Histone lysine-crotonylation in acute kidney injury

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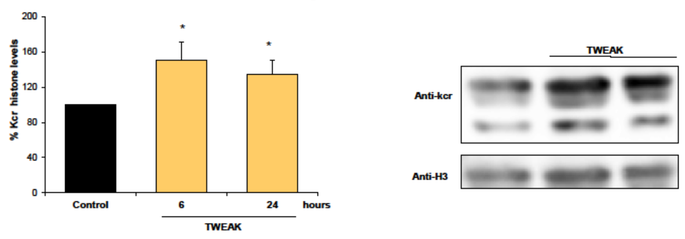
INTRODUCTION

Posttranslational modifications of proteins are key regulators of gene expression and are involved in kidney disease. Histone crotonylation is a recently described posttraslational modification, that is mechanistically and functionally different from histone lysine acetylation. Histone crotonylation was observed in kidney tissue, suggesting that it might play a role in epigenetic regulation of gene expression during kidney injury. Acute kidney injury (AKI) has a mortality of 50% and there is currently no satisfactory therapy, so this study may provide clues to design novel therapeutic approaches. Sirtuin-3 (SIRT3) is a histone deacetylase, recently identified also as a decrotonylase. The mitochondrial biogenesis regulator peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and SIRT3 regulate each other expression, and both play an important role in AKI.

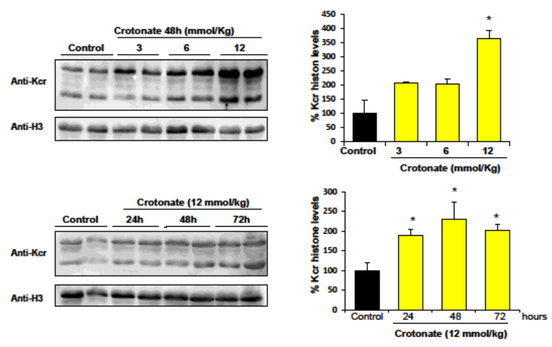
OBJECTIVES

TWEAK (tumor necrosis factor-like weak inducer of apoptosis) is a TNF superfamily cytokine that promotes kidney injury in the setting of AKI. Since TWEAK actions are mediated through the recruitment of signaling mechanism that include histone acetylation, we hypothesized that TWEAK may also modulate histones lysine-crotonylation. Furthermore, since epigenetic changes are also observed in AKI and histone deacetylase (HDAC) inhibitors may protect from kidney injury, we further hypothesized that histone lysine-crotonylation may be a contributor to and a therapeutic target in AKI. Thus, we explored histone crotonylation regulation and function in cultured kidney tubular epithelial cells and during kidney injury in vivo.

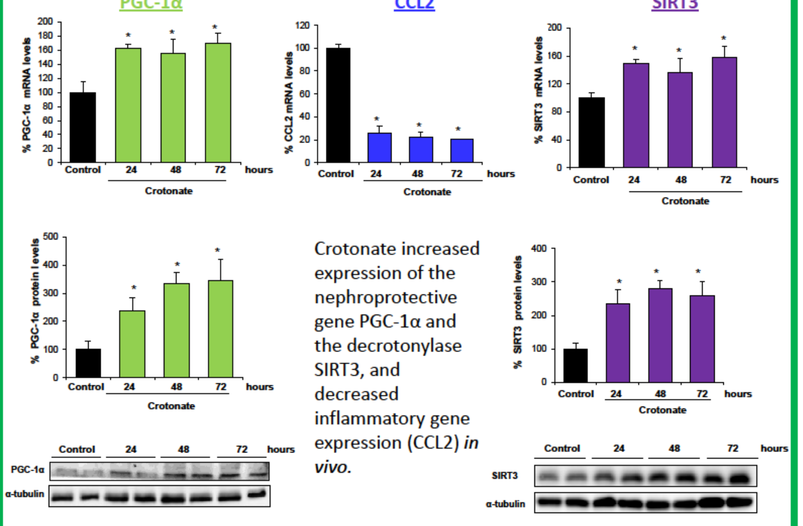
TWEAK increases histone crotonylation in cultured kidney tubular cells



Crotonate increases histone crotonylation and modulates regulated gene expression in mouse kidney



Systemic administration of crotonate increased histone crotonylation in mouse kidney in a dose- and time-dependent manner



Crotonate increased expression of the nephroprotective gene PGC-1α and the decrotonylase SIRT3, and decreased inflammatory gene expression (CCL2) *in vivo*.

METHODS

To asses the role of crotonylation in kidney injury, crotonate was used to increase histone crotonylation in cultured tubular cells or in the kidneys *in vivo*.

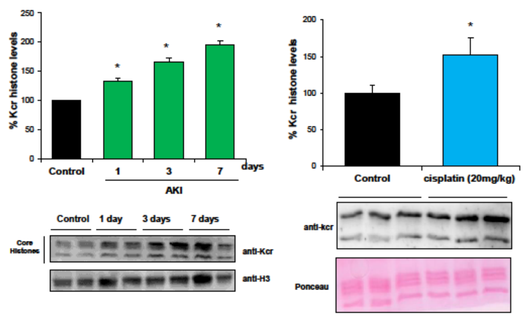
In vivo studies were performed in adult female C57Bl/6 mice (12 weeks old, 20 g). Systemic administration of folic acid (300 mg/kg in 0.3 mol/l sodium bicarbonate or vehicle) or crotonate (12 mmol/kg) was done by intraperitoneal injection.

