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Escalating dose evaluation of a prolyl hydroxylase domain inhibitor (PHDi), MK8617: safety, pharmacokinetics, and functionality in healthy rats.

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Introduction

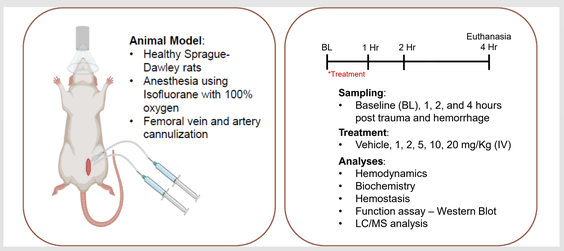
Prolyl hydroxylase domain inhibitors (PHDi) prevent degradation of hypoxia-inducible factor (HIF), enabling transient activation of cytoprotective pathways during hypoxia. This study aimed to evaluate the safety, pharmacodynamic activity, and dose-response functionality of MK-8617 (MK) in healthy rats over 4 hours to define two optimal dose levels for future prophylactic application in a trauma model.

Objective

Characterize the safety and efficacy of escalating doses of a PHDi, MK8617, in healthy rats through immunohistochemistry, changes in protein regulation, and LC-MS quantification over four hours. Characterize the functionality of MK by measuring the nuclear accumulation of HIF-1A and HIF-2A in blood cells and critical tissues of PHDi treated animals under normoxic conditions. Identify the range of MK dosage (low and high) through quantitative analysis of experimental outcomes for future prophylactic application in a trauma model.

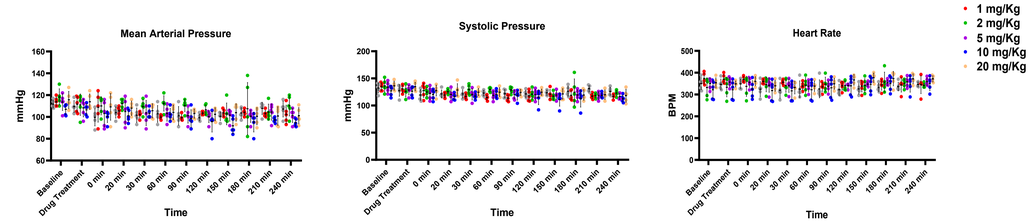
Methods

Escalating doses of MK (1, 2, 5, 10, 20 mg/kg) were administered intravenously to healthy adult rats (n = 5) under isoflurane anesthesia. Blood samples were collected at baseline (BL), 1, 2, and 4 hours and tissues were harvested at 4 hours after euthanasia, the white blood cells (WBC) were isolated using the Ficoll-Pague density gradient method. Nuclear protein extracts from WBC and liver were used to measure HIF-1A and HIF-2A levels via immunoblotting. Plasma concentrations of MK were determined using LC-MS. Tissues (lung, liver and kidney) were stained with hematoxylin and eosin (H&E) and examined for morphologic abnormalities.

