TOXICITY REPORT

ACUTE ORAL TOXICITY STUDY – MCR 1329

SUMMARY

Title: Acute oral toxicity study of MCR-1329 in rats

Test Compound: MCR-1329

Species/Strain: Rat/Wistar

Gender: Male

No. of Test Animals: 15

Duration and Frequency of treatment: Single Dose

Route of Administration: Oral Gavage

Maximum Dose level: 2000 mg/kg

Volume of administration: 1ml/kg

Vehicle: 0.5% sodium carboxymethyl cellulose in distilled water

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Mortality
Gross necropsy

Results of the Study: Administration of 2000mg/kg MCR-1329 showed no signs of toxicity or mortality during the test period

THE LD50 OF MCR-1329 IN RATS IS >2000MG/KG

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 423, 2001). Annex 2C of the guideline document was followed unless indicated otherwise. Accordingly, the initiation dose was 300mg/kg and extended upto 2000 mg/kg. Category 5 evaluation was precluded as a dose beyond 2000mg/kg was unlikely to be ever used in practice.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

PhD Contingency of Mr. Hardik Gandhi, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: MCR-1329

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: M.R. Yadav, P.P. Naik, H.P. Gandhi, B. S. Chouhan and R. Giridhar. Design and synthesis of 6,7-dimethoxyquinazoline analogs as multitargeted ligands for α1- and AII-receptors antagonism. Bioorg. Med. Chem. Lett., 23: 3959-3966

Appearance: Pale yellow powder

Storage: Room temperature, away from light

Safety requirement: Not Known

Expected Pharmacological Effect: Prevention of blood pressure elevation

Expiry Date: Not Known

Preparation: Required amount of MCR-1329 is weighed and suspended in 1 ml of 0.5% sodium carboxymethyl cellulose by triturition followed by vortexing immediately before administration.

ANIMALS USED FOR THE TEST

Species/Strain: Rat/Wistar Albino

Age at the commencement of test: 10-12 weeks

Body weight range: 200-230 g

Sex: Male

Total no. of Animals used: 15

Source of Animals: Zydus Research Centre, Ahmedabad, India

Acclimitization: 1 week

Randomization: 1 day prior to test compound administration

Justification for species and sex: Wistar albino rats are preferred for studies on compounds acting on the cardiovascular system. They present an appropriate mammalian system for replication of effects that might be observed upon administration of the test compounds to humans. Although the guideline suggests that female animals may be preferred, it was decided to evaluate the test drug in male animals to avoid the protective effects of estrogen upon the cardiovascular system which may become evident when female animals are used.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3)with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr. NO.	Substance	Dose (mg/kg)	Volume (ml/kg)	No. of Animals
1	0.5% Carboxymethyl cellulose (Vehicle)	–	1	03
2	MCR-1329 suspended in Vehicle	300	1	06*
3	MCR-1329 suspended in Vehicle	2000	1	06*

*3 animals dosed twice

Dose Selection: Since no previous information about the in vivo data regarding the test compound was available, Annex 2C of the guideline was followed for dose selection. No pilot study or dose-ranging study was performed.

Mode of administration: orally via gastric tube
Justification for route of administration (ROA): This ROA is the intended ROA for further preclinical and clinical studies.
Dosing Frequency: Single dose
Applied maximum dose and volume: 2000mg/kg; 1 ml/kg
Dosing protocol: The animals were fasted overnight before dosing. On the day of dosing, they were manually restrained to facilitate insertion of the gastric tube through which the test compound was administered in the said dose and volume. The date of administration was 28th September, 2011.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the date of dosing.

Body weight examination: Body weights of the animals were recorded on days 0, 7 and 14. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. The animals were starved during this period with access to water. No significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. During the entire post-treatment observation period special attention was paid to alteration of skin or fur, abnormal locomotion or breathing and changes in the eye. No untoward observations were made in this regard until the terminal day of the study.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until day 14.