In vitro experiments were done in murine tubular epithelial cells, by different techniques: RT-PCR, Western blot, ChIP-seq, cytometry or immunostaining.

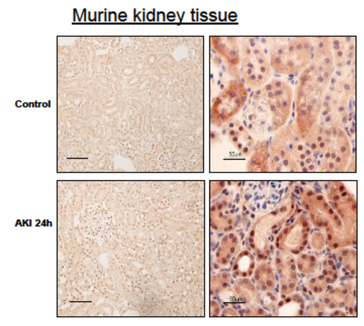
RESULTS

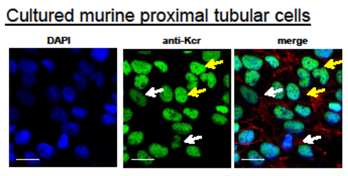
Histone crotonylation is increased in kidney tubular cells during acute kidney injury

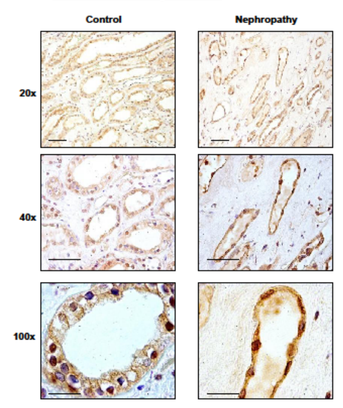
Intraperitoneal dministration of folic acid into mice increase histone crotonylation 1, 3 and 7 days after injury. Similar results were observed in cisplatin-induced AKI at 72h.



Nuclear localization of histone crotonylation was observed ***in vivo*** and ***in vitro***. As we can see by inmmunostaining there were different degrees of crotonylation suggesting that this is a regulated and dynamic process.

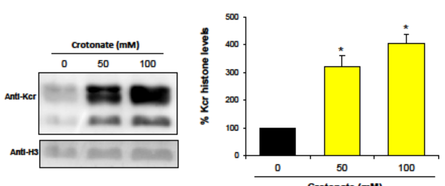




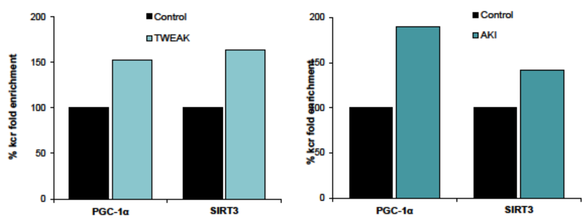


Immunohistochemistry also identified nuclear lysine-crotonylation in diseased human kidney tubular cells.

Crotonate increases histone crotonylation

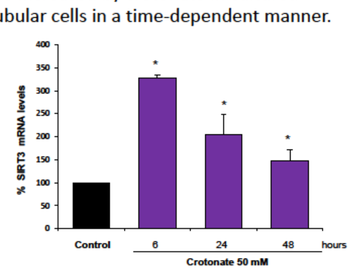


ChIP-seq disclosed enrichment of histone crotonylation at the genes encoding the mitochondrial biogenesis regulator PGC-1α and the sirtuin-3 decrotonylase in both TWEAK-stimulated tubular cells and in AKI kidney tissue.

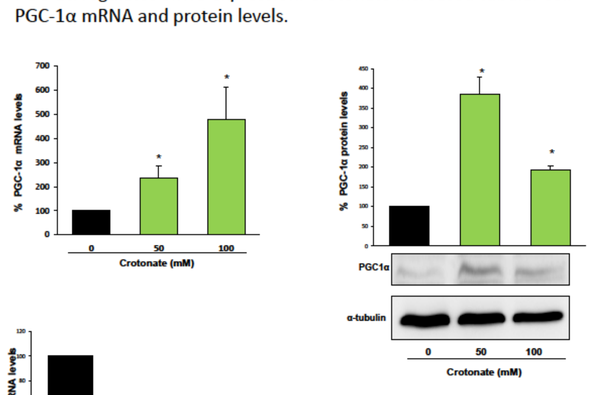


Crotonate elicits biological responses in cultured tubular cells

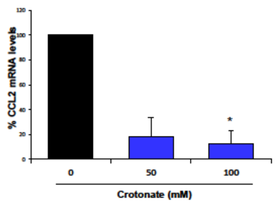
Decrotonylase SIRT3 expression mRNA levels are increased by crotonate in cultured tubular cells in a time-dependent manner.



