 and Enhancement of Relaxation Rates in Multiple Animal Species
- Report No. 135:** Protein Binding and Enhancement of Relaxation Rates in Rat Plasma with Unformulated MS-325
- Report No. 136:** Protein Binding and Enhancement of Relaxation Rates in Rabbit Plasma with Unformulated MS-325
- Report No. 137:** Protein binding and enhancement of relaxation rates in monkey plasma with unformulated MS-325
- Report No. PTR2002-03:** Determination Of The Dialyzability Of MS-325 In The Presence Of 4.5% Human Serum Albumin Using Commercially Available Dialysis Equipment
- Report No. PTR2003-01:** Effect of MS-325 on Mast Cell Degranulation
- Report No. A04820:** Neurotropic effects of ZK236018 in the Irwin test in mice after single intravenous administration
- Report No. 7L358:** General pharmacological study of MS-325
- Report No. A09829:** Electrophysiological Examination Of MS-325 On The hERG-Mediated Potassium Current
- Report No. A09148:** Effects Of MS-325 On Cardiac Action Potential In Isolated Guinea Pig Papillary Muscle
- Report No. A10333:** Effects of a Single Intravenous Administration of MS-325 on Cardiohemodynamics and ECG in Anesthetized Beagle Dogs
- Report No. RTAW-107:** A Cardiovascular Safety Assessment of MS-325 in Telemetered Cynomolgus Monkeys. A rising dose study
- Report No. RTAW-109:** A Study to Assess the Effect Of MS-325 On Renal Function In Anesthetized Cynomolgus Monkeys
- Report No. PTR2003-12:** Assessment Of MS-325 In Drug-Induced Rodent Model Of Renal Impairment
- Report No. PTR2004-019:** Assessment of MS-325 in a Cisplatin-Induced Rat Model of Renal Impairment
- Report No. PTR2003-38:** The Binding of Fluorescent Probes to Human Serum Albumin and their Displacement by MS-325 and the Isomers of MS-325

Drug Interaction

- Report No. 180:** MS-325 Competitive Binding Pilot Study In 4.5% Human Serum Albumin: The Effect Of Common Protein-Binding Drugs On Relaxation Rates
- Report No. 401:** MS-325 Competitive Binding Pilot Study In 4.5% Human Serum Albumin: The Effect Of Phenprocoumon On Relaxation Rates
- Report No. CHW6754-102:** Effect Of MS-325 On The In Vitro Protein Binding Of Digitoxin, Propranolol, Verapamil And Warfarin In Human Plasma
- Report No. CHW6754-108:** Effect Of MS-325 On The In Vitro Protein Binding Of Warfarin In Human Plasma

Pharmacokinetic

- Report No. 301:** Plasma Kinetics Of Unformulated MS-325 In Rats (153-Gd-MS-325)
- Report No. 302:** Plasma Kinetics Of Unformulated MS-325 In Rabbits (153-Gd-MS-325)
- Report No. 309:** Plasma Kinetics Of Unformulated MS-325 In Monkeys (153-Gd-MS-325)
- Report No. 400:** MS-325 Plasma Kinetics And Relaxation Rates In Baboons
- Report No. 6754-127:** Metabolism And Pharmacokinetics Of MS-325 Following Intravenous Administration To Monkeys
- Report No. PTR 2003-46:** Pharmacokinetics Of MS-325-Gd¹⁵³ In Non-Naive Cynomolgus Monkeys

Tissue Distribution/Mass Balance

Report No. 300: Whole Body Retention And Elimination Of Unformulated MS-325 In Rats (153-Gd-MS-325)

Report No. 303: Unformulated MS-325 Biodistribution And Excretion In Monkeys (153-Gd-MS-325)

Report No. RTAZ-0114: Limited Tissue Distribution And Mass Balance Of 153gd-MS-325 Following A Single Intravenous Administration To Non-Naive Cynomolgus Monkeys

Report No. RTAZ-113: Assessment Of Breast Milk Gadolinium Content Of Intravenously Administered 153 Gd-MS-325 In Lactating Rats

Metabolism

Report No. 320: Metabolism Of Unformulated MS-325 In Rat Bile, Urine And Blood Following IV Administration (153-Gd-MS-325)

Report No. 321: Metabolism Of Unformulated MS-325 In Monkey Blood, Urine And Feces Following IV Administration (153-Gd-MS-325)

Report No. PTR2003-02: MS-325 In Human Liver Microsomes

Report No. 6754-127: Metabolism And Pharmacokinetics Of MS-325 Following Intravenous Administration To Monkeys

Acute Safety Assessment

Report No. 210: A Single Dose Toxicity Study Of MS-325 In The Rat Via Intravenous Administration

Report No. 211: A Single Dose Toxicity Study Of MS-325 In The Cynomolgus Monkey Via Intravenous Administration

Report No. A06874: Systemic Tolerance Study In Rats After Single Intravenous Administration Of ZK236018 (MS-325) Over A Period Of 4 And 15 Days

Report No. 6622-102: Single Dose Intravenous Toxicity Study Of MS-32516 In Rats

Report No. RTAW 101: Single Dose Toxicity Study Of Angiomark (MS-325) Administered Via Injection Into The Lateral Ventricle Of The Brain In Sprague Dawley Rat]

Subacute Safety Assessment

Report No. 214: A 2-week toxicity study of MS-325 in the rat via intravenous administration with a 28-day recovery period

Report No. 215: A-2 week toxicity study of MS-325 in the cynomolgus monkey via intravenous administration with a 28-day recovery period

Mutagenicity

Report No. 200: Mutagenicity Test On MS-325-Dpi In Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay With Confirmatory Assay

Report No. 202: Mutagenicity Test On MS-325-Dpi In An In Vivo Mouse Micronucleus Assay

Report No. 19423-0-409OECD: Mutagenicity Test On MS-325-Dpi & MS-325-Dp2 In Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay

Report No. 19423-0-437OECD: Mutagenicity Test On MS-325-Dpi & MS-325-Dp2 Measuring Chromosomal Aberrations In Chinese Hamster Ovary (CHO) Cells

Report No. 19423-0-455OECD: Mutagenicity Test On MS-325-Dpi & MS-325-Dp2 In An In Vivo Mouse Micronucleus Assay

Reproduction Toxicity

Report No. A10565: Study Of Fertility And Early Embryonic Development To Implantation Of Sprague Dawley Rats With MS-325 By Intravenous Administration To The Animals Of The Fo-Generation

Report No. A10557: Study Of Embryo Fetal Development In Rats With MS-325 By Intravenous Administration

Report No. A10556: Study Of Embryo Fetal Development In Rabbits With MS-325 By Intravenous Administration

Report No. 14707-01: Study For Effects On Pre/Postnatal Development Including Maternal Function In Rats With MS-325 After Daily Intravenous Administration To The Dams Of The Fo Generation From Day 6 Of Gestation To Day 21 Post Partum

Local Tolerance & Other Toxicity

Report No. 204: In Vitro Hemolytic Potential & Blood Compatibility Testing Of MS-325 In Human Whole Blood & Plasma

Report No. 205: Guinea Pig Maximization Test With MS-325 (Method Of Magnusson And Kligman)

Report No. 206: Acute Intravenous And Perivascular Irritation Study Of MS-325 In Rabbits

Report No. FTR2003-01: Effect Of MS-325 On Mast Cell Degranulation

Report No. 5149-44203: Determination Of Potential Immunomodulating Effects Of MS-325 By Means Of The Plaque-Forming Cell Assay And Lung Macrophage Phagocytosis Test After 18 Days Of Intravenous Injection Of Male Wistar Rat.

Studies not reviewed within this submission:

b(4)

b(4)

3.2 PHARMACOLOGY

3.2.1 Brief summary

Gadofosveset trisodium, Vasovist™, binding to human serum albumin is supposed to prolong plasma half-life, retain the agent in the blood pool, and thus is expected to increase the relaxation rate of water protons in plasma, and imaging for up to at least one hour after administration into humans. Mechanistic studies conducted in vitro and in vivo, demonstrated that the drug binds in a specific and reversible fashion to albumin in different species, and increases water proton relaxation time, used as a surrogate for MRA imaging. Imaging was studied in 2 animal models, where Vasovist was shown to improve the images compared to pre-contrast measurement.

3.2.2 Primary pharmacodynamics

The potency of a MRI contrast agent is related to its magnetic efficiency, or relaxivity. A high relaxivity MRI contrast agent alters the water proton relaxation time (T1) more efficiently than a low relaxivity agent does. This results in a greater proton relaxation rate ($1/T1$, in units of s^{-1}) which is detected as a brighter MR image (higher signal intensity). The relaxation rate, or $1/T1$, is used in describing contrast agent effects because, unlike T1 relaxation times, relaxation rates can be added arithmetically to assess their cumulative effects.

In earlier stages of drug development, MS-325 was formulated as _____, but later was developed as a sodium salt, contained in the clinical formulation. Therefore many mechanistic studies included both _____ and sodium formulations tested side by side. A study in rabbits (Report 105) demonstrated that relaxation rates were similar for _____ and sodium formulations.

b(4)

3.2.2.1 IN VITRO PHARMACOLOGY STUDIES

Report 106: Assessment of unformulated MS-325 isomers A & B: effects on enhancement of relaxation rates in rabbit plasma. MS-325-Isomer A: Lot KM74-99

— MS-325-Isomer B: Lot KM74-104 — MS-325-DP1: Lot 00445 —
GdDTPA: Lot TJM24-132.

b(4)

Study objective: Comparison of unformulated isomers of MS-325 (Isomer A and Isomer B at the equilibrium ratio of — in female New Zealand White rabbits administered IV injection of 0.025 mmol/kg MS-325 or 0.10 mmol/kg GdDTPA. $\Delta 1/T1$ AUC values were determined as enhancement of relaxation rates.

b(4)

Results: AUC (MS-325) is 3-4X higher than AUC (GdDTPA) at all times and Isomer B is — fold more potent than Isomer A. The sponsor concludes that Isomers A and B of MS-325-DP1 are both potent.

b(4)

Reviewer's comments: Isomers A and B have relatively similar AUCs. However, when tested combined, they are more potent than the equivalent amount of MS-325.

Report 114: *In vitro* Magnetic Resonance Imaging of MS-325 and Some Commercially Available Contrast Agents in Human Plasma. MS-325-DP1, 250.56 mM, Lot 512016.

Study objective: Comparison of *in vitro* magnetic resonance imaging of MS-325 at a concentration of 0.10 to 40 mM (clinical plasma concentration: 0.43 mM), Magnevist 500 mM stock solution, Omniscan 500 mM, and ProHance 500 mM, in 3 mL human plasma phantoms. Regions of interest (ROIs) were determined inside the phantom, pixels within an ROI were averaged to produce a mean signal intensity for that ROI; the mean signal intensity for each sample was used as surrogate of MR angiographic imaging.

Results: Overall, the signal intensity was similar for all compounds except for MS-325. In the range of 0.10 to 2 mM, MS-325 signal intensity was approximately 2 fold greater than any other agent was. The maximum in the mean signal intensity (~1200) was reached with 1.5 to 3 mM of MS-325, and with 5 to 10 mM for the other contrast agents. Minimum in signal intensity was reached at 40 mM MS-325 and 50 mM for other agents. At 0.4 mM MS-325, anticipated clinical concentration, the signal intensity was about 75% of the maximum. At 4 mM of MS-325 and 10 mM for the 3 other agents, the signal intensity started to decline. This dual response (two-component) was attributed by the sponsor to a second relaxation time specific to gadolinium agents, which relaxes the T1-generated MRI signal, before the machine could detect the signal. The effect is more pronounced with MS-325, because its R1 and R2 relaxivities are higher than those of the other tested agents. The sponsor concludes that over the clinical range of 0.10 to 2 mM, MS-325 is more potent than the other contrast agents tested in this study are.

Reviewer's comments: The sponsor compared signal intensity for equal concentrations of Omniscan, Magnevist, ProHance and MS-325. However, the approved dose for Omniscan, Magnevist, and ProHance is 0.1 mmol/kg, and at this dose, the anticipated plasma concentration is 1.4 mM, *i.e.* 3.2 times that of MS-325 for a clinical dose of 0.03 mmol/kg. Thus, at equivalent clinical concentrations, 1.5 to 3 mM of MS-325 and 5 to 10 mM for the other contrast agents, an equal maximum signal intensity would be reached. Furthermore, in the range of 0.10 to 2 mM, MS-325 signal intensity was approximately 2

fold greater than the other agents, when it should be at least 3.2 times higher. Therefore, superiority of MS-325 may only result from its ability to bind to human serum albumin. The sponsor states that because the commercially available agents tested in this study do not bind to serum proteins, they rapidly leak to the extravascular space, hence the higher background and the weaker signal intensity observed with these compounds. On the other hand, the phantom used in this study did not simulate the extravascular system.

Report 130: *In vitro* Protein Binding and Enhancement of Relaxation Rates of Unformulated MS-325 in Human Plasma. ¹⁵³Gd-labeled-MS-325 (10 mM): Lot SD87-50; ¹⁵³Gd-labeled-GdDTPA (10 mM): Lot SD87-87; MS-325-DP1 (250.56 mM): Lot 512016; GdDTPA (300 mM): Lots SD87-87 and SD63-174.