Pathological Necropsy: At the end of the study period, the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Table1: Body Weight

Day 0	Body weight(in gms)*				
Animal No.	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	210	211	215	220	220
2	213	204	211	202	210
3	205	209	213	212	208
Day 7	Body weight(in gms)*				
Animal No.	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	218	216	223	231	235
2	224	213	224	215	222
3	215	220	221	223	227
Day 14	Body weight(in gms)*				
Animal No.	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	229	228	241	243	245
2	234	230	238	229	223
3	229	232	239	241	242

*results are rounded off to nearest whole number

Table2: Daily food intake

Day No.	Total food intake/cage/3 animals (in gms)*				
	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	31	33	29	33	35
2	33	34	33	29	33
3	31	33	36	30	37
4	29	32	32	35	32
5	30	31	33	35	31
6	33	34	31	36	35
7	35	38	32	37	33
8	36	35	30	34	33
9	32	33	29	33	34
10	31	28	29	34	33
11	29	31	30	34	32
12	35	33	33	37	36
13	36	30	35	32	32
14	32	35	36	31	29

*results are rounded off to nearest whole number

DEVIATIONS FROM THE GUIDELINE

Although the guideline suggests that female animals may be preferred, it was decided to evaluate the test drug in male animals to avoid the protective effects of estrogen upon the cardiovascular system which may become evident when female animals are used. No other deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. Hence it was concluded that the LD_{50} of MCR-1329 upon oral administration is >2000 mg/kg.

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End of Report

TOXICITY REPORT

REPEAT DOSE ORAL TOXICITY STUDY – MCR 1329

SUMMARY

Title: Repeat Dose oral toxicity study of MCR-1329 in rats with 14-day recovery period

Test Compound: MCR-1329

Species/Strain: Rat/Wistar

Gender: Male & Female

No. of Test Animals: 30

Duration and Frequency of treatment: Repeat dose; daily for 28 days

Route of Administration: Oral Gavage

Maximum Dose level: 10 mg/kg

Volume of administration: 1ml/kg

Vehicle: 0.5% sodium carboxymethyl cellulose in distilled water

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Hematology

Serum biochemistry
Urine biochemistry
Mortality
Gross necropsy

Results of the Study: Administration of 10mg/kg MCR-1329 for 28-days showed no signs of toxicity or mortality during the test period

The dose level of 10 mg/kg is safe for chronic administration in rats.

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 407, 1995). Text of the guideline document was followed unless indicated otherwise. Based on preliminary acute toxicology data and literature review of related class of compounds, 10 mg/kg was chosen to be the animal therapeutic dose for pharmacodynamic studies. Accordingly, this dose was selected for this toxicity evaluation, since this dose is twice that of maximum intended therapeutic dose.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

PhD Contingency of Mr. Hardik Gandhi, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: MCR-1329

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: M.R. Yadav, P.P. Naik, H.P. Gandhi, B. S. Chouhan and R. Giridhar. Design and synthesis of 6,7-dimethoxyquinazoline analogs as multitargeted ligands for α1- and AII-receptors antagonism. Bioorg. Med. Chem. Lett., 23: 3959-3966

Appearance: Pale yellow powder

Storage: Room temperature, away from light

Safety requirement: Not Known

Expected Pharmacological Effect: Prevention of blood pressure elevation

Expiry Date: Not Known

Preparation: Required amount of MCR-1329 is weighed and suspended in 1 ml of 0.5% sodium carboxymethyl cellulose by triturition followed by vortexing immediately before administration. Fresh sample of the test substance is required to be prepared daily.

ANIMALS USED FOR THE TEST

Species/Strain: Rat/Wistar Albino

Age at the commencement of test: 10-12 weeks

Body weight range: 200-230 g

Sex: Male & Female (nulliparous)

Total no. of Animals used: 30

Source of Animals: Zydus Research Centre, Ahmedabad, India

Acclimitization: 1 week

Randomization: 1 day prior to initiation of test compound administration

Justification for species and sex: Wistar albino rats are preferred for studies on compounds acting on the cardiovascular system. They present an appropriate mammalian system for replication of effects that might be observed upon administration of the test compounds to humans.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3)with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr. NO.	Substance	Dose (mg/kg)	Volume (ml/kg)	No. of Animals
1	0.5% Carboxymethyl cellulose (Vehicle)	–	1	10 (5 males/5 females)
2	MCR-1329 suspended in Vehicle	10	1	10 (males)
3	MCR-1329 suspended in Vehicle	10	1	10 (females)

*3 animals dosed twice

Dose Selection: Based on preliminary acute toxicology data and literature review of related class of compounds, 10 mg/kg was chosen to be the animal therapeutic doses for pharmacodynamic studies. Accordingly, this dose was selected for this toxicity evaluation, since this dose is twice that of maximum intended therapeutic dose.No pilot study or dose-ranging study was performed.