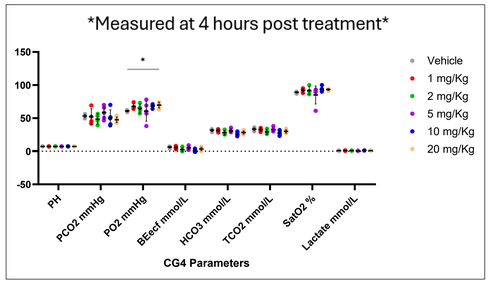


Results

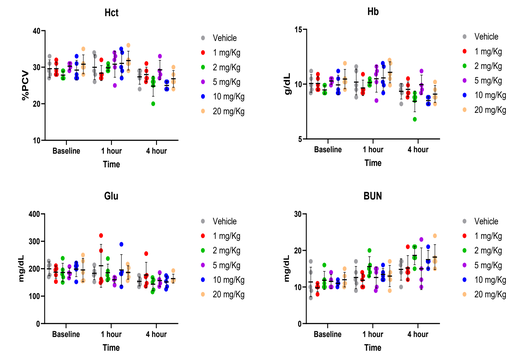
Hemodynamics



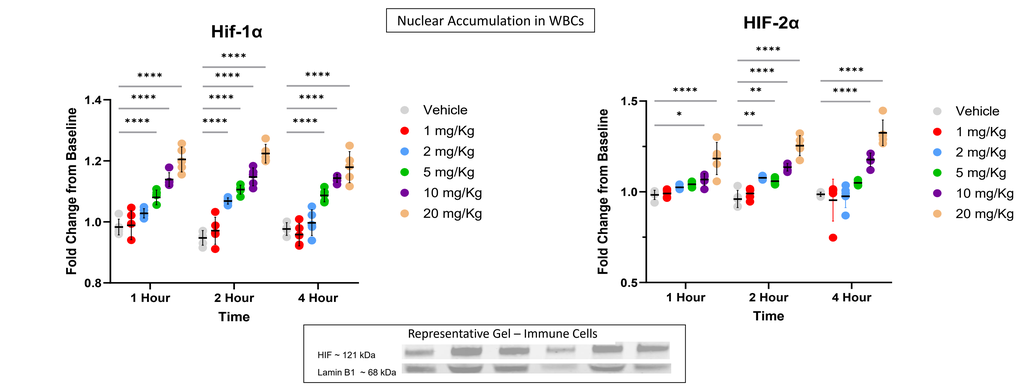
Biochemistry

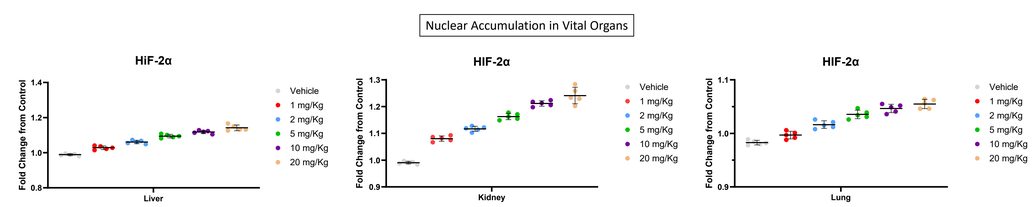


Hemostasis



Functional Assay – Western Blot

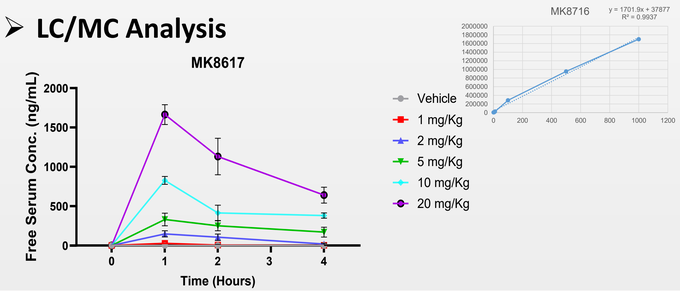




Summary

Escalating MK doses demonstrated no significant changes in hemodynamics; no evidence of acute toxicity was observed based on biochemistry parameters. MK plasma levels peaked at 1 hour, from 26.7 ± 8.81 ng/mL (1 mg/kg) to 1663.5±125 ng/mL (20 mg/kg). Nuclear extracts from blood cells and liver demonstrated a dose-dependent accumulation of HIF-1A and HIF-2A compared to controls. H&E staining revealed no significant evidence of morphologic abnormalities. Quantitative analysis identified 2 mg/kg as the lowest dose achieving sustained significant HIF levels for 2 hours and 20 mg/kg as the highest.

LC/MC Analysis



Conclusion

MK administration resulted in a dose-dependent nuclear accumulation of HIF-1A and HIF-2A in blood cells and critical tissues without acute histopathologic injury over four hours. The range of effective dose levels was identified: 2 mg/kg was the lowest dose for effective target engagement and 20 mg/kg achieved maximal HIF stabilization. This dose range maintained a favorable safety profile, supporting advancement of prophylactic MK administration in a pre-clinical trauma model.

Acknowledgement: This project was supported by combat casualty care program CO250008.

Disclaimer: Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AALAC International.

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