Study objective: To evaluate *in vitro* protein binding and enhancement of relaxation rates of unformulated MS-325 in human plasma and to determine the reversibility of the binding of MS-325 to human plasma. This study would also assess the effects of the binding, evaluated by relaxivity 1/T1 measurements, on efficiency. The binding study was performed in human plasma at concentrations ranging from 0.008 mM to 20 mM of MS-325, and from 0.02 mM to 14 mM for GdDTPA. Mean total and unbound CPM were determined in human plasma by ultrafiltration (Unbound and bound fractions) and mean human plasma 1/T1 values measured at 20 MHz.

Results: MS-325 binds predominantly to human plasma with a bound fraction of ~75% at concentrations ranging from 0.008 to 2 mM. At 0.2 mM, the fraction bound starts to decline in a continuous fashion until 20 mM. GdDTPA does not bind to plasma.

-In PBS, both agents showed linear concentration-dependency in relaxation rates, with MS-325 values 1.6 fold greater than Gd-DTPA values at any given concentration.

-In plasma, 1/T1 was concentration-dependent for GdDTPA at all doses, and for MS-325 only at doses lower than 0.10 mM. Influence of protein binding on relaxivity was shown in a plot of 1/T1 versus total gadolinium plasma concentration, where effect of MS-325 on 1/T1 is greater than that of Gd-DTPA. The sponsor concluded that, because of the binding properties of MS-325, the relaxation rates increase was not linear for concentrations of MS-325 greater than 0.1 mM. This indicates that below 0.1 mM the MS-325 is entirely bound to plasma proteins.

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

Report 132: Qualitative Analysis of the *In Vitro* Binding Affinity of Unformulated MS-325 to Human Serum Albumin. ¹⁵³Gd-MS-325 (10 mM): Lot SD87-50; MS-325-DP1 (250.56 mM): Lot 512016 (dilution SD-87-46).

Study objective: Evaluation of *in vitro* binding of unformulated MS-325 in 4.5% human serum albumin, dissolved in 10 mM PBS, at a concentration of 0.06-50 mM. Protein binding was assessed by ultrafiltration by determining CPM total from supernatant, CPM unbound from filtrate). Fraction unbound=CPM unbound/ CPM total.

Results: Between 0.06 and 2 mM, MS-325 fraction bound is 90% to ~60%. As the concentration of Gadolinium increases, the fraction bound decreases, and the average number of molecule bound to HSA increases. The sponsor concludes that with increasing concentrations of MS-325, the average number of molecules bound to HSA increases, suggesting that even when the fraction bound decreases, HSA continues to bind drug.

Reviewer's comments: Approximately 30% of MS-325 remains bound to HSA even at 50 mM MS-325.

Report 133: *In vitro* Protein Binding of Unformulated MS-325 and Enhancement of Relaxation Rates in Multiple Animal Species. ¹⁵³Gd-MS-325, 10 mM: Lot SD87-50; ¹⁵³Gd-MS-325, 9.8 mM: Lot KM-52-16; MS-325, ~10 mM: Lots KM52-114, KM39-206, SD63-198, SD87-20.

b(4)

Study objective: Evaluation of *in vitro* protein binding of unformulated MS-325 and enhancement of relaxation rates in rat, mouse, rabbit, monkey, human (3 individuals), 4.5% HSA, 4.5% dog albumin, and 4.5% pig albumin, at a final concentration of 0.10 mM of MS-325. Protein binding was determined by ultrafiltration and relaxivity titration by measurement of proton relaxation times determined at 20 MHz.

Results: MS-325 is bound at the same rate by human plasma and by 4.5% HSA. In the binding studies, the fraction bound was highest in rabbits and lowest in mouse, with the following ranking: rabbit>human>pig>4.5% HSA>monkey>dog>rat>mouse. Relaxivity R1 was highest in humans and lowest in mouse, and was as follows: human>pig>monkey>4.5%HSA>rabbit>dog>rat>mouse. The sponsor concludes that with the exception of the rabbit, the fraction bound and the enhancement of relaxivity appears to correlate in all species.

Reviewer's comments: Different lots of drugs were used, with different formulations containing either sodium or [redacted] Although Study 105 shows that for rabbits, [redacted] and sodium MS-325 have the same binding, no good correlation was found between relaxivity and binding in the rabbit. Only one concentration 0.1 mM of MS-325, which is well below the anticipated clinical plasma level, was used in the binding studies; additional concentrations, covering at least the clinical range, would have provided a better comparison between species. The sponsor concludes that because 4.5% HSA and human plasma bind the drug at the same rate, it indicates that the drug binds exclusively to albumin. Such statement should be confirmed in comparative studies using other plasma proteins. This is probably the reason why the binding was different between species, in addition to the fact that the number of albumin binding sites varies across species.

b(4)

Report 131: Specificity of Binding of Unformulated MS-325 to Plasma Proteins
Lot TJM24-185 (formulated with [redacted])

b(4)

The objective of this study was to determine if MS-325 is bound specifically to human serum albumin, using anion exchange chromatography.

Study design: The study was performed in fresh plasma obtained from a volunteer, and spiked with ^{153}Gd -labeled MS-325 at a concentration of 100 μM . Labeled-MS-325 was added to the chromatographic eluent at a final concentration of 5 μM , corresponding to the estimated free concentration (assuming 95% binding) for a total concentration of 100 μM MS-325 in plasma. When the plasma sample is injected onto the column, the major plasma components are separated, while MS-325 binds non-covalently to the components until equilibrium with the free drug concentration is reached. Increase in activity associated with the binding of labeled MS-325 is detected using a gamma detector.

Results: The chromatogram trace shows a positive peak of ^{153}Gd at the same retention time as HSA. Samples of α_1 -acid glycoprotein and γ -globulins were also injected onto the column resulting in their separation from HSA. There was no co-elution with either protein. Injection of unlabeled plasma resulted in a similar profile. However, a negative peak was detected at ^{153}Gd retention time of MS-325, indicating that the MS-325 is removed from the mobile phase and is binding to the injected plasma. The sponsor concludes that because all the activity is observed under the HSA peak, MS-325 binds exclusively to HSA.

Reviewer's comments: This study shows that in total plasma MS-325 binds preferentially to HSA. This was further confirmed by using two other plasma proteins known to bind drugs. MS-325 did not co-elute with either. Under the experimental conditions of this study MS-325 appears to bind specifically to HSA.

Report PTR2002-03: Determination Of The Dialyzability Of MS-325 In The Presence Of 4.5% Human Serum Albumin Using Commercially Available Dialysis Equipment. Lot 3095p19.

The objective of this study was to determine the efficacy of a standard clinical dialysis system in the removal of MS-325 from a 4.5% human serum albumin solution in order to determine whether MS-325 could be administered to patients undergoing hemodialysis.

Study design: A blood circuit was made of 4.5% HSA and 1 g/L urea in a volume of 6 L with normal saline. The solution was used to prime the tubing, and a 6L flask, resting on a magnetic stirrer device, acted as a reservoir and represented the corporal blood compartment. The solution circulated at 300 mL/min through the dialyzer. At time -12 min, 10.3 mL of a MS-325 stock solution was introduced into the blood circuit to achieve the intended clinical plasma concentration of 0.43 mM. Solutions collected pre- and post dialysis were analyzed for their concentrations in urea, used as a standard for the efficacy of the dialysis system, and MS-325.

Results: Urea was cleared in a time dependent manner, with a clearance rate of ~227 mL/min, and the time to remove 97% of the urea was projected to be 1.42 hours. MS-325 was cleared in a bi-exponential manner. The clearance rate was ~ 47 mL/min and the time to remove 97% of MS-325 was projected to be 10 hrs based on the absolute gadolinium concentration data, or 14.2 based on $AUC_{0-\infty}$. Urea was cleared 5 times faster than MS-325, and that was attributed to the binding of MS-325 to albumin. It was concluded that since MS-325 is dialyzable from 4.5% human albumin serum using commercially available dialysis device with 97% of the agent removed in 14.2 hours, MS-325 may be used in dialysis patients.

Reviewer's comments: The sponsor used a solution of human serum albumin to demonstrate that MS-325 can be cleared from plasma using a standard dialysis system. MS-325 was removed from a 4.5% human serum albumin solution, in a bi-exponential manner. The time to remove 97% of MS-325 was projected to be 10 to 14 hours, compared to 1.42 hours for urea. Vasovist dialysability was evaluated in humans, and results have shown that 3 successive dialysis sessions were necessary to eliminate [data from clin pharm] MS-325.

Report PTR2003-38: The Binding of Fluorescent Probes to Human Serum Albumin and their Displacement by MS-325 and the Isomers of MS-325

The objective of this study was to determine the site on albumin to which MS-325 binds, therefore providing information on potential interaction of MS-325 with other albumin binding molecules.

Human serum albumin (HSA) is an abundant transport protein found in plasma that binds a wide variety of drugs in two primary binding sites (I and II) and can have a significant impact on their pharmacokinetics. Site I is a large site on domain IIA capable of binding warfarin, dansyl-L-asparagine (Dasn) among others, which can be used as fluorescent probes to study this site. Site II is located on domain IIIA and binds diazepam, ibuprofen, naproxen, among others, and dansylsarcosine, which can be used as fluorescent probe to study this site.

Study design: The fluorescence of 2 μ M solutions of each of the fluorescent probes in PBS was measured with appropriate filters, while increasing the concentrations of HSA from 0 to 50 μ M. Dissociation constants were determined for each of the probes. A solution containing the fluorescent probe, the human serum albumin, and 400 μ M of test article (nothing, MS-325, Isomer A, Isomer B, or ibuprofen) was prepared. The experiment with MS-325 was performed 5 times with DS, Dasn, and twice with warfarin. The experiments with the isomers and ibuprofen were performed once with DS and Dasn.

Results: Dissociation constants K_d for DS, DASn, and warfarin were determined at 37C in PBS, and were ~5, 14, and 5 μ M respectively. MS-325 displaced DS with an inhibition constant K_i of ~85 μ M and Dasn with a K_i of 1500 ± 300 μ M, suggesting that the main binding of MS-325 is at site II. Both isomers were capable of displacing DS with similar

K_i values (~85 for Isomer A and ~76 μM for Isomer B). Likewise, they showed very weak displacement of Dasn, although higher affinity for binding site I was noted for Isomer B. Warfarin was not significantly displaced by MS-325 at concentrations of up to 400 μM

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

3.2.2.2. IN VIVO STUDIES

Report 135: Protein Binding and Enhancement of Relaxation Rates in Rat Plasma with Unformulated MS-325.

Report 136: Protein Binding and Enhancement of Relaxation Rates in Rabbit Plasma with Unformulated MS-325.

Report 137: Protein binding and enhancement of relaxation rates in monkey plasma with unformulated MS-325.

Study design: Sodium _____ MS-325 formulations and GdDTPA were used in the 3 studies and all MS-325 data were combined based on results of Study 105. Animals were administered bolus intravenous injections of MS-325 or Gd-DTPA, as follows: 1) *Sprague Dawley rats*: 0.025, 0.05, and 0.10 mmol/kg MS-325, and 0.05 and 0.10 mmol/kg GdDTPA, 2) *New Zealand white rabbits*: 0.025 and 0.10 mmol/kg MS-325, and 0.10 mmol/kg Gd DTPA, and 3) *Cynomolgus monkeys*: 0.10 mmol/kg MS-325 _____ and Gd-DTPA, and 0.025 mmol/kg MS-325 (sodium). Proton relaxation times were determined at 20 MHz using a _____ nuclear magnetic resonance process analyzer, and protein binding was determined by ultrafiltration

b(4)

Results:

1. Rats

At 0.025, 0.05, and 0.10 mmol/kg MS-325, $\Delta 1/T1$ at 5 and 10 min, are significantly higher than those obtained for GdDTPA at 0.1 mmol/kg. For an equal dose of 0.1 mmol/kg, MS-325 values are between 5.5-6 times greater than GdDTPA. However, for clinical equivalent doses, 0.025 mmol/kg for MS-325 and 0.1 mmol/kg Gd-DTPA, plasma $\Delta 1/T1$ were more similar. The fraction of MS-325 bound to plasma protein at 5, 20, and 60 min, is 0.775, 0.840, and 0.905 respectively. The sponsor concludes that rats are a good model for measuring the relaxation rates.

2. Rabbits

MS-325 dose-corrected plasma $\Delta 1/T1$ AUC was significantly greater than that of GdDTPA at 0.025 and 0.10 mmol/kg MS-325, including at clinically equivalent doses (0.025 mmol/kg MS-325 and 0.10 mmol/kg Gd-DTPA) where an approximately 20 fold difference was noted. Fraction bound is close to 97-99 %, which confirms the in vitro results where the comparison between different species showed that the greatest fraction bound was found in rabbit plasma. A slight decrease in the fraction bound was observed with higher doses of MS-325, which is expected to occur since the ratio bound drug/free drug will tend to decline when the free fraction is increased. This does not necessarily mean that the binding is less significant.