Mode of administration: orally via gastric tube
Justification for route of administration: This ROA is the intended ROA for further preclinical and clinical studies.
Dosing Frequency: Repeat dose, daily for 28 days
Applied maximum dose and volume: 10mg/kg; 1 ml/kg
Dosing protocol: This study was conducted under fed conditions. On the day of dosing, they were manually restrained to facilitate insertion of the gastric tube through which the test compound was administered in the said dose and volume. Different groups were administered on subsequent days to facilitate urine collection.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the last date of dosing.

Body weight examination: Body weights of the animals were recorded on days 0, 7, 14, 21 and 28. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. The animals were starved during this period with access to water. No significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. During the entire post-treatment observation period special attention was paid to alteration of skin or fur, abnormal locomotion or breathing and changes in the eye. No untoward observations were made in this regard until the terminal day of the study. Hematology, serum and urine biochemistry were performed on 28^{th} day.

Hematology: RBC and WBC count, total Hb and hemotcrit were calculated at the terminal stage of the study. Results are presented in Table3.

Serum biochemistry: Glucose, Total cholesterol, AST, ALT, ALP and creatinine were estimated and results are presented in Table4.

Urine biochemistry: Urine volume, pH, glucose and protein were estimated at the end of the dosing period and results are presented in Table5.

Tail-cuff pressure: At the end of the study period, tail cuff pressure was recorded to demonstrate the effect of MCR-1329 on normal blood pressure. Results are shown in Figure1.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until the end of the test period.

Pathological Necropsy: At the end of the study period (42 days), the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Organ weights: After dissecting the animals, major organs were weighed and their weights were recorded. Any major difference from control group was noted down and presented in the report (Table6).

Table1: Body Weight

Day No.	Mean Body weight per group (in gms)*			
	Control (male)#	Control (female)#	10 mg/kg (male)$	10 mg/kg (female)$
0	221	213	225	209
7	221	215	232	217
14	237	228	240	226
21	245	236	247	233
28	249	240	252	238

*results are rounded off to nearest whole number
#Mean of 5 observations
$Mean of 10 observations

Table2: Daily food intake

Day No.	Mean food intake per animal (in gms)*			
	Control (male)#	Control (female)#	10 mg/kg (male)$	10 mg/kg (female)$
0	11.6	12.3	11.5	12.5
3	11.3	11.7	11.9	11.7
7	12.1	11.6	11.3	10.5
10	11.3	12.0	11.6	11.1
14	10.7	11.2	11.6	12.0
17	11.5	10.9	12.1	12.1
21	11.3	10.8	11.4	11.6
24	12.0	11.4	11.8	10.9
28	11.1	11.7	10.9	11.4

*results are rounded off to first decimal
#Mean of 5 observations
$Mean of 10 observations

APPENDIX II- Repeat Dose Oral Toxicity Study of MCR-1329

Table3: Hematological data

Groups	RBC ($\times 10^6$ cells/mm^3)	WBC ($\times 10^3$ cells/mm^3)	Total Hb (gm/dl)	Hematocrit* (%)
Control (male)[#]	7.1	11.1	12.3	36.9
Control (female)[#]	7.8	10.4	11.6	34.8
10mg/kg (male)[$]	8.4	10.9	12.1	36.3
10 mg/kg (female)[$]	7.7	11.7	12.0	36.0

[#]Mean of 5 observations, [$]Mean of 10 observations, *hematocrit was derived by triplicating Total Hb values.

