Intramuscular dose of midazolam in rats producing exposure equivalent to recommended dose of Seizalam® (midazolam injection) in humans

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INTRODUCTION

Seizalam® is a midazolam HCl (MDZ) formulation approved by the FDA as a first-line treatment for status epilepticus (SE) in adults. MDZ is a BZ that acts as a positive allosteric modulator of synaptic $GABA_A$ receptors but is inactive on extrasynaptic $GABA_A$ receptors. The recommended dose of Seizalam® is 10 mg, administered by intramuscular (IM) injection. According to its label, Seizalam® at the recommended dose yields a C_{max} of 113.9 ± 30.9 (SD) ng/ml in humans.

Based on the assumption that C_{max} is the relevant parameter pharmacokinetic for therapeutic activity, we sought to estimate the dose of MDZ which when administered intramuscularly in rats would produce a plasma exposure equal to this value.

AIM

Our objective was to define the appropriate dose of MDZ to be used in studies in rats of novel SE treatments that may be administered in conjunction with Seizalam®. We dosed MDZ to rats by intramuscular injection, measured plasma MDZ concentrations at time intervals after administration, and determined pharmacokinetic (PK) parameters based on noncompartmental analysis (NCA) of plasma level data. We used two-compartment PK modeling to estimate the rat-equivalent dose to match the manufacturer's reported C_{max} with IM administration in humans.

MATERIALS & METHODS

Adult male Sprague-Dawley rats (SD; Charles-Rivers, 150–300 g) were used in the present study.

Male Sprague-Dawley rats (n=13) were implanted with a permanent cannula in the right jugular vein. After 7–10 days of post-operative care, animals were injected with a 0.9 mg/kg IM dose of MDZ (commercial 5 mg/mL solution for injection, Hospira). Blood was withdrawn at 0, 2, 5, 15, 30, 60, 120, 240, 300, 360, and 480 min after the MDZ injection.

The plasma was separated and MDZ levels were measured using LC-MS/MS (<LLOQ=50 pg/ml). A NCA was performed on the plasma level data from each animal to estimate the T_{max}, C_{max} and AUC. The plasma level data was also fit with a two-compartment PK model to obtain the clearance parameters for each individual and for the entire group.

Simulations were performed of 0.5, 0.7, 0.9, 1, 1.1 mg/kg doses using the PK parameters either from group model fitting or mean of individual model fitting to estimate the dose of MDZ.

RESULTS

MDZ Pharmacokinetic and Non-Compartmental Analysis

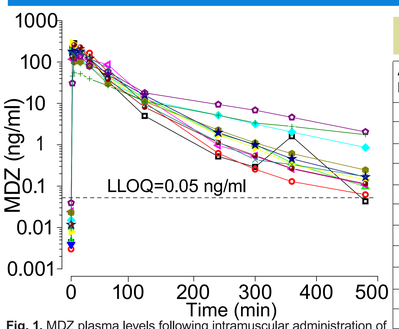


Fig. 1. MDZ plasma levels following intramuscular administration of MDZ (0.9 mg/kg) in rats. Blood was collected through permanently implanted jugular vein catheter at 0, 2, 5, 15, 30, 60, 120, 240, 300, 360 and 480 min. Data points represent values from individual animals as shown in Table 1.

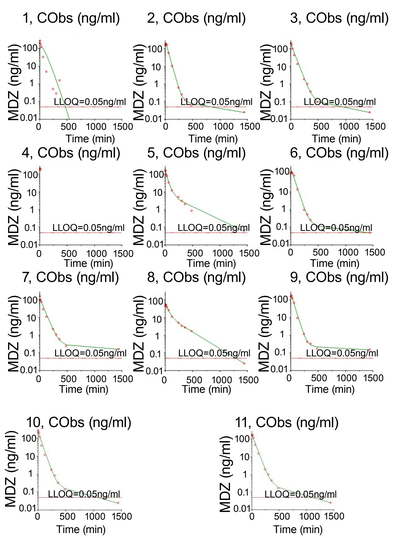
Table 1. Non-compartmental analysis for intramuscular MDZ in rats.