3. Monkeys

No significant difference was observed between $\Delta 1/T1$ at 0.025 and 0.1 mmol/kg. This is in contrast with rat and rabbit results, where a dose-dependent increase was observed. This may be due to biological differences between the 2 species as well as the anesthetizing procedures, which were different between the two studies. $\Delta 1/T1$ was greater than Gd-DTPA at all doses studied.

Reviewer's comments: The sponsor combined the results of sodium formulated-MS-325 with results obtained with _____ formulated MS-325, based on the fact that the potency was similar between the 2 formulations in a rabbit study. There is no evidence that the latter would be true in other species such as the ones used in this study. At 0.4 mM of MS-325 (anticipated clinical plasma concentration), $\Delta 1/T1$ is slightly greater than for Gd-DTPA at clinical equivalent concentration (1.4 mM).

Fraction bound to plasma protein was approximately 80-90 % for the sodium formulation, and 70-80% for the _____ formulation. This slight difference may not be biologically significant. Overall, binding was significant in the 3 animal models, with the highest values in rabbits.

Report 110: Magnetic resonance angiography dose-response with unformulated MS-325 in rabbits

Study design: This study evaluated the degree of vessel enhancement on magnetic resonance angiography (MRA). MS-325 was injected as an IV bolus to female New Zealand White rabbits, at 0.015, 0.025, 0.05, and 0.10 mmol/kg, respectively 0.16, 0.3, 0.5, and 1 time the intended clinical dose based on BSA. MS-325 was formulated with sodium or _____, and was compared to GdDTPA at 0.1 mmol/kg.

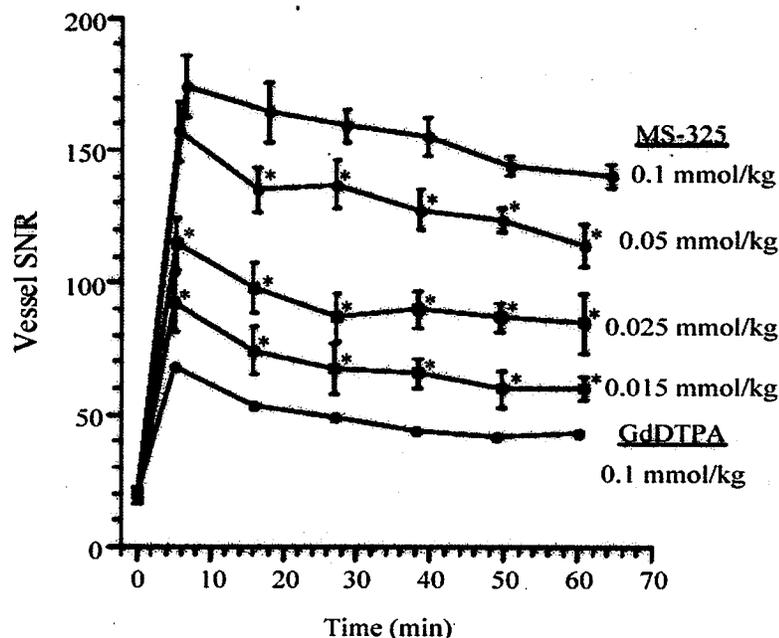
Image acquisition: The volume acquired included the lower extremities between the femoral artery bifurcation and the ankle. A flip angle of 60° was chosen for all pre- and post-contrast image acquisitions so that quantitative determination of MS-325-induced signal enhancement could be determined. A lower flip angle of 20°, which is optimized for MRA without a contrast agent, was also evaluated in some animals prior to MS-325 administration. The total image acquisition was approximately 9.5 minutes and was repeated for up to 60 minutes post-injection. The effect of contrast agent administration on enhancement of fine vessels (secondary and tertiary femoral branches) was assessed qualitatively. The effect of contrast agent administration on enhancement of large (femoral) vessels was assessed quantitatively. The signal intensity from the six ROIs of each tissue type was averaged to produce a mean signal intensity for muscle and vessel at each time point for the animal.

Results: On pre-injection imaging, using a flip angle of 20°, only the major vasculature (femoral and iliac arteries) is visible. When using a flip angle optimized for post-contrast imaging (60°), a decrease in vessel-to-background contrast was noted. Images obtained five minutes post-contrast with a flip angle of 60° at doses of 0.015, 0.025, 0.05 and 0.10 mmol/kg, showed that large vessel contrast and small vessel definition is increased in

comparison to image pre-contrast obtained using a flip angle of 20°.

The effects of Gd-DTPA at 0.1 mmol/kg and MS-325 at 0.025 mmol/kg were compared on vessel visualization as a function of time. With MS-325, small and large vessel visualization exists even up to one hour post-injection, whereas only the 5-minute post-contrast GdDTPA image shows significant vessel enhancement while small vessel visualization is poor.

Figure: MS-325 and GdDTPA MRA Signal to Noise Ratio (SNR) in Vessels



*p < 0.05 compared to 0.10 mmol/kg MS-325 at corresponding time point.

Conclusion: MS-325 produces high quality MRA images with persistent enhancement using a standard MRA protocol. In this study, a dose of 0.05 mmol/kg was found to be optimal in terms of vessel enhancement using the pulse sequence selected.

Reviewer's comments: In this study, MS-325 at doses as low as 0.015 mmol/kg allows improved visualization of vascular structures over that obtained with pre-contrast MRA. It also allows a brighter and longer visualization of small vessel than Gd-DTPA. The results were dose-dependent for MS-325 within 5 minutes of injection, differences were noted beyond that time interval. Although a dose dependent increase was observed in the intensity, the magnitude of the increase between the doses did not appear to be large, probably because of the MS-325 albumin binding properties.

Report 113: Comparison of Pig Renal Artery Stenosis Grading using MS-325 Magnetic Resonance Angiography, Non-contrast Magnetic Resonance

Angiography, and Contrast X-ray Angiography

The objective of this study was to compare the degree of stenosis determined by contrast x-ray angiography to that determined by magnetic resonance angiography (MRA) with and without MS-325.

Study design: Female Yorkshire pigs (n=8) were anesthetized with a ketamine (20 mg/kg), xylazine (2 mg/kg) and atropine (8 µg/kg). The animals were intubated and mechanically ventilated on halothane (1.25-1.5%) and 100% oxygen. A laparotomy was performed and one (n = 4) or two (n = 4) MR-compatible ameroid occluders (inner diameter = 3.5-4.0 mm) were placed around the left renal artery just distal to the aorta. This technique is expected to result in increasing levels of stenosis over a period of a few weeks. The abdomen was closed and the animals were allowed to recover.

-Contrast x-ray angiography: was performed just prior to surgery and at week 1 (n = 8), weeks 3-4 (n = 7), and weeks 5-6 (n = 7) post-surgery. A femoral cut-down was performed and a catheter inserted, through which Renografin ® was administered selectively to each renal artery.

-MR imaging: Animals were imaged in a supine position using a standard phased-array coil on [] whole body imager. Unless otherwise stated, image volumes included the mid-abdominal aorta, renal arteries, and kidneys.

b(4)

Results:

-One animal had complete occlusion at week 1 post-surgery, presumably due to thrombosis or spasm. This animal was eliminated from subsequent analysis.

-Contrast X-ray angiographic images provided high resolution, high contrast visualization of normal and stenotic arteries. This confirmed that the renal artery stenosis model consistently produced a gradually increasing degree of stenosis up to complete occlusion.

-Several artifacts were observed with MRA technique including annular or crescent profile (bright ring or broken ring surrounding signal void), diffuse profile (severely non-circular), and displaced profile (severely off-center).

The dynamic contrast 3D MRA acquisitions showed significant vascular enhancement. The images had a high degree of arterial weighting with only minor enhancement of venous structures. Arterial contrast-to-noise was significant, and structures with diameters as low as 1 mm, were visualized. The renal artery stenosis was visible in detail, with no severe signal loss apparent within or distal to the stenosis. Stenosis appearance agreed well with contrast x-ray images in the majority of cases.

The steady-state contrast 3D MRA images showed vascular enhancement. The images also had vessel to background contrast, within and distal to the renal artery stenosis. Venous structures were visible. However, motion artifact was present and appeared as slight blurring and ghosting of structures.

Vessel lumen measurements obtained with 3D MRA were not statistically different than those obtained from contrast X-ray angiography.

Conclusion: Due to its relatively high relaxivity and to its intravascular confinement, MS-325 should be appropriate for MRA of stenotic vessels in dynamic contrast imaging with less conclusive results in steady-state contrast imaging. Given that it was possible to image lumen diameters on the order of 1 mm, which represents the limit of current surgical intervention, MS-325-enhanced MRA may have potential in clinical diagnostic imaging.

Reviewer's comments: Agree with overall conclusions

3.2.3 Secondary pharmacodynamics

3.2.4 Safety pharmacology

Human Dose Multiples in safety and toxicology studies are calculated based on body surface area (BSA), unless otherwise stated.

Safety pharmacology summary:

1. Cardiovascular system

The effects of a single intravenous administration of MS-325 on cardiohemodynamics and ECG were assessed in anesthetized Beagle dogs (n=5/dose), at doses of 0.1, 0.3 or 1 mmol/kg (1.8, 5.4, and 18X the human dose based on body surface area (BSA)). Signs of cardiac arrhythmia were observed in all treated groups. Unifocal PVC (bigeminy) was noted in one dog given 1.8X, 100 min post-dose. Abnormally inverted downsloping T-waves followed by multifocal PVCs were observed in another dog of same group, 30 to 120 minutes post-dose. At 0.3 mmol/kg (5.4X), one animal showed ECG unifocal PVCs and premature atrial complexes 20 and 25 min post-treatment. At 1 mmol/kg (18X), 1 dog exhibited unifocal PVCs (Bigeminy), and 1 dog showed abnormally inverted downsloping T-waves, 2.5 min post-dose. The effect observed is likely drug-related.

In the 0.1 mmol/kg group, Bazett's QTc intervals did not vary significantly. When compared to control values, QTc intervals were increased by up to 20 ms in 1/5 dogs at 0.3 mmol/kg (5.4X the HD), and by up to 27 ms in 1/5 dogs at 1 mmol/kg (18X the HD).

b(4)

Dose-dependent and transient changes were observed for various hemodynamic parameters. A statistically significant increase was observed in central venous pressure at all doses. Cardiac output and stroke volume increase of 20-35% was observed at 2.5 to 5 min post-dose at 5.4X and 18X, but not at the 1.8X dose. Although not statistically significant, increase in left ventricular end-diastolic pressure was observed at 40 and 120 min post-dose for 1.8X and 5.4X the human dose. At 18 times the HD, mean aortic pressure and total peripheral resistance were reduced by 20-40%. Overall, cardiohemodynamic parameters changes were dose-dependent and occurred immediately

after injection. The only drug-related effect observed at 1.8 times the human dose was the increase in mean central venous pressure.

In another study, 4 telemetered monkeys were each intravenously administered doses of 0.1, 0.3, 1.0, 2.0, and 3.0 mmol/kg (1, 3.2, 11, 22, and 32X the human dose based on body surface area) with a washing period of approximately 7 days between dosing. Effects were usually seen immediately after injection. Increases of 26-31% in mean heart rate were detected within 5 min at doses of 1 and 2 mmol/kg. Taken individually, the response to treatment varied between animals. In one monkey the percent changes were more marked than in the 3 other monkeys. At 0.1 mmol/kg, a 43% increase in heart rate was observed in one monkey at 10 and 15'. The value returned to baseline within 1 hour. For the 2 other monkeys, a 20% increase in HR was observed immediately post-dose and the return to baseline value was observed within 15'. The response was dose-dependent up to 1 mmol/kg. At highest doses of 2 and 3 mmol/kg, the effects on heart rate, and on pressure were less severe than those observed with low doses, perhaps because the monkeys were adapting to increasing doses of the drug.

Variations observed in systolic, diastolic and mean arterial pressure were similar, with a maximum decrease of ~35-50%. In general, the MAP, SAP, and DAS returned to normal base values within 1 to 2 hours post treatment. More pronounced effects were observed at 0.3 mmol/kg, the treatment affecting one monkey more severely with a MAP down to ~60% at 10-15'.

All monkeys receiving 32X the human dose vomited during or immediately following dosing. Changes in hematology parameters were slight and transient.

Because the effects on cardiovascular system were all transient in this monkey study, and the dose-dependent pattern could not be demonstrated for doses higher than 1 mmol/kg, the NOAEL was established by the sponsor at >3 mmol/kg (32X the human dose). However, this may not reflect the toxicity, although reversible, of MS-325, therefore the NOEL was not established in this study. On the other hand, the NOEL for clinical effects can be established at 2 mmol/kg (22X the human dose).

At 23 times the human dose, MS-325 did not affect the hERG channels. MS-325 blocked hERG potassium channels by 70 and 80% at 113 and 236 times the human plasma concentration anticipated for a clinical dose of 0.03 mmol/kg. The positive control, Terfenadine data were obtained in a previous experiment.

MS-325 did not affect cardiac action potential in isolated guinea pig papillary muscle at doses of up to 2.3 times the human plasma concentration at 0.3, 1, and 3Hz. The only finding that can be attributed to MS-325 is the increase of upstroke velocity (up to 20%), which tended to decrease marginally over time in the control group (~10%).