Table4: Serum Biochemistry

Groups	Turbidity	Appearance	Total Cholesterol	Glucose (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dl)
Control (male)[#]	NIL	Pale Yellow	132.3 ± 5.8	100.3 ± 4.7	67.2 ± 2.1	21.9 ± 1.6	91.5 ± 6.8	0.45 ± 0.04
Control (female)[#]	NIL	Pale Yellow	140.0 ± 2.7	110.0 ± 5.9	70.3 ± 5.6	24.7 ± 3.1	89.0 ± 4.9	0.51 ± 0.07
10mg/kg (male)[$]	NIL	Pale Yellow	144.3 ± 8.2	111.2 ± 4.8	65.8 ± 6.01	24.3 ± 2.4	87.9 ± 6.9	0.55 ± 0.05
10 mg/kg (female)[$]	NIL	Pale Yellow	138.8 ± 5.9	105.4 ± 3.3	66.8 ± 3.9	27.2 ± 2.0	94.1 ± 5.4	0.52 ± 0.09

[#]Mean of 5 observations, [$]Mean of 10 observations

Table5: Urine Analysis

Groups	Mean volume	Appearance	Turbidity	Mean pH	Glucose	Protein
Control (male)[#]	5.7	Pale Yellow	NIL	6.7	<10mg/dl	NIL
Control (female)[#]	5.9	Pale Yellow	NIL	6.8	<10mg/dl	NIL
10mg/kg (male)[$]	5.5	Pale Yellow	NIL	6.5	<10mg/dl	NIL
10 mg/kg (female)[$]	5.8	Pale Yellow	NIL	6.6	<10mg/dl	NIL

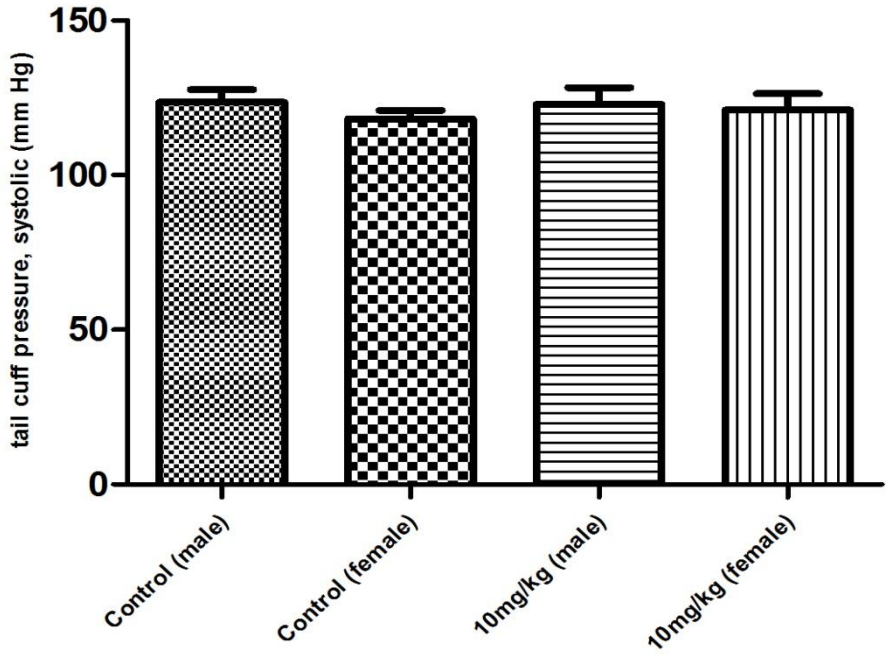
[#]Mean of 5 observations, [$]Mean of 10 observations

Table6: Organ weights at 42 days

Group	Mean organ wet weight (in gms)					
	Brain	Heart	Lung	Liver	Spleen	Kidney*
Control (male)#	2.01 ± 0.13	1.10 ± 0.06	1.31 ± 0.07	7.43 ± 0.54	0.48 ± 0.07	1.80 ± 0.05
Control (female)#	1.86 ± 0.04	0.66 ± 0.08	0.90 ± 0.11	5.98 ± 0.67	0.39 ± 0.04	1.35 ± 0.05
10mg/kg (male)$	2.13 ± 0.08	0.98 ± 0.04	1.25 ± 0.06	6.89 ± 0.91	0.51 ± 0.04	1.75 ± 0.07
10 mg/kg (female)$	1.93 ± 0.03	0.84 ± 0.06	1.06 ± 0.07	6.01 ± 0.42	0.42 ± 0.06	1.57 ± 0.05

*Both the capsules were weighed, #Mean of 5 observations, $Mean of 10 observations

Figure1: Tail cuff pressure comparison between groups*



*For control groups, n=5; for test groups, n=10.

