

UNCLASSIFIED Daprodustat Is Elevated in Plasma, Muscle and Kidney After IV Injection in Rat

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The use of limb tourniquet (TK) for effective hemorrhage control has reduced mortality and improved survival. However, when left in place for prolonged periods of time, TK application leads to severe tissue hypoxia, increasing the risk for muscular injury, rhabdomyolysis (RM), and acute kidney injury (AKI) due to the release of intracellular contents of damaged myocytes into the systemic circulation. RM-induced AKI was reported to occur in 34.3% of critically injured US service members and was strongly associated with mortality if left untreated. Daprodustat, a prolyl hydroxylase domain inhibitor (PHDi), is FDA-approved for anemia in dialysis patients. PHDi drugs stabilize the hypoxia-inducible factor (HIF) complex, stimulating endogenous erythropoietin production, angiogenesis, and anaerobic glycolysis while preventing lactate production. Recent studies indicate that PHDis can improve outcomes in rodent models of trauma and hemorrhage, likely by enhancing hypoxic resilience. Though, a critical knowledge gap exists regarding the drug's distribution and concentration in plasma and key tissues after administration.

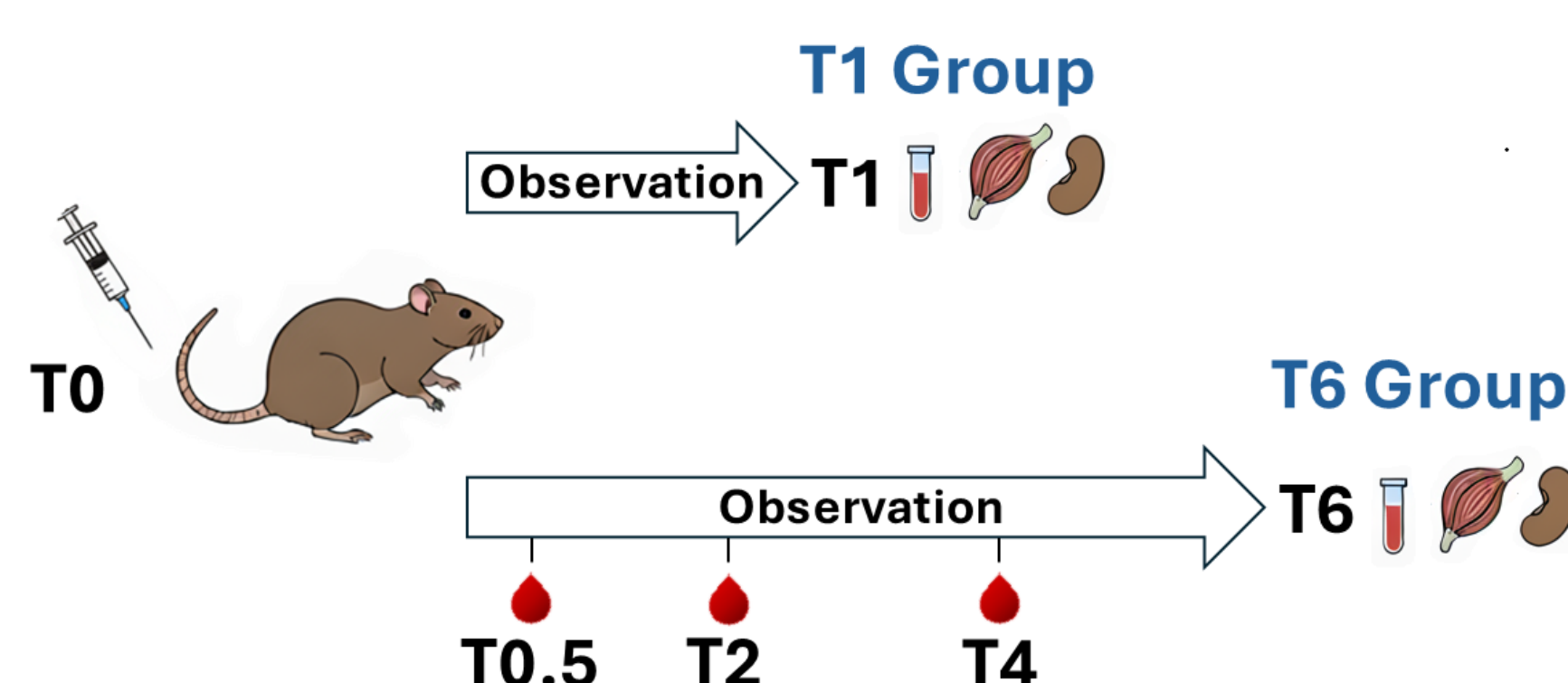
Objectives

The objective of this study is to measure Daprodustat concentrations in rat plasma, muscle, and kidney following intravenous injection at 2 key timepoints.