2. Central nervous system

Neurotropic effects of MS-325 were assessed in the Irwin test in mouse (n=3/dose) at doses of 20, 39, 78, 156, 313, 625, 1250, and 2500 $\mu\text{mol/kg}$ (0.05, 0.11, 0.21, 0.42, 0.85, 1.7, 3.4, and 6.8 times the human dose based on BSA). Transient (less than 2 min) signs of hyperexcitability were observed immediately following administration of doses $\geq 3.4\text{X}$ the clinical dose in all of the animals. At 6.8X the human dose, running fits as manifestation of a convulsive action with preceding pallor and subsequent tremor, loss of righting reflex and dyspnea was observed in 1/3 animal. NOEL for the Irwin's test was established at 1.7 times the human dose.

A single dose toxicity study of MS-325 administered via injection into the lateral ventricle of the brain in rats suggested the possible brain toxicity of the test article if given IV to patients with a compromised brain blood barrier (BBB). This toxicity consisted, amongst others, of vocalization, tremors, eye squint, and salivation.

3. Renal system

Three studies were conducted to evaluate the effects of MS-325 on renal function: one in normal monkey, and 2 in a renally impaired rat model. These studies were considered inadequate in part because of failure to establish a stable model (rat study), and methodological inadequacies (monkey study). None of these studies focused on the effect of an impaired kidney on the fate of Vasovist following its administration to renally impaired animals. Such a study would have provided information on the effects of renal impairment on the drug pharmacokinetic parameters.

Overall, the renal system was inadequately investigated. According to the clinical pharmacology reviewer, human PK data showed that Vasovist elimination half-life was extended up to 70 hrs (normal: 16 hrs) in subjects with severe renal impairment, thus exposing this population to increased risk compared to the non-renally impaired subjects. Availability of clinical data ameliorates the lack of adequate preclinical data in renally impaired animal model.

Cardiovascular and pulmonary effects:

Report A10333: Effects of a single intravenous administration of MS-325 on cardiohemodynamics and ECG in anesthetized Beagle dogs (conducted in 2001 by Schering AG, Berlin, Substance code number: ZK 236018, 3095p19, 0.25 mmol/L)

Volume #, and page #:	Vol. 20, page 5-737
Conducting laboratory and location:	Schering AG, Berlin
Date of study initiation:	June 14, 2001
GLP compliance:	Yes () No (x)
ICH guidelines compliant	
QA report:	Yes () No (x)

Drug, lot #, and % purity: Gadofosveset Trisodium
(ZK236018)/3095p19/99.3%
 Batch number: 200046499
 Animal species/strain/sex per dose: Beagle dogs/3 males + 2 females/dose
 Weight: 11.0-16.4 kg
 Doses/vehicle: 0.1, 0.3, and 1 mmol/kg, using a dosing volume of 4 ml/kg. Vehicle was 0.9% w/v saline.
 Duration/route: Single i.v. administration, 30 ml/min

Dose mmol/kg	0.1	0.3	1	NOEL <0.1
Human dose multiple	1.8	5.4	18	<1.8

The objective of this study was to evaluate the effects of MS-325 on cardiohemodynamic system and ECG in anesthetized dogs.

Study design:

Four groups of 5 Beagle dogs (3 males, 2 females) were anesthetized with thiopental (25 mg/kg) and tramadol (2mg/kg) and instrumented for recording of hemodynamic parameters and ECG. Each dose group was given the test substance in single doses of 0.1, 0.3 or 1 mmol/kg intravenously (respectively 1.8, 5.4, and 18X the intended clinical dose), at a volume of 4 ml/kg and a rate of 30 mL/min via catheter. Control animals received 4 mL/kg of saline.

Baseline data were recorded following a 60 min equilibration period. Thereafter, vehicle or test compound were administered, and hemodynamic parameters /ECG recorded at intervals up to 120 min. The following parameters were measured: Mean aortic blood pressure (PM), left ventricular end-diastolic pressure (LVEDP), central venous pressure (PCV), mean pulmonary artery pressure (PMPU), heart rate (HR), maximum rate of rise of left ventricular pressure (dP/dtmax), cardiac output (CO), stroke volume (SV), total peripheral resistance @, pulmonary vascular resistance (RPU), and ECG parameters including RR-, PR-, QT-time interval and Bazzett's QTc. Tracings of ECG were obtained as well. Changes of the cardiohemodynamic and ECG data versus pre-treatment were calculated and compared with the volume control group (one way analysis of variance – ANOVA and, if appropriate, Dunnett's method, 5 % significance level).

Blood samples were collected to determine gadolinium concentration in heparinized plasma, before administration and at 2.5, 5, 10, 20, 30, 45, 60, 90 min, and 2, 3, and 4 h after start of the administration of test compound.

Results:

ECG results: At 0.1 mmol/kg, 2/5 dogs showed signs of cardiac arrhythmia. In one animal, unifocal ventricular contractions (PVCs), expressed as alternate rhythms (Bigeminy) were observed at 100 minutes after start of application. In the other animal,

abnormally inverted downsloping T-waves followed by multifocal PVCs were observed 30 to 120 minutes after application). At 0.3 mmol/kg, one animal showed ECG unifocal PVCs and premature atrial complexes 20 and 25 minutes after the start of treatment. At 2.5 minutes after administration of 1 mmol/kg, 1 animal exhibited unifocal PVCs, expressed as alternate rhythms (Bigeminy) whereas another animal showed abnormally inverted downsloping T-waves.

In the 0.1 mmol/kg group, Bazett's QTc did not vary significantly. In the 0.3 mmol/kg group (5.4X the HD), an increase of up to 20 ms was observed in 1/5 dogs, within 15 minutes following administration. In 1/5 dogs of the highest dose group (18X the HD), an increase of 23-27 ms was observed between 15 and 20 minutes post-dosing. Overall, significant increases were noted in 2/10 dogs dosed with either 0.3 or 1 mmol/kg, which are 5.4 and 18 times the human dose. □

b(4)

Hemodynamic results: Hemodynamic effects were dose-dependent and were usually observed shortly following MS-325 administration. They were not correlated with peak plasma levels and were therefore considered incidental by the sponsor. However, they are likely drug-related.

Increase in central venous pressure was observed at all doses. This was the only significant change observed at 0.1 mmol/kg, since the increase in LVEDP was limited at one time point (40'), and therefore may be regarded as sporadic. Cardiac output and stroke volume increase of 20-35% was observed at 2.5 to 5 min post-dose at 5.4X and 18X, but not at the low dose. Although not statistically significant, increase in left ventricular end-diastolic pressure was observed at 40 and 120 min post-dose for 1.8X and 5.4X the human dose. At 18 times the HD, mean aortic pressure and total peripheral resistance were reduced by 20-40%. Overall, cardiohemodynamic parameters changes were dose-dependent and occurred immediately after injection. The only drug-related effect observed at 1.8 times the human dose was the increase in mean central venous pressure.

Pharmacokinetics results: Summary table of PK results (by sponsor)

Appears This Way
On Original

Parameters	[unit]	Dose mmol/kg					
		0.1		0.3		1	
C_{max}	[mmol/L]	1.12	± 0.18	3.48	± 0.71	9.77	± 1.70
DF (C_{max})		1		3.09		8.69	
DF (C_{max}/D)		1		1.03		0.87	
T_{max}	[min]	2.5	± 0	2.5	± 0	2.5	± 0
AUC _(2.5min-240min)	[mmol*min/L]	60.0	± 3.53	160	± 25.4	392	± 60.2
$C_{av(2.5-240min)}$	[mmol/L]	0.25	± 0.01	0.67	± 0.11	1.63	± 0.25
DF (AUC _(2.5-240min))		1		2.67		6.52	
DF (AUC/D)		1		0.89		0.65	

C_{max} : maximum Gd-concentration in serum observed after drug administration

DF (C_{max}): Dose factor ($C_{max(x \text{ mmol/kg})} / C_{max(0.01 \text{ mmol/kg})}$)

T_{max} : first observation point

AUC_(2.5-240min): area under the concentration versus time curve from first to last point of time

$C_{av(2.5-240min)}$: average plasma concentration from first to last point of time

DF (AUC_(2.5-240min)): Dose factor ($AUC_{(x \text{ mmol/kg})} / AUC_{(0.01 \text{ mmol/kg})}$)

DF (AUC/D): Dose normalized dose proportionality factor
 $[(AUC_{(x \text{ mmol/kg})} / AUC_{(0.01 \text{ mmol/kg})}) / (D_{(x \text{ mmol/kg})} / D_{(0.01 \text{ mmol/kg})})]$

There was a dose dependent increase of the systemic exposure. The results of PK studies conducted on the dog in parallel with ECG and hemodynamics showed that there was no correlation between peak concentration and effects observed at all doses. Plasma levels were reached at 5 minutes. Elimination half-life was 1.5 to 2 hours.

Conclusion: The sponsor concludes that transient changes, mainly observed immediately after administration, were dose-dependent. The effects detected at 0.3 mmol/kg were considered marginal. Arrhythmia and T-wave changes can be attributed to the drug, since they were not observed in the vehicle group. Single intravenous administration of 0.1, 0.3 and 1 mmol/kg of MS-325 in females and males resulted in a dose-dependent increase of the systemic exposure, however, the AUC values were proportional to the dose, with the increase of AUC smaller than that of the dose.

Reviewer's comments: For some parameters, the values at 0.5 and 1 min were missing. Arrhythmia was observed in all treated groups, but not in vehicle control group. Therefore, this effect is likely drug-related even though it was not possible to determine a dose-dependency. These effects were detected in 2/5 dogs in the 0.1mmol/kg group, 1/5 in the 0.3mmol/kg group, and 1/5 in the 1mmol/kg group. Arrhythmias manifested as unifocal ventricular contractions, expressed as alternate rhythms (Bigeminy) in 1 dog of low dose group, 1 dog in middle dose group, and 1 dog in high dose group. In the low dose group, abnormally inverted downsloping T-waves followed by multifocal PVC at 30-120 min). Thus, no NOEL can be established. According to the clinical reviewer, unifocal arrhythmia may be non-specific and multifocal PVCs are usually related to drug toxicity. However, arrhythmia was found in animals of each treated group, and not in the control group. Furthermore, the associated abnormally inverted downsloping T waves and the premature atrial complexes are supportive of a cardiac toxicity caused by MS-325.

There were dose-dependent and transient changes of various hemodynamic parameters especially directly after administration. Increase in the central venous pressure was the only effect significant change observed at 1.8 times the human dose. At higher dose effects were more pronounced and dose-dependent.

The results of PK studies showed that there was no correlation between peak concentration and effects observed at low dose. Blood was collected at different time points after administration with a last collection at 4 hours post-dose. Because it binds to plasma albumin, MS-325 half-life is much longer (16 hours in humans) than that of previous gadolinium compounds, and half-life in dogs was determined to be 1.5 to 2 hours. In this view, longer time points should have been included for ECG and hemodynamic parameters evaluation. Arrhythmia was not detected in the other cardiovascular study (Study 7L358) conducted in anesthetized dogs. It is of note that in that study, hemodynamic parameters and ECG were monitored for only 30 minutes after administration, whereas such effects were observed longer after the administration of the drug in the present study. Although no positive control was used in this study, and longer time points should have been used, cardiotoxic effects were detected for low doses of MS-325. Therefore, based on the findings of this study, [

b(4)

]

Study RTAW-107: A cardiovascular safety assessment of MS-325 in telemetered cynomolgus monkeys. A rising dose study (Study conducted by [

b(4)

], Test article: Lot b3095p39, GLP compliant)

The objective of this study was to determine the cardiovascular safety of MS-325 when administered intravenously to telemetered cynomolgus monkeys.

Study design: The study consisted of one group of 2 males and 2 females. Each monkey had previously been telemetered. Animals were administered saline on Day 1. MS-325 was I.V. administered, at 12 mL/kg and 30 mL/min, on Days 8, 15, 22, 29, and 36, at doses of 0.1, 0.3, 1.0, 2.0, and 3.0 mmol/kg (respectively 1, 3.2, 11, 22, and 32X the intended clinical dose). Sponsor did not specify the injection method (manually or power injector). Blood pressure and ECG, direct blood pressure (systolic, diastolic and mean arterial pressure, derived), heart rate, temperature, and activity were monitored post-dosing. Blood samples were collected for hematology and serum chemistry evaluation, and for Gadolinium determination. Body weights were recorded at Days 1 and 43, and monkeys were videotaped for at least 3 hours post-dose.

Data were assessed as percent change from baseline and were statistically analyzed using analysis of variance (ANOVA) and Dunnett's t-test or other statistical analysis tests comparing the effects of vehicle and test article.

Key findings: Changes in hematology parameters were slight and transient. The NOEL for cardiovascular effects was not established. The NOEL for clinical effects can be established at 2 mmol/kg (22X the human dose).

Results

No animal died in the study.

Body weights

There was no significant drug-related change.

Clinical signs

At 3 mmol/kg, monkeys (3) that received the totality of the dose vomited during or immediately following dosing. The 4th monkey did not receive the full dose due to a technical incident.