DEVIATIONS FROM THE GUIDELINE

The guideline mentions the use of a range finding test or a limit test with a dose of 1000 mg/kg but since such a dose level is unlikely and corresponding human dose may never be applied in practice, we preferred using a dose of 10 mg/kg. No other deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. The most plausible side effect related to the mechanism of action of MCR-1329 is hypotension. This

effect was not evident from the tail-cuff recordings. Biochemical estimations did not suggest any major digression from normal values. Urinary output and hematological data appeared normal. Hence it was concluded that chronic administration of MCR-1329 at a dose level of 10mg/kg was safe.

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End of Report

TOXICITY REPORT

ACUTE ORAL TOXICITY STUDY – MCR 788

SUMMARY

Title: Acute oral toxicity study of MCR-788 in rats

Test Compound: MCR-788

Species/Strain: Rat/Wistar

Gender: Male

No. of Test Animals: 15

Duration and Frequency of treatment: Single Dose

Route of Administration: Oral (diet-admixture)

Maximum Dose level: 2000 mg/kg

Volume of administration: -NA-

Vehicle: Pelleted chow

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Mortality
Gross necropsy

Results of the Study: Administration of 2000mg/kg MCR-788
showed no signs of toxicity or mortality during the test period

THE LD50 OF MCR-788 IN RATS IS >2000MG/KG

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 423, 2001). Annex 2C of the guideline document was followed unless indicated otherwise. Accordingly, the initiation dose was 300mg/kg and extended upto 2000 mg/kg. Category 5 evaluation was precluded as a dose beyond 2000mg/kg was unlikely to be ever used in practice.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

1. PhD Contingency of Mr. Hardik Gandhi, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda
2. CSIR-SRF Contingency of Mr. Hardik Gandhi, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: MCR-788

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: Compound Data-Sheet of MCR-788 by Palash Pal, Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Appearance: White powder

Storage: Room temperature, away from light

Safety requirement: Not Known

Expected Pharmacological Effect: Prevention of cholesterol accumulation and improvement of lipid profile

Expiry Date: Not Known

Preparation: Required amount of MCR-788 is weighed and admixed in the diet based on daily requirement and quantity of food consumed by each individual animal [e.g. if an animal (B.W. 200g) consumes 15g of food pellets on day1, then to administer a dose of 1000mg/kg the compound-feed admixture is prepared so as to contain 200mg test compound per 15g or 1.34g test compound per 100g of feed). The modified food pellets were formed everyday and stored at 2-8°C until provided to the animals.

ANIMALS USED FOR THE TEST

Species/Strain: Rat/Wistar Albino

Age at the commencement of test: 10-12 weeks

Body weight range: 200-230 g

Sex: Male

Total no. of Animals used: 15

Source of Animals: Zydus Research Centre, Ahmedabad, India

Acclimitization: 1 week

Randomization: 1 day prior to test compound administration

Justification for species and sex: Wistar albino rats are preferred for studies on compounds acting on the cardiovascular system. They present an appropriate mammalian system for replication of effects that might be observed upon administration of the test compounds to humans. Although the guideline suggests that female animals may be preferred at the starting of the study, it was decided to evaluate the test drug in male animals to avoid the protective effects of estrogen upon the

cardiovascular system which may become evident when female animals are used.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3) with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr. NO.	Substance	Dose# (mg/kg)	Volume (ml/kg)	No. of Animals
1	Control group – Pelleted chow	–	1	03
2	MCR-788 suspended in Vehicle	300	1	06*
3	MCR-788 suspended in Vehicle	2000	1	06*

#admixed in the diet; *3 animals dosed twice

Dose Selection: Since no previous information about the in vivo data regarding the test compound was available, Annex 2C of the guideline was followed for dose selection. No pilot study or dose-ranging study was performed.

Mode of administration: orally via feed admixture

Justification for route of administration (ROA): This ROA is the intended ROA for further preclinical studies.

Dosing Frequency: Single dose

Applied maximum dose and volume: 2000mg/kg; NMT 15g diet-admix

Dosing protocol: The animals were fasted overnight before dosing. On the day of dosing, the normal diet was replaced

by modified diet as indicated in the *preparation* section. Over the day, food intake was measured every 2 hrs and the modified diet was replaced with normal Pelleted diet as soon as the required amount of drug was consumed.
The date of administration was 4th September, 2013.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the date of dosing.