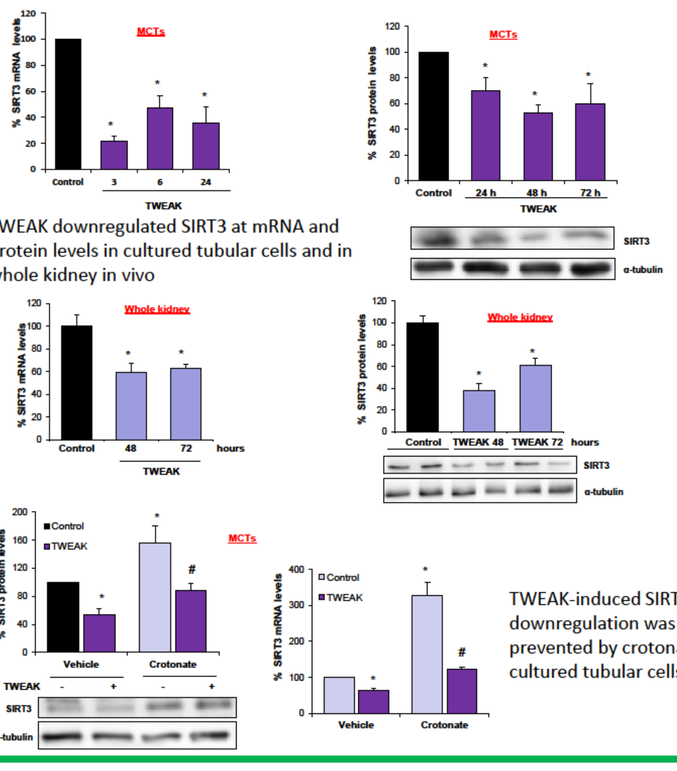
PGC-1α regulates SIRT3 expression. Crotonate increased tubular cell PGC-1α mRNA and protein levels.



CCL2 encodes the MCP-1 chemokine, a promoter of kidney injury. Crotonate decreased tubular cell CCL2 mRNA in cultured cells.



TWEAK downregulates SIRT3 and this is prevented by crotonate

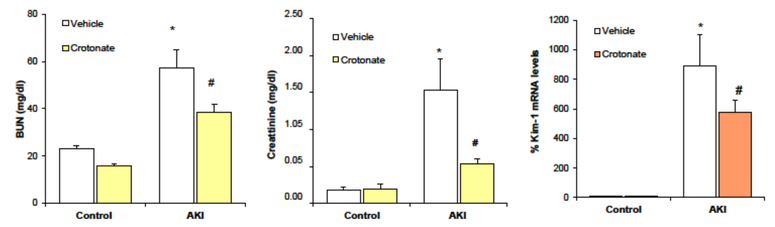


TWEAK downregulated SIRT3 at mRNA and protein levels in cultured tubular cells and in whole kidney in vivo

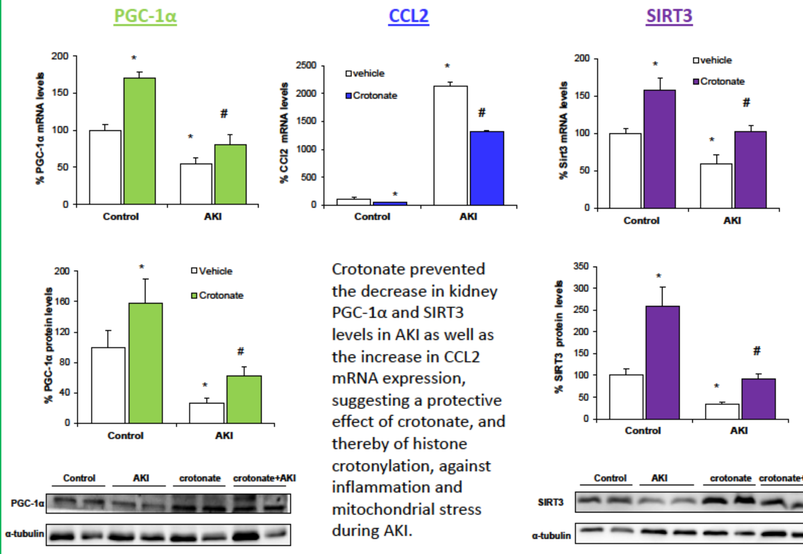
TWEAK-induced SIRT3 downregulation was prevented by crotonate in cultured tubular cells.

Crotonate protects from experimental AKI

Mice were pretreated with 12 mmol/kg crotonate and 24 hours later was induced by a folic acid overdose and mice were euthanized at 72h, when renal failure peaks.



Crotonate resulted in lower serum levels of BUN and creatinine, markesr of renal dysfunction severity, and in lower KIM-1 mRNA levels, a marker of kidney injury.



Crotonate prevented the decrease in kidney PGC-1α and SIRT3 levels in AKI as well as the increase in CCL2 mRNA expression, suggesting a protective effect of crotonate, and thereby of histone crotonylation, against inflammation and mitochondrial stress during AKI.

CONCLUSIONS

For the first time we have shown that the pattern of histone crotonylation changes during AKI and in cultured tubular cells stressed by an inflammatory cytokine, suggesting a role of histone crotonylation in kidney injury. Furthermore, we have shown that the degree of kidney cell histone crotonylation may be manipulated therapeutically by administering crotonate and that increasing overall histone crotonylation was nephroprotective.

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