Serum chemistry

-Significant changes were noted in glucose levels pre- and post-dose. Dose-unrelated fluctuations in the triglycerides were also observed.

-Alk was increased by up to 60% in 2 females at doses of 2 and 3 mmol/kg. BUN reduction was observed in all groups including control animals, however, it was slightly more pronounced at high doses 11X and up. This effect occurred on average 3 days after administration of test article and was reversible in most cases.

Hematology

Hemoglobin decreased by up to 10% in all groups including control animals. There was no drug-related significant effect. Very similar changes were found in hematocrit. The most remarkable effect was observed in lymphocyte percentage with a 90% drop, 4 hours following 2 or 3 mmol/kg administration in all animals. This effect was also observed at 0.3 and 1 mmol/kg to a lesser extent (~50%), and was reversible within 24-48 hours.

Hemodynamic parameters:

The effects were usually seen immediately after injection. Significant changes (26-31% increase) in mean heart rate were detected within 5 min at doses of 1 and 2 mmol/kg. Taken individually, the response to treatment varied between animals. In one monkey the percent changes were more marked than in the 3 other monkeys. At 0.1 mmol/kg, a 43% increase in heart rate was observed in one monkey at 10 and 15'. The value returned to baseline within 1 hour. For the 2 other monkeys, a 20% increase in HR was observed immediately post-dose and the return to baseline value was observed within 15'. The response was dose-dependent up to 1 mmol/kg. At highest doses of 2 and 3 mmol/kg, the effects on heart rate, and on pressure were less severe than those observed with low doses, perhaps because the monkeys were adapting to increasing doses of the drug. Variations observed in systolic, diastolic and mean arterial pressure were similar, with a maximum decrease of ~35-50%. In general, the MAP, SAP, and DAS returned to normal base values within 1 to 2 hours post treatment. More pronounced effects were observed at 0.3 mmol/kg, the treatment affecting one monkey more severely with a MAP down to ~60% at 10-15'.

The cardiology report of the qualitative ECG was attached as an appendix and consisted of a letter from a DVM. It was stated that the Eggs from all monkeys were within normal limits during the pre-dosing and the post-dosing periods, and that all monkeys remained in the sinus rhythm throughout the study; it is also stated that at doses up to 3.0 mmol/kg, MS-325 caused no qualitative effects on Egg's recorded by telemetry in this study.

Reviewer's comments:

The significance of the variations in pre-dose glucose levels is unclear, since monkeys were fasted at least 4 hours before each blood sampling. Decrease in hemoglobin and hematocrit may, in part, result from frequent blood collection, since this effect was observed in control animals as well. However, the decrease was slightly more pronounced at high doses suggesting its possible relation to the drug. The drop in the percent of lymphocytes following administration of MS-325 at doses of 0.3 mmol/kg and up (>3X clinical dose) is transient, thus necrosis of lymphoid tissues, which is known to induce such increase, is not likely. On the other hand, lymphocytes are usually affected in laboratory animals.

MS-325 affects heart rate. Such an effect has been reported for gadolinium agents and is often attributed to the high osmolality characterizing this class of compound. It is also possible that potential release of free gadolinium from Vasovist, even if minimal, could contribute to the observed effects on the cardiovascular system.

Study 104] A safety study to monitor the cardiovascular effects of unformulated MS-325 given intravenously to cynomolgus monkeys

b(4)

The objective of this study was to measure the cardiovascular effects of an intravenous injection of MS-325 in cynomolgus monkeys.

MS-325: Lot ZT47-50 (300mM), 1091 mOsM/kg, formulated with _____

b(4)

Study design: Adult male Cynomolgus monkeys (n=2) were anesthetized with isoflurane, mechanically ventilated with medical grade room air, and surgically instrumented for measurements of cardiovascular parameters. These parameters included systemic systolic, diastolic, and mean arterial pressure (SAP, DAP, and MAP), central venous pressure (CVP), left ventricular pressure (LVP), cardiac output (CO), dP/dt (change in LVP/change in time, an indirect measure of cardiac contractility), and core body temperature. Hemodynamic parameters and electrocardiograms (ECGs) were monitored continuously for at least 3 time points prior to injection, at the time of injection, and at 5, 15, 30, and 60 minutes post-injection. Serial blood samples were collected for arterial blood gas values, hematology, clinical chemistry values, and serum iron concentrations. Blood was collected at 1, 2, 5, 20, 30, and 60 minutes and 2, 5, 6, 8, 12, and 24 hours post injection. Creatinine clearances were calculated.

After a baseline period, animals were sequentially treated with 3.5 mL of vehicle control, followed by 0.025mmol/kg and 0.25 mmol/kg of MS-325 (0.27 and 2.7X the human dose). The recovery period following each treatment was 30, 60, and 90 minutes respectively.

Results:

-Both animals had one or more premature ventricular contractions (PVCs) attributed by the sponsor to irritation of the endocardial surface by the cardiac catheters.

-ECGs did not appear to vary significantly over time or with treatment. MS-325 did not seem to affect QTc intervals in 1 monkey (T6714). There was a slight increase in QTc in the second monkey; however, a significant amount of tracing was missing for the latter, therefore data could not be properly interpreted. In addition, ECGs obtained in 4 monkeys from the previous study [] were included in this report. Appended to the report is a review of these ECG records by a veterinarian.

b(4)

-Heart rate increased mildly over time in both animals (10-15%) at 1 hr post-injection for 2.7 times the human dose. Body temperatures increased slightly over time in one animal, and remained relatively unchanged in the other animal.

-Pulmonary arterial pressure, mean arterial pressure and left ventricular pressure measured displayed mild variability over time. Central venous pressure was within the normal range for one animal, although it was found to be low throughout for the other one. Central venous pressure, cardiac output, and dP/dt and appeared unaffected by treatment for both animals.

-Urine creatinine clearance was increased by 2 fold in 1 monkey, and 7 fold in the second one at 2.5 hrs post-injection. No explanation was provided for these changes and there was no follow-up on these findings.

Blood gases result: Blood gases were not significantly affected by treatment. The most significant variations occurred following saline administration, between time point 0 and 35', as shown in the following tables, thus ruling out a drug related effect.

Table 1. Blood gas results for Monkey 63-323 (provided by sponsor)

Time point	pH	PO2 (mmHg)	PCO2 (mmHg)	TCO2 (mmol/L)	HCO3- (mmol/L)	B-EX (mmol/L)
-0:30	7.52	129.6	30.1	25.4	24.5	3.0
0:00	7.50	130.8	29.6	24.1	23.2	1.6
0:05	7.45	120.2	33.6	24.5	23.5	0.7
0:30	7.41	105.7	35.9	24.0	22.9	-0.6
0:35	7.39	99.9	36.6	23.6	22.4	-1.4
1:00	7.38	98.1	36.1	22.4	21.3	-2.7
1:30	7.37	93.2	36.3	22.3	21.2	-2.9
1:35	7.39	98.1	35.5	22.7	21.6	-2.2
2:00	7.38	98.3	34.1	21.1	20.1	-3.7
2:30	7.37	95.0	34.7	21.3	20.3	-3.6
3:00	7.38	107.1	32.5	20.1	19.1	-4.5

Table 2. Blood gas results for Monkey T6714 (provided by sponsor)

Time point	pH	PO2 (mmHg)	PCO2 (mmHg)	TCO2 (mmol/L)	HCO3- (mmol/L)	B-EX (mmol/L)
-0:30	7.47	105.7	33.8	25.7	24.7	2.1
0:00	7.44	107.9	38.3	27.2	26.0	2.5
0:05	7.40	98.4	40.0	26.2	25.0	0.8
0:30	7.41	106.2	38.5	25.4	24.3	0.3
0:35	7.39	98.9	40.9	26.2	24.9	0.5
1:00	7.39	98.0	40.7	26.2	25.0	0.7
1:30	7.40	100.3	37.4	24.2	23.1	-0.8
1:35	7.40	92.5	41.4	26.9	25.6	1.2
2:00	7.39	93.7	39.8	25.6	24.4	0.2
2:30	7.38	96.3	39.3	24.6	23.4	-1.0
3:00	7.38	94.9	38.3	24.0	22.9	-2.3

Reviewer's comments: The limited number of animals does not allow for proper statistical analysis. The recovery periods between 2 dosings are too short, and some parameters did not return to baseline before the initiation of the next dose. Therefore, the findings may be considered as the result of cumulative doses of MS-325. Due to some technical errors, ECGs were not recorded for one animal at several time points. In addition, SAP and DAP were recorded intermittently and only MAP was recorded continuously and reported. Overall, little, although valuable information was provided in this study.

The significant changes in urine creatinine clearance may not be accurate need to be followed-up and creatinine clearance should have been measured over 24 hours. Both animals had one or more premature ventricular contractions (PVCs) attributed by the sponsor to irritation of the endocardial surface by the cardiac catheters. Arrhythmia was observed in the cardiovascular safety study in Beagle dogs. However, because such effects were not observed in the other monkey study, and because of the low number of monkeys in the present study, one may conclude that the PVCs may be drug-related in dogs but not in monkeys.

Study Report 7L358: Effects on the cardiovascular and respiratory systems

The effects on cardiovascular system were assessed in 4 anesthetized beagle dogs administered i.v. injection of solvent or 0.1, 0.3, and 1 mmol/kg MS-325 (1.8X, 5.4X, and 18X the human dose) followed by a 30' observation period. There was no significant effect on heart rate, mean blood flow of the carotid artery and ECG. A dose of 1 mmol/kg transiently (between 1 and 5' post-dose) decreased mean blood pressure by 15%, increased respiration rate by 38-25%, and decreased ventilation volume by 11-14%. The NOEL was established at 0.3 mmol/kg (5.4X the intended clinical dose).

Report No. A09829: Electrophysiological examination of MS-325 on the HERG-mediated potassium current (study conducted by []
Substance code number: ZK 236018 (3095p19, 0.25 mmol/L)

b(4)

The objective of this study was to examine the effect of MS-325 on the hERG-mediated potassium current in Chinese hamster ovary cells (CHO) stably expressing this channel.

Study design as described by sponsor: The experiments were performed using the voltage clamp technique in a recombinant cell line (Chinese hamster ovary cells) stably expressing the HERG channel to study inhibition of the inward rectifier potassium current. Potassium currents were activated at a frequency of 0.1 Hz. Currents were recorded in the absence and presence of test compound. DMSO was used as a vehicle. MS-325 was added to the incubation medium to reach concentrations of 10, 50, or 100 μM for 6 min (stimulus 14 to 50). The 5 min time interval between 2 min and 6 min (stimulus 20 to 50) was considered for the evaluation of the test solution effect. The amplitude of the potassium currents gradually decreased in some experiments over time, even in control conditions (called "run-down").

Table 1. Test solution preparation

Test Solution (ml)	Vehicle Buffer (ml)	Final	
		Concentration (mmol/L)	Human Plasma Level Multiple
1.00	24	10	23X
5.00	20	50	116X
5.00	7.5	100	233X

(Table prepared by reviewer)

Study results: In presence of $10 \times 10^{-3} \text{M}$, $50 \times 10^{-3} \text{M}$, $100 \times 10^{-3} \text{M}$ of MS-325, the outward current amplitudes at -40 mV were reduced at high concentrations, with an IC_{50} of $44.8 \pm 3.6 \times 10^{-3} \text{M}$, and a Hill coefficient (n_H) of 1.8 ± 0.2 , indicating an effect of MS-325 on the hERG-mediated potassium current. Concentration-response for the block of the hERG channel was obtained for Terfenadine at 0.001, 0.01, and 0.1 μM , with an IC_{50} of $26.51 \pm 8.56 \text{ nM}$ and a n_H of 0.81 ± 0.20 . In most of the experiments, the reversibility of effects could not be recorded with MS-325 because the patch was disrupted. However, such study was possible for the $50 \times 10^{-3} \text{M}$ cell, where the effect recorded was shown to be nearly fully reversible to 96% of the control current amplitude during wash period.

Table 2. Relative current amplitude

Test compound	Relative current amplitude
Vehicle	0.95 ± 0.01
10 mM (23X)	0.98 ± 0.04
50 mM (116X)	0.29 ± 0.14
100 mM (233X)	0.21 ± 0.02

(Table prepared by reviewer)

Table 3. Effects of test compounds on the HERG-mediated potassium current (provided by sponsor)

Test compound	$\text{IC}_{50} \pm \text{SEM (M)}$	$n_H \pm \text{SEM}$
---------------	-------------------------------------	----------------------

MS-325	$44.8 \pm 3.6 \times 10^{-3}$	1.8 ± 0.2
Terfenadine*	$26.51 \pm 8.56 \times 10^{-3}$	0.81 ± 0.20

Estimated half-maximal inhibition concentrations (IC_{50}) and corresponding Hill coefficients (n_H). SEM= standard error of the mean.