Body weight examination: Body weights of the animals were recorded on days 0, 7 and 14. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. The animals were starved during this period with access to water. No other significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. Since the animals were provided with modified diets they initially showed a mild aversion towards diet consumption but later on consumed the diets at will. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. During the entire post-treatment observation period special attention was paid to alteration of skin or fur (to identify any signs of excess free cholesterol deposition and consequent xanthomatosis), eyelids (to check for meibomian gland atrophy due to ACAT inhibition), abnormal locomotion or breathing and changes in the eye.

No untoward observations were made in this regard until the terminal day of the study.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until day 14.

Pathological Necropsy: At the end of the study period, the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. The liver is the most susceptible internal organ to be affected by these category of compounds. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Table1: Body Weight

Day 0	Body weight(in gms)*				
Animal No.	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	224	221	202	216	218
2	204	224	228	219	214
3	211	214	209	204	221
Day 7	Body weight(in gms)*				
Animal No.	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	229	226	210	222	223
2	211	218	232	225	220
3	217	220	215	212	228
Day 14	Body weight(in gms)*				
Animal No.	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	236	231	217	227	230
2	218	224	239	233	228
3	224	226	227	219	241

*results are rounded off to nearest whole number

Table2: Daily food intake

Day No.	Total food intake/cage/3 animals (in gms)*				
	Control	300mg/kg (set1)	300mg/kg (set2)	2000mg/kg (set1)	2000mg/kg (set2)
1	28	30	33	31	30
2	31	31	29	35	31
3	34	29	28	31	32
4	33	33	30	30	33
5	29	33	32	28	28
6	32	32	34	33	34
7	32	31	31	30	29
8	35	35	33	29	33
9	33	34	33	32	34
10	31	29	35	33	35
11	30	30	29	31	32
12	28	30	28	29	29
13	29	31	30	30	33
14	32	31	33	29	34

*results are rounded off to nearest whole number

DEVIATIONS FROM THE GUIDELINE

Although the guideline suggests that female animals may be preferred, it was decided to evaluate the test drug in male animals to avoid the protective effects of estrogen upon the cardiovascular system which may become evident when female animals are used.

The guideline does not indicate that the test compound may be admixed with the diet. However, the guideline does allow administration of smaller fractions in suitable vehicle over a 24-hr period. Taking this statement into consideration, we administered the test compound as an admixture with the feed where feed may be considered as the *suitable vehicle* and the small fractions will depend on the amount of feed consumed by the animal (size of the fractions not being mentioned in the guideline).

No other deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. Hence it was concluded that the LD_{50} of MCR-788 upon oral administration is >2000 mg/kg.

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End of Report

TOXICITY REPORT

REPEAT DOSE ORAL TOXICITY STUDY – MCR 788

SUMMARY

Title: Repeat Dose oral toxicity study of MCR-788 in rats with 14-day recovery period

Test Compound: MCR-788

Species/Strain: Rat/Wistar

Gender: Male & Female

No. of Test Animals: 30

Duration and Frequency of treatment: Repeat dose; daily for 28 days

Route of Administration: Oral (diet-admixture)

Maximum Dose level: 60 mg/kg

Volume of administration: -NA-

Vehicle: Pelleted chow

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Hematology

Serum biochemistry
Urine biochemistry
Mortality
Gross necropsy

Results of the Study: Administration of 60mg/kg MCR-788 for 28-days showed no signs of toxicity or mortality during the test period

The dose level of 60 mg/kg is safe for chronic administration in rats.

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 407, 1995). Text of the guideline document was followed unless indicated otherwise. Based on preliminary acute toxicology data and literature review of related class of compounds, 60 mg/kg was chosen to be the animal therapeutic dose for pharmacodynamic studies. Accordingly, this dose was selected for this toxicity evaluation, since this dose is twice that of maximum intended therapeutic dose.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

1. PhD Contingency of Mr. Hardik Gandhi, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda
2. CSIR-SRF Contingency of Mr. Hardik Gandhi, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: MCR-788

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: Compound Data-Sheet of MCR-788 by Palash Pal, Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.
Appearance: White powder
Storage: Room temperature, away from light
Safety requirement: Not Known
Expected Pharmacological Effect: Prevention of cholesterol accumulation and improvement of lipid profile
Expiry Date: Not Known
Preparation: Required amount of MCR-788 is weighed and admixed in the diet based on daily requirement and quantity of food consumed by each individual animal [e.g. if an animal (B.W. 200g) consumes 15g of food pellets on day1, then to administer a dose of 1000mg/kg the compound-feed admixture is prepared so as to contain 200mg test compound per 15g or 1.34g test compound per 100g of feed). The modified food pellets were formed everyday and stored at 2-8°C until provided to the animals.