Animal ID	T_{max} (min)	C_{max} (ng/ml)	AUC_{last} (min*ng/ml)	AUC_{INF_obs} (min*ng/ml)	AUC_{0-480} (min*ng/ml)
1	5.00	284.17	8878.90	8974.62	8975.42
2	15.00	190.15	10338.80	10358.02	10299.59
3	2.00	206.25	9119.24	9127.41	9068.52
4	5.00	216.47	2824.16	–	–
5	2.00	163.87	7982.68	8003.89	7689.09
6	5.00	127.70	9712.48	9752.51	9639.97
7	2.00	161.61	6574.57	6611.19	6380.23
8	5.00	54.99	5486.50	5492.20	5095.90
9	15.00	176.03	9870.45	9909.78	9714.61
10	2.00	284.85	10064.82	10076.36	9999.78
11	15.00	178.08	9722.60	9732.96	9651.33
12	15.00	138.86	10537.20	10542.48	10097.97
13	5.00	257.39	9528.01	9540.77	9471.61

Table 2. Non-compartmental analysis parameters statistics (Animal # 4 was excluded for AUC_{0_480} and AUC_{INF_Obs} calculations due to insuffient time-point data).

Parameter	Unit	N	Mean	SD	SEM	Min	Median	Max
AUC_{0_15}	Min*(ng/ml)	13	2288.40	758.76	210.44	731.67	2376.56	3252.84
AUC_{0_480}	Min*(ng/ml)	12	8840.33	1623.24	468.59	5095.90	9555.79	10299.59
AUC_{INF_Obs}	Min*(ng/ml)	12	9010.18	1555.77	449.11	5492.20	9636.87	10542.48
AUC_{last}	Min*(ng/ml)	13	8510.80	2267.09	628.78	2824.16	9528.01	10537.20
C_{max}	ng/ml	13	187.72	64.45	17.88	54.99	178.08	284.85
T_{max}	Min	13	7.15	5.60	1.55	2.00	5.00	15.00

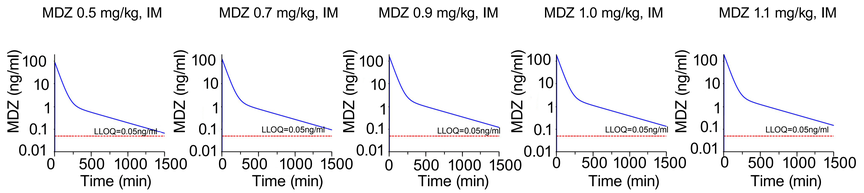
Individual Two Compartment PK Modeling

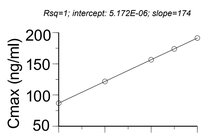


Animal ID	Ka (1/min)	V1(L/Kg)	V2 (L/Kg)	CL (L/kg/h)	CL2 (L/kg/h)
1#	0.024	0.00	2.07	3.87	11.26
2	0.39	3.41	0.71	5.21	0.05
3	29.14	4.80	0.71	5.27	0.05
4#	0.66	3.83	596.84	2.14	0.74
5	38.23	5.72	6.76	6.64	1.87
6	0.36	4.36	1.87	5.53	0.10
7	21.12	7.31	19.19	6.93	0.60
8	0.61	13.87	10.80	9.96	5.07
9	0.55	4.03	14.97	5.27	0.36
10	8.44	3.80	0.57	4.69	0.07
11	1.47	4.77	0.77	5.36	0.09
12#	0.34	8.33	2396.21	3.38	1.11
13	0.49	3.78	0.74	5.30	0.09
Mean	10.08	5.59	5.71	6.02	0.83
SD	14.20	3.13	6.95	1.55	1.59

V1: Volume of the central compartment; V2-volume of peripheral tissue compartment; Cl: clearance from central compartment
CL2: Inter-compartmental distribution; Ka: absorption rate constant
Animal 1, 4 and 12 were excluded from the Mean and SD calculations due to the fitness issue.

