In Vitro ADME, Pharmacokinetics, and Bone Marrow Accumulation of IMAE, a Candidate for Acute Radiation Syndrome Prevention

Sandeep K. Singh, PhD[1], Daniel Draney, PhD[2], Patrick Dussault, PhD[2], Rebecca E Oberley-Deegan, PhD[3], Daryl J Murry, PharmD[1]

[1]Department of Pharmacy Practice and Science, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE, 68198, USA. [2]Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE, 68588-0304, USA. [3]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198, USA.

NATIONAL STRATEGIC RESEARCH INSTITUTE at the University of Nebraska

Background

- Exposure to whole-body irradiation causes DNA damage and accelerates ROS production, which triggers bone marrow (BM) cell death resulting in acute radiation syndrome (ARS).
- 1-(1H-indol-3-yl)-2-(methylamino) ethan-1-ol (IMAE) is a novel racemic compound under investigation for prevention of ARS.
- IMAE has shown protection against radiation damage in mice at single per-oral (PO) doses of 200 to 400 mg/kg administered 15 minutes prior to radiation exposure.
- A validated LC-MS/MS assay is required for accurate quantitation and preclinical pharmacokinetics (PK) characterization of IMAE.
- Systemic and BM exposure of IMAE is necessary to evaluate its therapeutic efficacy in a mouse model of ARS and further optimize dose and dosing schedule for preventing ARS.

Methods

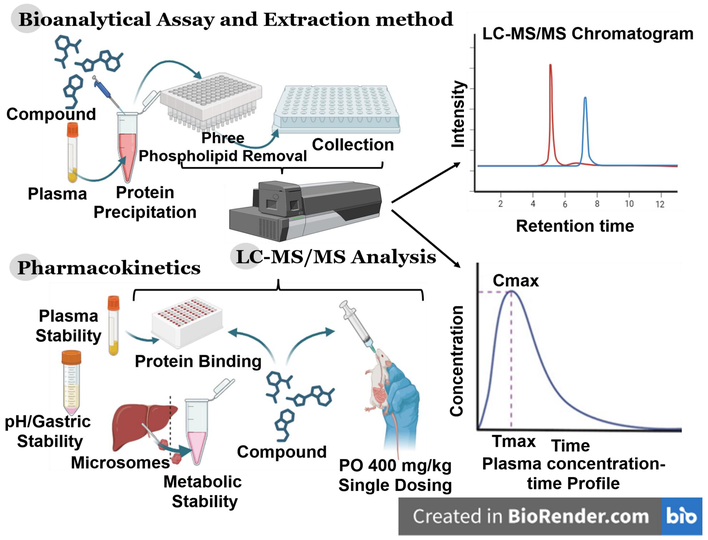
LC–MS/MS Conditions

- **Mobile Phase Compositions:**
- **A:** 0.1 % v/v Formic acid (FA) in Water
- **B:** 0.05% v/v FA in Acetonitrile: Methanol (50:50 v/v)
- **Elution program / Flow Rate:** Gradient Flow; 0.3 mL/min
- **LC Column:** Phenomenex® Synergi Polar-RP (150×2 mm, 4µm)

Table 1. Optimized MS/MS parameters for IMAE and Baclofen-d4 (internal standard; I.S.).

Analytes	MRM* transition m/z (Q1→Q3)	Q1 (V)	CE (V)	Q3 (V)	RT (min)
IMAE	191.00/173.05	-23	-8	-12	3.4
Baclofen-d_4 (I.S.)	218.10/155.10	-30	-19	-16	3.7

***MRM** multiple reaction monitoring.



Results: Method Development

Fig 1. LC-MS/MS overlay chromatogram of extracted IMAE (200 ng/mL) and baclofen-d4 (I.S.; 2.5 µg/mL) from blank mouse plasma.

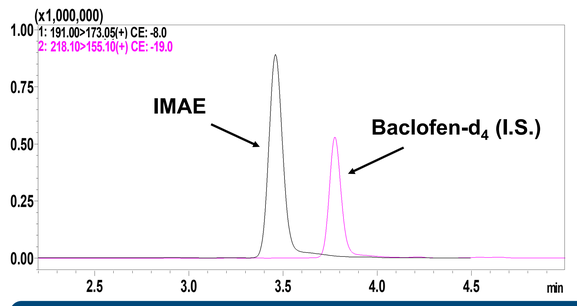
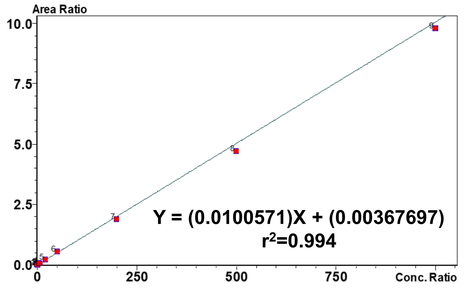


Fig 2. Concentration (x axis) and area ratio (y axis) calibration curve for IMAE (0.5–1000 ng/mL) in blank mouse plasma.