ANIMALS USED FOR THE TEST

Species/Strain: Rat/Wistar Albino
Age at the commencement of test: 10-12 weeks
Body weight range: 200-230 g
Sex: Male & Female (nulliparous)
Total no. of Animals used: 30
Source of Animals: Zydus Research Centre, Ahmedabad, India
Acclimitization: 1 week
Randomization: 1 day prior to initiation of test compound administration
Justification for species and sex: Wistar albino rats are preferred for studies on compounds acting on the cardiovascular system. They present an appropriate mammalian system for replication of effects that might be

observed upon administration of the test compounds to humans.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3)with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr. NO.	Substance	Dose# (mg/kg)	Volume (ml/kg)	No. of Animals
1	Control group – Pelleted chow	—	1	10 (5 males/5 females)
2	MCR-788 suspended in Vehicle	60	1	10 (males)
3	MCR-788 suspended in Vehicle	60	1	10 (females)

#admixed in the diet; *3 animals dosed twice

Dose Selection: Based on preliminary acute toxicology data and literature review of related class of compounds, 60 mg/kg (or less) was chosen to be the animal therapeutic doses for pharmacodynamic studies. Accordingly, this dose was selected for this toxicity evaluation, since this dose is twice that of maximum intended therapeutic dose. No pilot study or dose-ranging study was performed.

Mode of administration: orally via feed admixture

Justification for route of administration (ROA): This ROA is the intended ROA for further preclinical studies.

Dosing Frequency: Repeat dose, daily for 28 days

Applied maximum dose and volume: 60mg/kg; NMT 15g diet-admix

Dosing protocol: This study was conducted under fed conditions. On the day of dosing, the normal diet was replaced by the modified diet and continued for 28 days. The amount of modified diet replaced each day was dependent upon the diet consumed by each animal on the previous day. Different groups were inducted on subsequent days to facilitate urine collection.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the last date of dosing.

Body weight examination: Body weights of the animals were recorded on days 0, 7, 14, 21 and 28. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. No significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. During the entire post-treatment observation period special attention was paid to alteration of skin or fur, abnormal locomotion or breathing and changes in the eye. No untoward observations were made in this regard until the terminal day of the study. Hematology, serum and urine biochemistry were performed on 28th day.

Hematology: RBC and WBC count, total Hb and hemotcrit were calculated at the terminal stage of the study. Results are presented in Table3.

Serum biochemistry: Glucose, Total cholesterol, AST, ALT, ALP and creatinine were estimated and results are presented in Table4.

Urine biochemistry: Urine volume, pH, glucose and protein were estimated at the end of the dosing period and results are presented in Table5.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until the end of the test period.

Pathological Necropsy: At the end of the study period (42 days), the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Organ weights: After dissecting the animals, major organs were weighed and their weights were recorded. Any major difference from control group was noted down and presented in the report (Table6).

Table1: Body Weight

Day No.	Mean Body weight per group (in gms)*			
	Control (male)#	Control (female)#	60 mg/kg (male)$	60 mg/kg (female)$
0	219	207	225	214
7	225	212	231	222
14	234	221	239	233
21	241	226	245	238
28	248	235	253	244

*results are rounded off to nearest whole number
#Mean of 5 observations
$Mean of 10 observations

Table2: Daily food intake

Day No.	Mean food intake per animal (in gms)*			
	Control (male)#	Control (female)#	60 mg/kg (male)$	60 mg/kg (female)$
0	11.5	10.9	11.7	11.2
3	11.9	11.4	11.3	11.4
7	10.8	11.4	11.9	11.9
10	12.3	11.9	11.1	12.1
14	12.0	10.8	11.0	11.7
17	11.7	11.3	11.9	11.3
21	10.9	12.1	11.8	11.9
24	11.3	12.3	11.3	12.0
28	11.7	11.7	11.2	10.9