Method

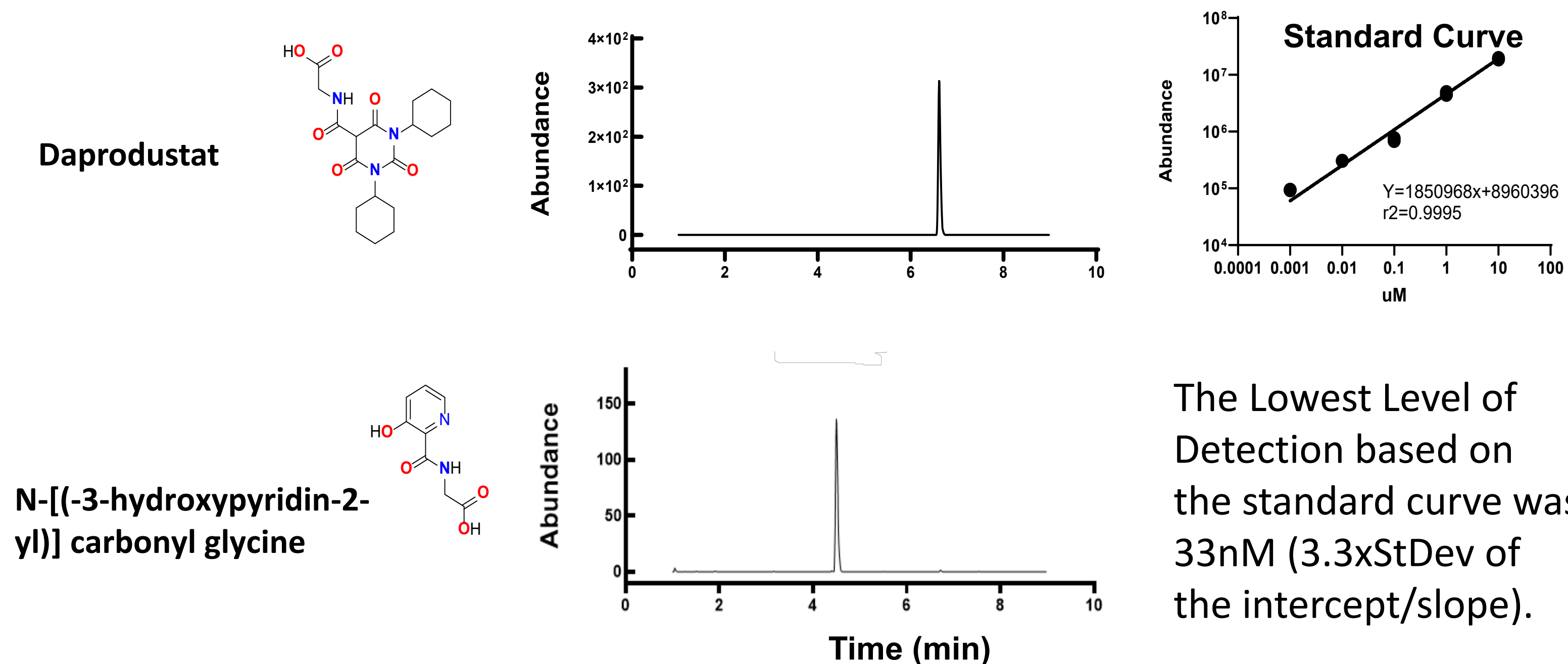
22 Sprague-Dawley rats (375-425g) pre-cannulated for carotid artery were purchased from an approved vendor.

- Rats were anesthetized with Isoflurane and received Daprodustat (7mg/kg) or vehicle control intravenously (IV) at a volume of ~0.3 mL (n=5-6/group).
- Citrated blood was collected at 1 hour (**T1** group) and 0.5, 2, 4, and 6 hours (**T6** group).
- Kidney and muscle tissues were collected at 1- and 6-hour endpoints.



