

Oxidative DNA Damage in Tamoxifen Injected THP^{fl/+} ROSA^{Cre/+} Mice

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Background and Hypothesis:

Tamm-Horsfall protein (THP) is an important regulator of urinary and systemic homeostasis expressed exclusively in the kidney. Complete knockout of THP has been shown to lead to systemic oxidative damage in a mouse model. To develop a more clinically relevant model, we generated a tamoxifen inducible knockout/heterozygote mouse using the Cre/Lox system. ***We hypothesize that inducing a heterozygous state would increase levels of oxidative damage in mice.***

Methods:

Experimental mice were generated by breeding THP^{fl/fl} mice with ROSA^{CreERT2/CreERT2} mice to develop the inducible heterozygote THP^{fl/+}Rosa^{CreERT2}. These mice, along with controls (THP^{fl/fl}) were treated with daily intraperitoneal injections of 75 mg/kg tamoxifen for 5 days. Serum samples were obtained from mice at baseline and 1, 2 and 3 weeks from the first injection, while kidneys were harvested at 1 or 3 weeks. PCR of kidney genomic DNA demonstrated excision of the floxed allele in mice expressing Cre-ERT2. Western Blot analysis of kidney lysates was used to measure kidney THP, while circulating THP was measured by ELISA. Oxidative DNA damage was measured in the kidney and circulation by ELISA.

Results:

Though kidney THP levels decreased in mice expressing Cre-ERT2, circulating levels of THP remained stable, with evidence of transient increases at 1 or 2 weeks for most animals. Mice expressing Cre-ERT2 had significantly increased oxidative DNA damage within the kidney and there was a trend toward increased oxidative DNA damage in the serum, though larger sample sizes are required to verify this finding.

Conclusion:

Despite decreased THP in the kidney, mice maintained normal levels of circulating THP. However, higher levels of oxidative damage were found in both the kidney and circulation. Together, these results suggest that THP levels in the serum are tightly controlled and that an acute loss of THP leads to rapid increases in oxidative damage.