*results are rounded off to first decimal
#Mean of 5 observations
$Mean of 10 observations

APPENDIX IV- Repeat Dose Oral Toxicity Study of MCR-788

Table3: Hematological data

Groups	RBC ($\times 10^6$ cells/mm^3)	WBC ($\times 10^3$ cells/mm^3)	Total Hb (gm/dl)	Hematocrit* (%)
Control (male)[#]	8.1	10.5	11.9	37.0
Control (female)[#]	7.2	10.9	12.5	39.1
10mg/kg (male)[$]	7.4	10.2	12.0	34.8
60 mg/kg (female)[$]	7.9	11.1	12.1	40.2

[#]Mean of 5 observations, [$]Mean of 10 observations, *hematocrit was derived by triplicating Total Hb values.

Table4: Serum Biochemistry

Groups	Turbidity	Appearance	Total Cholesterol	Glucose (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dl)
Control (male)[#]	NIL	Pale Yellow	135.2 ± 10.2	90.3 ± 5.9	65.3 ± 10.2	24.9 ± 5.3	88.4 ± 13.1	0.50 ± 0.09
Control (female)[#]	NIL	Pale Yellow	128.3 ± 14.3	111.8 ± 10.1	68.5 ± 9.4	27.3 ± 3.9	93.2 ± 14.7	0.53 ± 0.11
10mg/kg (male)[$]	NIL	Pale Yellow	122.8 ± 9.6	109.5 ± 7.9	67.4 ± 11.3	28.4 ± 6.2	91.6 ± 10.2	0.45 ± 0.06
60 mg/kg (female)[$]	NIL	Pale Yellow	125.6 ± 11.2	100.2 ± 12.4	69.8 ± 10.3	29.2 ± 4.2	95.3 ± 16.2	0.54 ± 0.10

[#]Mean of 5 observations, [$]Mean of 10 observations

Table5: Urine Analysis

Groups	Mean volume	Appearance	Turbidity	Mean pH	Glucose	Protein
Control (male)[#]	6.1	Pale Yellow	NIL	6.5	<10mg/dl	NIL
Control (female)[#]	5.7	Pale Yellow	NIL	6.9	<10mg/dl	NIL
10mg/kg (male)[$]	5.5	Pale Yellow	NIL	6.6	<10mg/dl	NIL
60 mg/kg (female)[$]	5.9	Pale Yellow	NIL	6.8	<10mg/dl	NIL

[#]Mean of 5 observations, [$]Mean of 10 observations

Table6: Organ weights at 42 days

Group	Mean organ wet weight (in gms)					
	Brain	Heart	Lung	Liver	Spleen	Kidney*
Control (male)#	1.83 ± 0.15	0.96 ± 0.09	1.27 ± 0.14	7.54 ± 1.10	0.45 ± 0.05	1.78 ± 0.29
Control (female)#	2.11 ± 0.18	0.078 ± 0.05	1.13 ± 0.10	6.79 ± 0.96	0.51 ± 0.03	1.65 ± 0.33
10mg/kg (male)$	2.21 ± 0.22	1.04 ± 0.09	0.97± 0.15	7.92 ± 1.03	0.53 ± 0.01	1.53 ± 0.27
60 mg/kg (female)$	1.99 ± 0.08	0.83 ± 0.1	1.19 ± 0.09	8.02 ± 1.23	0.55 ± 0.08	1.80 ± 0.31

*Both the capsules were weighed, #Mean of 5 observations, $Mean of 10 observations

DEVIATIONS FROM THE GUIDELINE

The guideline mentions the use of a range finding test or a limit test with a dose of 1000 mg/kg but since such a dose level is unlikely and corresponding human dose may never be applied in practice, we preferred using a dose of 60 mg/kg.

Doses were administered in the form of diet-admixture as this was the intended route for further preclinical studies.

No other deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. The most plausible side effect related to the mechanism of action of MCR-788 is free cholesterol deposition and associated xanthomatosis. This effect was not evident from visual observations nor did the cholesterol levels suggest any such effects. Biochemical estimations did not suggest any major digression from normal values. Urinary output and hematological data appeared normal. Hence it was concluded that chronic administration of MCR-788 at a dose level of 10mg/kg was safe.

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End of Report