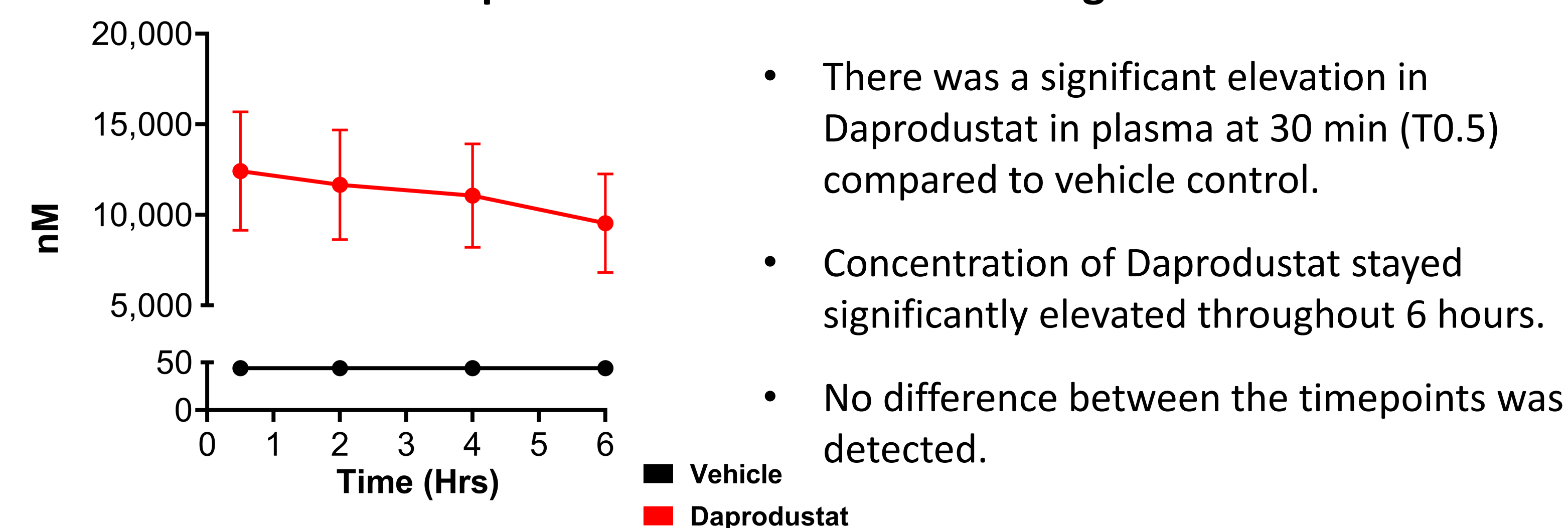
Results

LC-MS/MS Chromatographs of Daprodustat and Internal Control



The Lowest Level of Detection based on the standard curve was 33nM (3.3xStDev of the intercept/slope).