*Data from validation with Terfenadine on 22-June-2001

Reviewer's comments: The sponsor concluded that MS-325 did not affect potassium channels. Indeed, MS-325 does not block potassium currents at doses of 10 mmol/L equivalent to ~23 times the human plasma levels achievable at the clinical dose of 0.03mmol/kg; however, at higher concentrations (113 and 236X) the block on potassium currents was somewhat dose-dependent (70 and 80% block). The IC_{50} was established at 45 mM. Terfenadine data were obtained from an experiment performed 4 months prior to the present study. Thus, no positive control was tested the same day to validate the system. However, the dose-response effect indicates that the system was able to detect blockage of K channels. Therefore, the purpose of the study was achieved.

Report A09148: Effects of ZK 236018 (Epix) on cardiac action potential in isolated guinea pig papillary muscle (conducted in 2001 by Shering AG, Berlin, Substance code number: ZK 236018, 3095p19, 0.25 mmol/L)

The objective of this study was to assess the effects of ZK 236018 (MS-325) on intracellularly recorded action potential parameters in the guinea pig isolated papillary muscle preparation.

Study design:

Male guinea pigs papillary muscles were continuously superfused with Tyrode solution at 35°C, and were electrically paced via silver electrodes at 0.3 Hz, 1 Hz, and 3 Hz. Each muscle (n=6) was impaled with 3 M KCl- filled glass micropipettes to monitor membrane potential. Following equilibration, 3 muscles were exposed for 30-35 min to MS-325 at 0.01, 0.1, and 1mmol/L (0.023, 0.23, and 2.3X the clinical plasma concentration, respectively) and 3 muscles to saline at corresponding volumes. Measured parameters included action potential duration at 30%, 60%, and 90% repolarization (APD_{30} , APD_{60} , and APD_{90}), maximum rate of rise of the upstroke (V_{max}), upstroke amplitude (AP-Amp), and diastolic membrane potential (MP). For each concentration, changes in action were determined as the mean of 10 measurements per time point and stimulation frequency. *Di-Sotalol* (100 μ mol/L) was used as positive control.

Results: Tables were provided with a summary of the results. *Di-Sotalol* significantly increased the action potential duration at 30, 60 and 90% as expected, but did not affect membrane potential, action potential amplitude, and upstroke velocity. Following MS-325 treatment, membrane potential and action potential amplitude were stable; no effect was observed on MP and AP-Amp at all concentrations tested. For up to 1 mmol/L, MS-325 did not significantly affect repolarization of action potential in isolated guinea pig papillary muscle. The only finding that can be attributed to MS-325 is the increase of

upstroke velocity (up to 20%), which tended to decrease marginally over time in the control group (~10%).

Reviewer's comments: Although MS-325 induced some changes in the ADP30, 60 and 90%, these changes are rather small, thus do not appear to be significant. Moreover, the previous study on hERG, more specific for K channels involvement in the QT prolongation, did not demonstrate an effect of MS-325 on the action potential amplitude at concentration of 23 times the human concentration. The effects of MS-325 on the papillary muscle at doses within the proposed clinical dose could also be regarded as specific to gadolinium compounds. Although the highest concentration tested was clinically relevant (2.3X clinical concentration), the 2 low doses (0.023X and 0.23X) seem irrelevant in view that at the highest dose no significant effect was detected.

Neurological effects:

Study Report A04820: Neurotropic effects of ZK 236018 in the Irwin test in mice after single intravenous administration (Formulation batch number 3095p19)

The purpose of this study was to assess the potential effects of MS-325 on central nervous system.

Study design: Nine groups of 3 mice each (1 male and 2 females or vice versa per group) were administrated IV injections of saline or 20, 39, 78, 156, 313, 625, 1250, and 2500 $\mu\text{mol/kg}$, in a volume of 0.1 ml/10g. These doses are ~0.05, 0.11, 0.21, 0.42, 0.85, 1.7, 3.4, and 6.8 times the proposed clinical dose of 0.03 mmol/kg, based on body surface area. Neurological effects including 62 symptoms (vegetative, neurological and behavioral) were assessed at 30 min, 4 and 24 hr post-injection.

Results: A table summarizing the findings observed between 30 min and 24 hours post dosing, was provided with no individual results. Immediately following administration of doses $\geq 1250 \mu\text{mol/kg}$, all animals presented signs of hyperexcitability including splayed toes, immobility and accelerated respiration. In one animal treated with 2500 $\mu\text{mol/kg}$, running fits as manifestation of a convulsive action with preceding pallor and subsequent tremor, loss of righting reflex and dyspnea was observed. All of the above effects lasted less than 2 min. A slightly bluish-red tail was observed in 2 out of 3 animals in both the 1250 and 2500 $\mu\text{g/kg}$ group at 30 min, and lasted until 24 hr after injection. The NOEL was established at 625 $\mu\text{mol/kg}$, which is equivalent to 1.7 times the human intended dose, because at this dose, local irritation was reversible at 30 min post-injection.

Reviewer's comments: Transient (less than 2 min) drug-related signs of hyperexcitability described as splayed toes, immobility and accelerated respiration were observed immediately following administration of doses $\geq 3.4\text{X}$ the clinical dose in all of the animals. At the highest dose, equivalent to 6.8X the human dose, more severe effects were reported in one animal; however, these were also transient and the animal recovered

within 2 minutes post-dosing. On the other hand, the local tissue irritation occurring at all doses, was only reversible at doses 1.7 times the clinical dose, whereas they persisted at doses \geq than 1250 $\mu\text{g}/\text{kg}$ (3.4 times the human dose). This observation is in accordance with the rabbit local tolerance toxicity study data, where irreversible perivascular irritation was reported at doses equivalent to 4 times the clinical dose for a 96 hr observation period. In conclusion, the CNS-related effects appear to be transient.

Study Report 7L358: General pharmacological study of MS-325 (conducted in 10/97 in Japan in compliance with Japan GLPs, Lot PG-141-008; 0.25 mmol/ml, provided

b(4)

The purpose of this study was to evaluate the general pharmacology effects of MS-325.

Overall study design: Male Wistar rats, male Hartley guinea pigs, male Japanese White Variety rabbits, and male Beagle dogs were used in this study. The effects of MS-325 were evaluated on general behavior, on central and autonomic nervous systems, water and electrolyte balance, body temperature, cardiovascular and respiratory systems, and smooth muscle. Effects of the drug were also assessed on erythrocyte membranes using a hemolysis test, on the blood coagulation system in rats, on platelet aggregation in rabbits, and on metal ion plasma levels. For in vivo studies, MS-325 administration was performed at a rate of 1ml/min in rats, 3ml/min in rabbits, and 10ml/min in dogs. Control animals were administered saline solutions at the highest test volume. In-vitro effects of MS-325 were tested at final concentrations of 10^{-6} , 10^{-5} , and 10^{-4} M.

1. Effects on general behavior

Study design: Six dose groups of rats, solvent and 0.05, 0.1, 0.3, 1, and 3 mmol/kg of MS-325, were used in this study. Animals were observed for general behavior (Irwin's method) before treatment, and at 0.25, 0.5, 1, 2, and 4 hours after IV bolus administration.

Major findings: At 1 mmol/kg (5.4X) slightly depressed respiration was observed in 1 animal. At 3 mmol/kg (16X) body position, body tone, and respiration were slightly to very slightly lowered. All signs disappeared within 2 hours. The NOEL was 0.3 mmol/kg (1.6X the human dose).

2. Effects on the central nervous system

Study design: Groups of 6 to 10 rats (n=244) were used in this study. Animal groups each received solvent, 0.1, 0.3, or 1 mmol/kg. A subcutaneous administration of PTZ (Pentylentetrazol) 60mg/kg produces convulsions in 96% mice whereas a dose of 20 mg/kg produces no convulsions (Internet search).

Results: No effect was observed on pain threshold, body temperature, spontaneous motor activity, electrical stimulation threshold, electrically-induced tonic flexor, extensor, or clonic convulsions or coma, or on PTZ-induced clonic or tonic extensor convulsions or death. The NOEL was >1 mmol/kg ($>5.4\text{X HD}$).

A dose of 1 mmol/kg significantly decreased by 30% the convulsive threshold dose of PTZ¹, and the effect was transient effect (60'). There was a 23% increase in pentobarbital induced sleeping times. The NOEL was established at 0.3 mmol/kg (1.6X the HD).

3. Effects on the autonomic nervous system and smooth muscle

Effects on the autonomic nervous system and smooth muscle were studied in vitro on segments of ileum isolated from guinea pigs (n=6, 3 segments/pig). The ilea were treated with solvent, 10^{-6} , 10^{-5} , 10^{-4} M MS-325. No effect was observed on acetylcholine, histamine, or barium induced contraction in ileum preparations. The NOEL was $> 10^{-4}$ M (>0.3 X the anticipated human plasma concentration).

4. Effects on the cardiovascular system

The effects on cardiovascular system were assessed in 4 anesthetized beagle dogs administered i.v. injection of solvent or 0.1, 0.3, and 1 mmol/kg MS-325 (1.8X, 5.4X, and 18X the human dose). There was no significant effect on heart rate, mean blood flow of the carotid artery and ECG. A dose of 1 mmol/kg transiently (between 1 and 5' post-dose) decreased mean blood pressure by 15%, increased respiration rate by 38-25%, and decreased ventilation volume by 11-14%. The NOEL was established at 0.3 mmol/kg (5.4X the intended clinical dose).

5. Effects on the gastrointestinal tract

Four groups of rats (6/dose) were administered vehicle, 0.1, 0.3, or 1 mmol/kg MS-325. Passage was measured 30 minutes after oral administration of charcoal. There was no effect on the gastrointestinal motility of charcoal at any of the doses tested. The NOEL was >1 mmol/kg (>5.4 X the human dose).

6. Effects on water and electrolyte excretion

Six groups of rats (6/dose) were each given solvent, 0.1, 0.3, or 1 mmol/kg MS-325. At 0.1 and 0.3 mmol/kg, K^+ increased by $\sim 30\%$, and Cl⁻ decreased by $\sim 20\%$. At 1 mmol/kg, volume of urine increased by 1.5-2.5 fold for 1 hr post-dose; at the same dose, there was a 3 fold increase of Na^+ and 1.6 fold increase of K^+ in the urinary excretion for 2 hrs post-injection. Over a 6 hour period, the total excretion of urinary Na^+ was 1.6 fold that of control, whereas there was no effect on K^+ and Cl⁻. The NOEL was < 0.3 mmol/kg (1.6X the human dose).

7. Effects on blood

A hemolysis test was performed on 5 rabbits. Four samples were collected from each rabbit. There was a 3 to 5 % hemolysis in presence of MS-325 at 10^{-6} , 10^{-5} , and 10^{-4} M, compared to 0.5% in the controls. However, this effect was not significant. Therefore, NOEL was established at $>10^{-4}$ M (>0.3 X the anticipated clinical plasma concentration of 0.43mM).

A coagulation test was performed in rats. Four groups of rats were each I.V. treated with solvent, 0.1, 0.3, and 1 mmol/kg MS-325 (0.54X, 1.6X, and 5.4X the human dose). Immediately after administration, a dose of 0.3 mmol/kg produced a 12% increase in Activated Partial Thromboplastin Time (APTT). At 1 mmol/kg, there was a 13-18%

increase in Prothrombin Time (PT) that resolved at 30' post-dose, and a 27-44% increase in APTT, which returns to baseline values within 20'.

ADP- or collagen-induced platelet aggregation was evaluated in rabbits. Four samples from each rabbit were treated with solvent, 10^{-6} , 10^{-5} , and 10^{-4} M. No effect was detected at any of the concentrations tested, thus NOEL was established at $>10^{-4}$ M.

8. Effects on metal ion concentrations in blood.

The effects of MS-325 on metal ion concentrations were evaluated in rabbits treated with solvent, and MS-325 at 0.1, 0.3, and 1 mmol/kg (1.1X, 3.3X, and 11X the intended human dose). Blood was collected at 10, 20, 30, and 60' after MS-325 injection. No effect could be detected on Ca^{++} , Mg^{++} , Fe^{++} . NOEL was established at >1 mmol/kg. However, there were no pre-treatment data, and the results were compared to controls only.

Results summary: Based on body surface area, the NOEL in rat was respectively 1.6X to 5.4X the human dose for studies on behavior and central nervous system. In the cardiovascular system in dog study and gastrointestinal system in rat study, the NOEL was respectively established at 5.4X and >5.4 X the clinical dose. For all in vitro studies, the NOEL was higher than the highest dose tested of 10^{-4} M. The NOEL could not be established in rats for the effects on water and electrolyte excretion, but it was lower than 1.6X the human intended dose.

In general, there was no effect of MS-325 at 0.1 and 0.3 mmol/kg in the animal studies. The highest dose of MS-325 (1 mmol/kg) slightly depressed the respiration and abdominal muscle tone in the general behavior test, decreased the threshold in PTZ-treated rats, lowered mean blood pressure, increased urine volume as well as excretion of urinary Na^{+} and K^{+} , and decreased excretion of urinary Cl^{-} . The changes observed were 13 to 45% increases or decreases compared to the control. They were reversible with the exception of the slight decrease in Cl^{-} excretion. At 1 mmol/kg, PT and APTT were transiently increased in rat immediately after administration, and returned to normal values within 20 to 30 minutes post-dose.