Results: In Vitro Characterization

Table 2. Preclinical **in vitro** PK study of IMAE.

Parameters (Units)	IMAE Conditions / Incubation*	IMAE Results (Mean± SD, n=3)
Kinetic Solubility (µg/mL)	PBS (pH 7.4, 2 h)	58.4 ± 2.8
Stability (%)	SGF (pH 1.2, 2 h)	94.8 ± 2.9
	SIF (pH 6.8, 2 h)	97.6 ± 1.6
	PBS (pH 7.4, 2 h)	99.1 ± 0.6
	Mouse Plasma (4 h)	99.5 ± 0.7
Plasma Protein Binding (%, PPB)	Mouse Plasma	11.0 ± 1.8
Half-Life (min.)	Mouse Liver Microsomes	70.4 ± 1.9
Intrinsic Clearance (Hepatic, mL/min/kg)	Mouse Liver Microsomes	53.2 ± 1.5

***PBS** phosphate buffered saline; **SGF** simulated gastric fluid; **SIF** simulated intestinal fluid.

Results: In Vivo Pharmacokinetics

Fig 3. Plasma concentration versus time profile of IMAE in CD2F1 mice following a single 400 mg/kg PO dose.

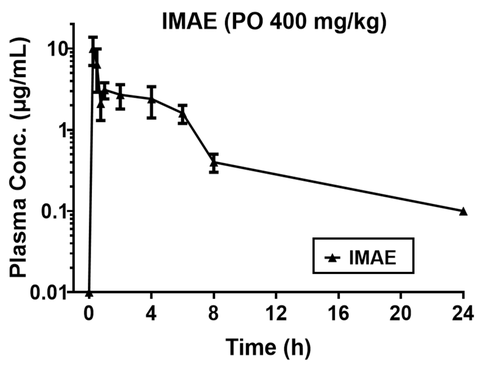


Table 3. PK parameters of IMAE in CD2F1 mice following a single 400 mg/kg PO dose.

Pharmacokinetic Parameters*	IMAE (400 mg/kg PO)
T_{max} (h)	0.3 ± 0.1
C_{max} (µg/mL)	11.0 ± 3.6
$AUC_{(0\text{-}24h)}$ (h × µg/mL)	20.8 ± 4.1
$AUC_{(0\text{-}\infty)}$ (h × µg/mL)	21.7 ± 3.6
$t_{1/2}$ (h)	4.9 ± 1.2
$V_{d/F}$ (L/kg)	137.6 ± 55.2
$CL_{/F}$ (L/h/kg)	18.8 ± 3.1

*T_{max} time of maximum plasma concentration; C_{max} maximum plasma concentration; **AUC** area under the concentration-time curve, $t_{1/2}$ terminal half-life; $V_{d/F}$ apparent volume of distribution, $CL_{/F}$ apparent clearance.

Table 4. Bone marrow (BM)/Plasma (PL) ratio of IMAE following a single PO 400 mg/kg dose in CD2F1 mice.

Time (h)	IMAE (Mean± SD, n=5) BM Conc. (µg/g)	IMAE (Mean± SD, n=5) Plasma Conc. (µg/mL)	BM/PL ratio
2	3.6±1.3	2.7±0.9	**1.3**
8	1.2±0.7	0.4±0.1	**3.0**
24	0.1±0.0	0.1±0.0	**1.0**

Summary and Future Directions

- A sensitive LC-MS/MS method was successfully validated for IMAE quantitation in mouse plasma and BM.
- IMAE was stable **(>95%)** in gastric pH and mouse plasma at specified incubation conditions.
- IMAE exhibits cytochrome P450 (CYP) and non-CYP mediated metabolic degradation in mouse liver microsomes.
- IMAE was detected in the plasma and BM up to 24 h post single PO administration at 400 mg/Kg in male CD2F1 mice.
- The maximum concentration (Cmax) and overall systemic exposure (AUC) of IMAE was 11.0 ± 3.6 µg/mL and 20.8 ± 4.1 h × µg/mL, respectively.
- IMAE intravenous PK studies in mice (5 mg/kg) are ongoing for determining systemic bioavailability.
- PK model development and dosing simulations incorporating drug efficacy for translation to human dosing are in progress.

References

- Obrador, E., et al., Radioprotection and radio mitigation: from the bench to clinical practice. Biomedicines, 2020. 8(11): p. 461.
- US Food and Drug Administration Centre for Drug Evaluation and Research (FDA), Guidance for Industry-Bioanalytical Method Validation. 2022, Silver Spring, MD: Department for Health and Human Services, US.

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