Daprodustat Concentration Changes in Plasma



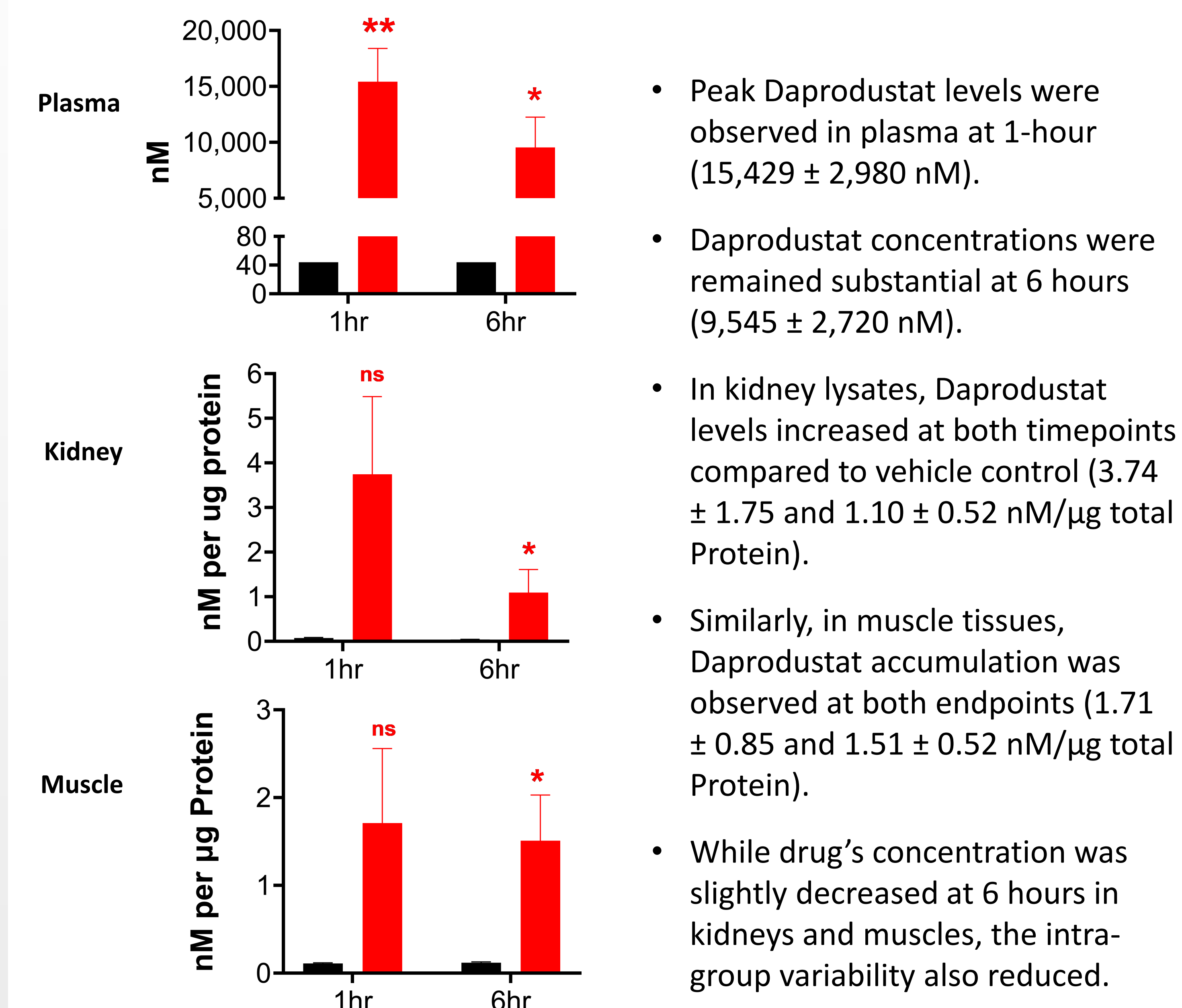
LC/MS

Daprodustat concentrations in plasma and tissue homogenates were determined by Liquid Chromatography Tandem Mass Spectroscopy method (LC-MS/MS Ultimate 3000, ThermoFisher Scientific):

- 100mg of kidney or muscle was homogenized and sonicated.
- Samples were run on Kinetex Polar C18, 2.6μ, 2.1x150mm column (Phenomenex, Torrance, California, USA) at 250μl/min with an increasing acetonitrile/0.1% formic acid gradient.
- 10uM of N-[(3-hydroxypyridin-2-yl)] carbonyl glycine was used as an internal control for monitoring extraction and measurement.
- Plasma levels over time were analyzed by Oneway ANOVA and Tukey post-test.
- A two-tailed, unpaired Welch's t-test was used to compare the level of Daprodustat in plasma, muscle, and kidney with Vehicle for T1 and T6 groups.
- Data presented as Mean ± SE. Significance * = P<0.05

Results

Daprodustat Concentration in Plasma, Muscle, and Kidney Compared with Vehicle Control



Conclusion

- The significant concentrations of Daprodustat measured in kidney and muscle confirm its bioavailability in key tissues following IV administration.
- The sustained plasma levels over 6 hours demonstrate a durable pharmacokinetic profile.
- The presence of Daprodustat in circulation supports its potential for systemic prolyl hydroxylase inhibition indicates that a single dose may offer a prolonged therapeutic window for acute indications.
- Future work will leverage these findings to investigate Daprodustat's potential to mitigate muscle and kidney injuries in a rodent model of rhabdomyolysis.

This project was funded by MRDC. Research was conducted in compliance with 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 the AAALAC International.



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