Reviewer's comments: MS-325 had no remarkable effect on the CNS, autonomic nervous system, smooth muscle, cardiovascular system, gastrointestinal tract at doses ranging from 1.6X to 5.4X the intended clinical dose. In addition, most of the observed changes were rather mild and were transient, except for the effect on electrolytes excreted in the urine. This increased excretion may be due to the osmolality of MS-325 (2 to 3 times that of the saline control). However, in absence of controls of similar osmolality, one cannot completely rule out the implication of the drug. Elevated APTT may affect bleeding time, and potentially produce hemorrhage if high doses of MS-325 are administered. This effect has been noted in monkeys as well.

MS-325 causes a slight increase of pentobarbital-induced sleep in rats (~23%) and a decrease of the threshold of PTZ-induced convulsions. The effect of MS-325 on the amount of PTZ required to induce a seizure event provides evidence of its neuroexcitatory potential. This is in accordance with the results of the previous Irwin's

test in mice, where hyperexcitability was noted immediately following injection of the drug.

The concentrations of MS-325 used in the in-vitro studies (0.1mM, 0.01mM, and 0.001mM) are lower than the achievable plasma level of 0.43 mM MS-325 in humans at the intended clinical dose. In that view, this part of the study does not provide useful information on the effects of the drug at clinically relevant doses. Likewise, effects on metal ion concentrations in blood and on coagulation and platelet aggregation were not measured pre-dose, therefore, post-treatment cannot be properly analyzed.

Renal effects:

Study RTAW-109-02-387: A study to assess the effects of MS-325 on renal function in anesthetized cynomolgus monkeys. (Study conducted by [] GLP, no information on the stability of the formulated test article. Lot number b3095p19, 0.25 mmol/L)

b(4)

Exposure to contrast MRI agents has been shown to result in renal vacuolation that could influence renal function. The objective of this study was to assess the effects of MS-325 on renal function following a single intravenous administration.

Study design: The study was comprised of 12 non-naïve female cynomolgus monkeys weighing 2.4-3.0 kg at the time of study initiation. All animals were anesthetized on Day 1, and catheters were placed to allow for urine collection, material administration, and blood collection. Once urine flow was confirmed, the animals were infused with 5% dextrose in water containing ~1 mCi of Tc^{99m}-DTPA (technetium 99m-diethylenetriaminepentaacetic acid) and ~0.5 mCi of I¹³¹-PAH (Iodine 131 para-aminohippurate) in a volume of 150 mL at a rate of 100 mL per hour. Intravenous administration of test or control article was initiated 15-30 minutes later at a volume of 12mL/kg and at a rate of 30 mL/min. Group 1 received saline while Groups 2 and 3 received MS-325 at 0.03 and 2.0 mmol/kg, respectively. These doses are respectively 0.3X and 22X the intended clinical dose based on body surface area.

Urine was collected over 2 periods of 30 minutes each and blood samples were collected every 15 minutes to allow for the determination of the PAH and DTPA levels by radioisotope analysis of Tc^{99m} and I¹³¹ respectively. Hematocrit was determined at each blood sample time point. This procedure was repeated on Day 8 without test material administration; only this time sample collection was initiated immediately following radioisotopes injection.

Meribundity/mortality checks were performed twice daily, clinical observations and incision site observations once daily; body weights were recorded before treatment and at necropsy. Physical examinations were performed once before the initiation of the study. Whole blood and urine samples for all animals were analyzed for radioactivity of Tc^{99m}-DTPA and I¹³¹-PAH in a gamma scintillation counter set up for dual channel analysis. Duplicate values obtained for radioactivity were used to calculate PAH clearance (renal

blood clearance), DTPA clearance (glomerular filtration rate [GFR]), effective renal blood flow (ERBF), actual renal blood flow (RBF), and filtration fraction (FF). On Day 8, the animals were euthanized and the kidneys were examined at the time of necropsy, *in situ*, and collected for histopathological evaluation.

Results and comments:

Mean body weight values were slightly decreased (~0.1 kg) in the Group 3 compared to control; the sponsor attributed this difference to fasting. This change seems negligible.

Clinical observations: There was no description of what the clinical observations consisted of. However, sponsor reported no effect in the course of the study.

Pathology: Animal 2104 (Group 2) did not recover after anesthesia and was euthanized moribund on Day 1 following dose administration and sample collection procedures. At examination, macroscopic findings included hemorrhage and cerebral swelling with mild cerebellar herniation into the foramen magnum. The sponsor attributed these findings to the monkey anesthetic history. However, there was no evidence that MS-325 did not impact the finding.

Several microscopic changes within the kidneys were variably present among the animals, such as mineralization, lymphocyte infiltrates, and dilated tubules. Findings including minor inflammatory, degenerative or artifactual changes commonly observed in *Cynomolgus* Monkeys.

Animal No. 3105 presented with pyelonephritis attributed by the sponsor to an ascending bacterial infection secondary to prolonged and repeated urinary bladder catheterization.

Hematocrit: Hematocrit showed significant variations in all groups with no consistent pattern in the changes, perhaps due to the procedure used for sample collection. These data are not interpretable although the sponsor concluded that the values were unaffected by test article administration. Plasma creatinine levels, and BUN, which were not evaluated here, would be more meaningful for the study of renal function.

Mean renal blood clearance and renal blood flow values for all treatment groups (compared to the control group) exhibited a similar rate of decrease from the 30 to the 60 minute time point on Day 1 and a similar rate of increase from the 30 to 60 minute time point on Day 8. For the 30 min time point, mean values on Day 8 were notably lower than on Day 1.

Mean glomerular filtration rate values for all treatment groups (compared to the control group) showed no remarkable change on Day 1 or Day 8. Mean values on Day 8 for both the 30 and 60 minute time points were lower for all treatment groups compared to Day 1 values at the same time points.

Mean filtration fraction values for all groups including the control group showed a similar rate of increase from the 30 to 60 minute time point on Day 1 and a similar rate of decrease from the 30 to 60 minute time point on Day 8.

Conclusion: Differences described in renal blood clearance and glomerular filtration rate were attributed by the sponsor to the procedural change and not to the test article as values within the same day of evaluation were comparable to the control group. Because the radioisotopes were immediately administered prior to sample collection on Day 8, Day 1 values cannot be directly compared to Day 8 values. Therefore, the sponsor concluded that within the same day of evaluation, the mean values were comparable between treated and control groups, indicating that the test article does not affect the renal function of cynomolgus monkey at doses tested (0.27X, and 22X the clinical dose).

Reviewer's comments: This study was poorly designed.

1. Doses tested (0.27X and 22X the clinical dose) were not adequate and sponsor should have included intermediate doses to establish a dose-response.
2. The interval of time of 1 hour selected for the monitoring of renal function is not adequate, when the pharmacokinetic studies showed that half-life of MS-325 in monkey is approximately 3 hrs compared to 16 hours in humans. Moreover, the drug appears to undergo a two-phase elimination that is not complete at 60 minutes in monkeys.
3. Because of the procedural change (administration of radioisotopes immediately prior to sample collection on Day 8), Day 1 values cannot be directly compared to Day 8 values. At some time points, the counts were extremely low for analysis.

Overall, this study did not adequately explore the effect of MS-325 on renal function.

Study PTR 2003-12: Assessment of MS-325 in Rodent Model of Drug-Induced Renal Impairment.

MS-325 is proposed in the detection of diseases of the vasculature including renal artery stenosis. The objective of this study was to evaluate the impact of MS-325 at multiples of the intended human dose in a rodent model of renal impairment.

Study design: Single doses of 0.3, 1.0 and 2.0 mmol/kg of MS-325 were studied following impairment induced by cisplatin or cyclosporine. These doses are respectively 1.6X, 5.5X, and 11X the human dose based on body surface area.

1. Cisplatin-induced nephrotoxicity: Thirty-six rats were each administered cisplatin 2mg/kg, i.v. daily for 5 days. Four days after the last dose of cisplatin, urine volume, and plasma and urine creatinine levels were measured for calculation of GFR. Two rats died from the cisplatin treatment. Animals were divided in 4 groups, each receiving an IV administration of saline or MS-325 at 0.3, 1, and 2 mmol/kg, after which the measurements for GFR were repeated. Animals were then euthanized, kidneys were removed, weighed and histological examined.

Results: Plasma creatinine levels ranged from 0.31 – 0.70 mg/dL, while calculated GFR ranged from 4.27 to 12.57 mL/min/kg. Elevation of plasma creatinine levels by more than 20% of the baseline levels ($0.39 + 0.06$, $n=17$), compared to the saline control group, was

considered indicative of renal impairment. This was the case for the 5 rats used subsequently in the saline group, 5 rats in the 0.3 mmol/kg MS-325 group, 2 rats in the 1 mmol/kg MS-325 group, and 4 rats in the 2.0 mmol/kg group. Due to the high number of deaths, the study was suspended.

Reviewer's comments: The sponsor decided that the number of animals with demonstrated renal impairment, in the MS-325 dose groups, was insufficient to draw conclusions about the impact of MS-325 on impaired kidney function. I disagree with the sponsor: except for the 1 mmol/kg group, which comprises only 2 rats with measurable renal impairment, the number of rats in the other groups (5,5,4) would have allowed for acceptable analysis of the results. Histological examination should have been conducted independently of the results. Valuable information may have been obtained from both the microscopic and macroscopic evaluation of the kidney.

2. Cyclosporine-induced nephrotoxicity: Twenty-two rats were each treated with cyclosporine 50 mg/kg, i.p. daily for 4 days. One rat died following cyclosporine treatment. Urine volume and plasma and urine creatinine levels were measured for calculation of GFR. Animals were divided in 4 groups, each receiving an IV administration of saline or MS-325 at 0.3, 1, and 2 mmol/kg, after which the measurements for GFR were repeated.

Results: Following the cyclosporine treatment, plasma creatinine levels were slightly elevated from baseline values, 0.36 ± 0.09 to 0.40 ± 0.09 mg/dL. Glomerular filtration rates declined from 8.6 ± 4.4 to 4.5 ± 1.1 mL/min/kg. Following saline or MS-325 administration, plasma creatinine levels in the surviving animals were further elevated at 0.58 ± 0.13 , 0.49 ± 0.01 ; 0.66 ± 0.13 , and 0.58 ± 0.05 mg/dL in the saline, and 0.3, 1.0, and 2.0 mmol/kg MS-325-treated groups, respectively. In addition, a marked decrease in GFR, ranging from 0.31 to 3.18 mL/min/kg, was apparent in the surviving animals. Eight of the 22 rats died prior to completion of the study, seven after treatment with MS-325. There was no dose-relationship to the deaths in each group (3 deaths at 0.3 mmol/kg, 3 at 1.0 mmol/kg and 1 at 2 mmol/kg). The sponsor believes that renal function continued to decline after cessation of the cyclosporine treatment and throughout the remainder of the study, implying that deaths can be attributed to cyclosporine. Based on this outcome, the sponsor concludes that it was clear the cyclosporine treatment regimen failed to produce stable renal impairment, and therefore it was not possible to evaluate the impact of MS-325 on impaired kidney function.

Reviewer's comments: Although this abbreviated report contains incomplete data, serious concerns stem from this study outcome.

1. According to the sponsor, renal impairment in rats has been demonstrated in animal studies (articles are referenced) following the administration of 20 – 50 mg/kg/day, i.p. of cyclosporine over a period of approximately two-weeks. Thus, it may seem unlikely that a much shorter treatment of cyclosporine, 50mg/kg/day for 4 days, although at the high end of the range, would produce such a high number of deaths in this study. This hypothesis is even weaker with the fact that no death occurred in the saline group. Even though there was no strict correlation between dose and number of

deaths per group, the fact that these deaths occurred only in the treated groups should have been addressed by the sponsor. What is of concern is that no NOEL was established and the lowest dose, at which 3 deaths were reported, is only 1.6 times the intended clinical dose.

2. Plasma creatinine levels and GFR were reported for the surviving animals, which excludes the most affected animals from the analysis, possibly resulting in the lack of significant difference between control and treated groups.

The following study PTR-2004-019 was submitted as a follow-up to the sponsor commitment to repeat the study on a better established model of renal impairment.

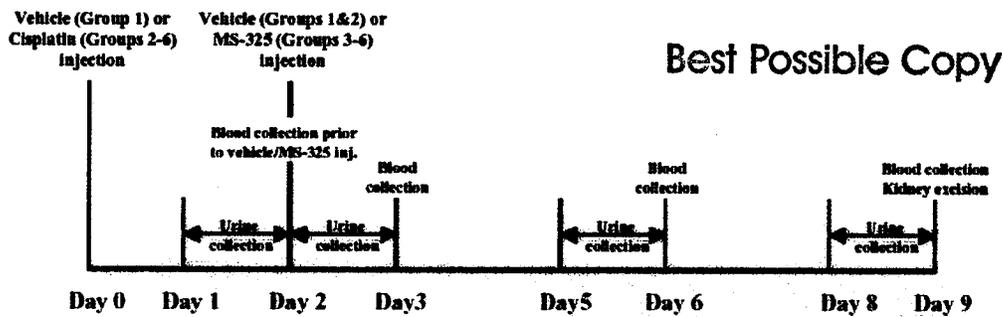
Study PTR-2004-019: Assessment of MS-325 in a Cisplatin-Induced Rat Model of Renal Impairment. (Blood and urine analysis was conducted by [] and histopathological analysis was performed by [])

b(4)

Study objective: The objective of this study was to evaluate the effect of 0.03, 0.1, 0.3 and 1 mmol/kg MS-325, respectively 0.16X, 0.5X, 1.6X, and 5X the proposed human dose, on renal function in a Cisplatin-induced model of renal impairment in the rat. It was submitted as a supplement to the NDA. Cisplatin causes proximal tubular nephrosis as well as changes in clinical parameters which can be manifested by polyuria, diminished Na⁺ excretion, a reduction in creatinine clearance (and elevation of BUN and serum creatinine), proteinuria, and enzymuria.