Simulation Using Mean Individual PK Parameters

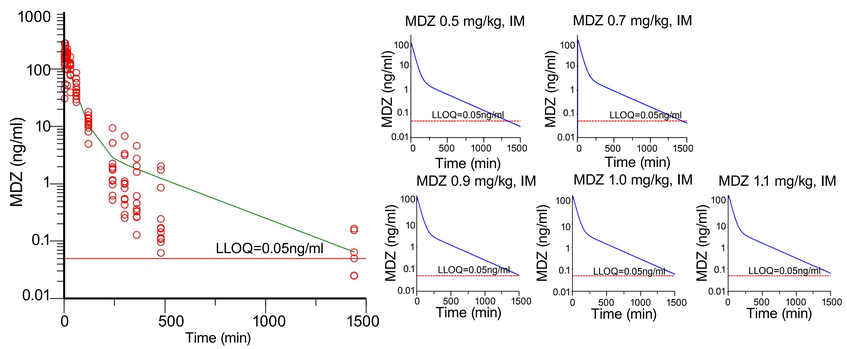




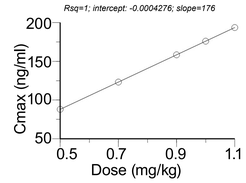
Dose (mg/kg)	C_{max} (ng/ml)
0.5	87.01
0.7	121.81
0.9	156.61
1	174.01
1.1	191.41

Interpolated Target	Dose (mg/kg)	C_{max} (ng/ml)
C_{max} – SD	0.48	83
Target C_{max} (FDA-approved label)	**0.66**	**113.9**
C_{max} + SD	0.83	144.8

Grouped Two-Compartment PK Modeling and Simulation



Variable	Unit	Estimate	SEM	CV%	2.5%CI	97.5%CI	Var. Inf. factor
Ka	1/min	0.72	0.26	36.13	0.21	1.23	0.30
V1	L/kg	5.00	0.50	10.08	0.00	0.01	9.97E-07
V2	L/kg	6.00	0.73	12.25	0.01	0.01	2.16E-06
CL	L/kg/h	6.65	0.39	5.80	9.82E-05	0.00	1.44E-10
CL2	L/kg/h	1.38	0.20	14.84	1.62E-05	2.97E-05	4.47E-11



Dose (mg/kg)	C_{max} (ng/ml)
0.5	88.01
0.7	123.21
0.9	158.42
1	176.02
1.1	193.62

Interpolated Target	Dose (mg/kg)	C_{max} (ng/ml)
C_{max} – SD	0.47	83
Target C_{max} (FDA-approved label)	**0.65**	**113.9**
C_{max} + SD	0.82	144.8

DISCUSSION

NCA of the plasma level measurements in each animal yielded a mean C_{max} value of 187.7 ± 64.5 (SD) ng/ml with T_{max} values in the range of 2–15 min (mean, 7.2 ± 5.6 min).

The mean AUC0-480 min was 8840.3 ± 1623.2 min*ng/ml. The levels remained greater than 115 ng/ml for at least 15 min after dosing in 10 of 13 animals tested.

Using the PK parameters from the group model fitting, the dose of MDZ required to reach the C_{max} of 113.9 ng/ml is 0.647 mg/kg (simulated C_{max} is 158.5 ng/ml at 0.9 mg/kg dose).

When using the mean of the individual PK parameters,a dose of 0.655 mg/kg MDZ is required to reach the targeted C_{max} (simulated C_{max} is 156.61 ng/ml at 0.9 mg/kg dose).

CONCLUSION

In conclusion, a MDZ dose of 0.65 mg/kg, IM, in rats is estimated to yield the same plasma exposure (C_{max}) as obtained with the recommended Seizalam® dose in humans and is proposed as the appropriate dose to be administered in rat models to mimic the human dosing.

Disclosure: None

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