Study design: Six groups of 8 Wistar derived male rats weighing 200 ± 20 g and caged individually were employed. The control group received vehicle alone. The remaining five groups were treated with a single dose of cisplatin (7.5 mg/kg) by intraperitoneal injection on day 0; the cisplatin-treated groups were challenged with intravenous administration of vehicle or MS-325 at doses of 0.03, 0.1, 0.3 and 1 mmol/kg on day 2. Blood samples were on days 2, 3, 6 and 9. Serum electrolytes (Na⁺, K⁺ and Cl⁻), creatinine, BUN, albumin, ALT and AST were measured. Urine was collected over 24 hours on days 1-2, 2-3, 5-6 and 8-9 after dosing with cisplatin (days -1, 0, 4 and 7 after MS-325). Urine volume was measured, and assayed for Na⁺, K⁺ and Cl⁻ concentrations, creatinine, proteins, β-NAG. Urine volume, urinary electrolytes and proteins were calculated and expressed per 100 g of animal body weight. Kidneys were weighed following sacrifice on day 9 and shipped to the Sponsor for histopathological examination.

Figure 1. Timeline for renal impairment experiment



Results: Significant changes were induced in rats renal function following a single administration of 7.5 mg/kg cisplatin including pronounced polyuria, reduced excretion of urinary electrolytes, marked increase in absolute urinary protein excretion, reduced creatinine clearance, increase in serum BUN and creatinine. There was no degree of impairment defined as mild, moderate, and severe to reflect human situations.

Results of the histopathological analysis indicated that administration of MS-325 did not affect the incidence but induced a slight increase in severity of tubular nephrosis, regardless of dose, compared to the vehicle control.

Significant deficiencies were identified in the study. The sponsor assigned 8 animals per dose, however, the control group that was treated with cisplatin was reduced to a group of 4 animals, because one animal died during the study, and 3 did not show any signs of nephrotoxicity as measured by several parameters (BUN, creatinine). Likewise, in the high dose group, 3 animals were not affected by the Cisplatin treatment. Sponsor could not detect differences between results obtained in cisplatin-treated rats and cisplatin/MS-325 treated rats. Therefore, it was concluded that MS-325 did not cause any significant alterations in Cisplatin-induced renally impaired rats compared to Cisplatin-induced renal impairment alone.

Reviewer's comments: I do not agree with the sponsor's conclusion. In comparing the change rates in renal function parameters, there was a clear, albeit not dose-dependent, effect of MS-325 on serum creatinine plasma levels and BUN of cisplatin treated rats. The highest dose of MS-325 failed to produce the highest toxicity. On Day 3, there was a dose-dependent increase of serum creatinine levels, which began to decrease on Day 6, returning to normal values on Day 9, indicating that the renal function impairment was reversible. A similar pattern was observed in BUN changes. Urinary protein excretion data were not interpretable. Although the results were not clear-cut, because of the reduced number of control animals, the study provides partial evidence that renally impaired rats were more affected by MS-325 than normal rats were.

Overall conclusion for the renal system evaluation:

The sponsor showed that MS-325 aggravated the impaired renal function. Nonetheless, an important issue that should have been addressed in this study is the effect of an impaired kidney on the fate of the drug following its administration to the animal. Such study would determine the effects of renal impairment on the drug pharmacokinetic parameters. Overall, the renal system was inadequately investigated.

According to the clinical pharmacology reviewer, human PK data showed that Vasovist elimination half-life was extended up to 70 hrs (normal: 16 hrs) in subjects with severe renal impairment, thus exposing this population to increased risk compared to the non-renally impaired subjects. Availability of clinical data ameliorates the lack of adequate preclinical data in renally impaired animal model.

Gastrointestinal effects:

GI study conducted in Study 7L358 (see above in Section: "Neurological effects")

Abuse liability:

Not applicable

Other:

Not applicable

3.2.5 Pharmacodynamic drug interactions

Summary:

The effect of MS-325 on protein binding of digitoxin, propranolol, verapamil, and warfarin was studied in vitro in human plasma. The percent of unbound digitoxin, propranolol, and verapamil was not affected. According to the clinical pharmacology reviewer, no interaction was found between Warfarin and Vasovist in human trials, whether Vasovist was administered before or after Warfarin.

Clinically relevant concentrations (1X to 10X the maximum therapeutic dose) of Phenprocoumon did not change significantly the 1/T1 effect of MS 325. Ibuprofen was shown to decrease MS-325 percent 1/T1 by 10% whereas a reduction of ~50% was observed with naproxen. The clinical relevance of the in vitro changes in 1/T1 is yet to be demonstrated since according to the clinical pharmacology reviewer, the changes in relaxation rates measured in human subjects did not accurately predict efficacy as measured by imaging.

Study CHW6754-102: The effect of MS-325 on the in vitro protein binding of digitoxin, propranolol, verapamil, and warfarin in human plasma. (GLF)

The objective of this study was to evaluate the effect of MS-325 on the protein binding of digitoxin, propranolol, verapamil, and warfarin in human plasma.

Study design

The protein binding of digitoxin, propranolol, verapamil, and warfarin at 28, 75, 263, and 950 ng/mL respectively, was determined with and without 0.3mM and 0.9mM of MS-

325, in human plasma (pH 7.0 to 7.8) from three individuals. The plasma was fortified with MS-325, a mixture of radiolabeled and non radiolabeled ligand, or radiolabeled ligand alone, followed by 20min incubation at 37°C. Protein binding of digitoxin and warfarin was determined by ultrafiltration. Because of nonspecific binding observed with the previous method, the protein binding of propranolol and verapamil was determined by equilibrium dialysis after 4hr incubation. The percent of each ligand bound to the plasma in the presence and absence of two concentrations of MS-325 (0.3 and 0.9 mM) was calculated and compared using analysis of variance with repeated measure. Determinations were performed in triplicate.

Results

The test material was stored at room temperature for 7 days, after which it was transferred to storage at 4°C.

Table 1. Mean percent unbound of propranolol and verapamil, after various times of dialysis in human plasma.

Time (hr)	Propranolol	Verapamil
2	10.8	6.93
4	13.5	11.6
6	15.6	12.6
8	17.7	17.0
18-24	16.6	15.4

Table 2. Mean percent of unbound ligand in the presence or absence of MS-325

	MS-325		
	0	0.29 mg/mL	0.88 mg/mL
Digitoxin (\pm SD)	2.42 \pm 0.055	2.49 \pm 0.117	2.28 \pm 0.240
Propranolol	13.3 \pm 0.21	13.7 \pm 0.36	13.8 \pm 0.52
Verapamil	11.0 \pm 1.59	10.6 \pm 0.57	11.1 \pm 0.95
Warfarin	0.53 \pm 0.012	0.76 \pm 0.049	0.71 \pm 0.012

The percent of unbound digitoxin, propranolol, and verapamil was not affected by either concentrations of MS-325. The percent of unbound warfarin increased significantly by 43% and 34% in the presence of 0.3mM and 0.9mM of MS-325 respectively. The effect observed does not show a dose-dependent pattern, perhaps because the maximum plasma protein binding for MS-325 is reached with 0.3 mM.

Reviewer's comments: This finding indicates that warfarin unbound fraction is significantly increased in presence of MS-325, suggesting a competition for the albumin binding site between warfarin and MS-325. This could potentially affect both safety and efficacy of MS-325. Because of warfarin narrow therapeutic index, and based on in vitro findings, potential increased toxicity may result from the combination of the drugs. However, the clinical pharmacology reviewer communicated that human data did not demonstrate such an interaction between warfarin and Vasovist.

Study CHW6754-108: The effect of MS-325 on the in vitro protein binding of warfarin in human plasma. (GLP)

The purpose of this study was to confirm the increase of unbound warfarin in presence of MS-325 found in the previous study (Report 102). The protein binding was determined by ultrafiltration and the radiopurity of the radiolabeled ligand in the ultrafiltrate was examined by HPLC. This should demonstrate that the ultrafiltrate is constituted with the ^{14}C warfarin and not with the ^{14}C . Human plasma was pooled from 2 individuals, and the protein binding of 950ng/mL of [^{14}C]warfarin was determined in the absence and the presence of 0.3mM and 0.9mM MS-325.

Results

-The binding of warfarin was not significantly altered by the presence of MS-325, mean percent unbound fraction ranged from 0.813% to 0.886%.

-Of the amount of radioactivity that was recovered in the ultrafiltrate (unbound fraction), 82, 81, and 71% of the radioactivity was in the form of [^{14}C]warfarin in presence of 0, 0.3, and 0.8mM of MS-325.

Reviewer's comments: The findings in this study conflict with findings of the previous study, in spite of the similar experimental conditions. Therefore, the effect of MS-325 on warfarin binding to plasma protein is equivocal. These findings were communicated to the clinical reviewer who stated that human data did not demonstrate such interaction between warfarin and Vasovist.

Study 180: MS-325 competitive binding pilot study in 4.5% human serum albumin: the effect of common protein-binding drugs on relaxation rates.

This study was performed to investigate the potential of commonly used drugs, known to be highly protein-bound, to displace MS-325 and therefore lower its relaxation rate $1/T_1$. MS-325 was tested at 0.1 and 0.6mM, which are approximately a third and a double the clinical plasma level respectively.

Compounds tested	Stock solution	Concentrations tested ($\mu\text{g/mL}$)
Warfarin	PBS	2.0, 20, 200
Ibuprofen	4.5% Has	0.55, 5.5, 55, 550
Digitoxin	Ethanol	0.01, 0.1, 1.0
Diazepam	-	0.6, 6.0, 60
Ketoprofen	Ethanol	2.3, 23, 230
Naproxen	PBS	7.5, 75, 750, 7500
Diclofenac	Ethanol	2.5, 25, 250
Piroxicam	0.1N NaOH	5, 50, 500

Study design

MS-325 solutions were made by adding appropriate volumes of MS-325 directly to the 4.5%HSA solution to form a 0.6mM, and then by diluting the latter to obtain the 0.1mM

solution. Drug solutions were prepared by adding an appropriate volume of the stock solution to the MS-325/HSA solution to achieve the highest concentration tested (100X the clinically relevant concentration), and then by diluting the latter to achieve 10X, 1X, or 0.1X solutions. In all cases, the dilution was less or equal than 1%.

Proton relaxation rates were measured by NMR in triplicates at 20 MHz. The longitudinal (T1) relaxation time was determined using an inversion recovery pulse sequence with several experimental time points.

Results

-At 1X the clinical concentrations, warfarin, ibuprofen, digitoxin, diazepam, ketoprofen, naproxen, diclofenac, and piroxicam decreased the percent 1/T1 by approximately 0.5, 1, 2, 3, -4, 13, -3, and -2% respectively. Results were similar in presence of both 0.1 and 0.6mM MS-325.

-At 10X their therapeutic concentration, ibuprofen and naproxen decreased the percent 1/T1 by 10% and ~50% respectively.

-At 100X the clinical concentration, the effects were more remarkable, although not clinically significant.

Reviewer's comments

-The sponsor stated that at clinical concentration (1X), ibuprofen decreased the percent 1/T1 by more than 10% based on this study. In fact, the results show that ibuprofen decreased that value by only 1%, which in my opinion is not significant. On the other hand, a significant 13% decrease was noted at the clinical concentration of naproxen.

-The changes observed with ibuprofen and naproxen may affect the efficacy of MS-325, as well as its safety. The question remains as to the clinical relevance of the *in vitro* changes in 1/T1. Upon discussion with the clinical pharmacology team, it was stated that the changes in relaxation rates measured in human subjects did not accurately predict the efficacy of imaging. Should the impact on the efficacy be insignificant, the safety issues will still remain and will need to be addressed.

Study 401: MS-325 competitive binding pilot study in 4.5% human serum albumin: the effect of phenprocoumon on relaxation rates.

Phenprocoumon is a drug with indications similar to warfarin and it is commonly used in Europe. The objective of this study was to determine the effects of phenprocoumon on the 1/T1 effect of MS-325 in 4.5% HSA, hence MS-325 efficacy *in vitro*. Clinically relevant (clinical and 10X the maximum therapeutic dose) as well as higher (100X the maximum therapeutic dose) concentrations were studied.

The study design was similar to the previous one (Report 180), and proton relaxation rates were measured by NMR in triplicates at 20 MHz and 64 MHz.

Results: