Vitamin K eliminates uremic posttranslational modifications of gamma-glutamyl carboxylase

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BACKGROUND

CKD patients exhibit a functional vitamin K deficiency and prominent vascular calcifications[1,2]. Vitamin K dependent carboxylation by gamma-glutamyl carboxylase (GGCX) is essential to activate the vasoprotective matrix gla protein[3]. GGCX activity was shown to be decreased in uremic rats and this was reversible by high intake of vitamin K[4]. Here we aimed to study the underlying mechanism.

METHODS

Two models of experimental uremia were investigated.

a) Adenine nephropathy: CKD was induced in 5 male Wistar rats by adenine intake (0.75%) +/- vitamin K over a period of 7 weeks.

b) 5/6 nephrectomy (Nx): 3 wildtype (C57BL/6) mice underwent a surgical one step procedure accompanied by high phosphate diet over 9 weeks.

GGCX was isolated from aortic, liver and kidney samples by polyacrylamide electrophoresis and enzymatic digestion. Fragments were identified and analysed by mass spectroscopy.

RESULTS

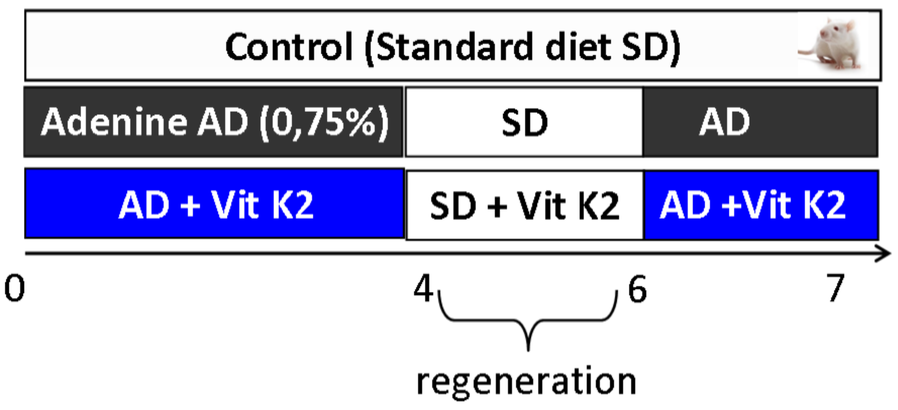

Figure 1: Adenine (AD) nephropathy in rats, control and standard diet (SD), protocol over 7 weeks.

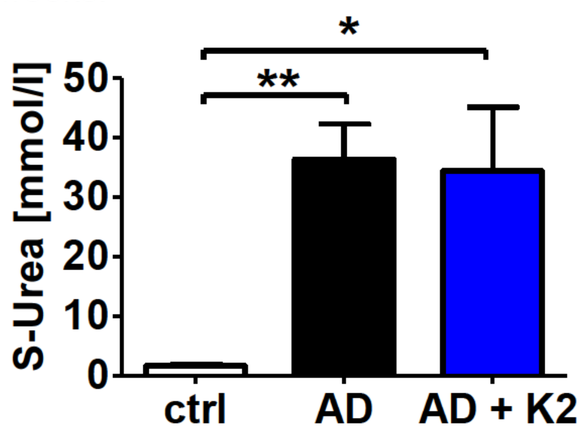

Figure 2: Adenine (AD) nephropathy results in 21-fold increase of serum urea; * $p < 0.05$; ** $p < 0.001$

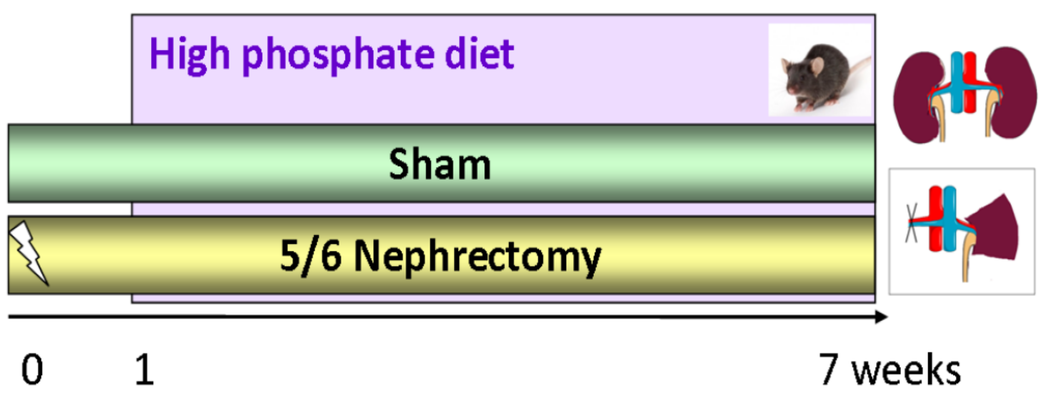

Figure 3: 5/6 Nephrectomy in mice: one step surgery and high phosphate diet over 7 weeks.

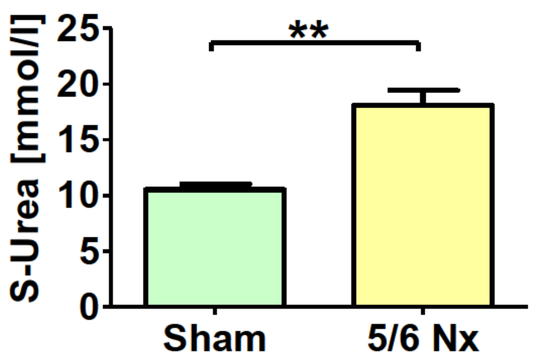

Figure 4: 5/6 Nephrectomy results in a 1.8-fold increase of serum urea; ** $p < 0.001$

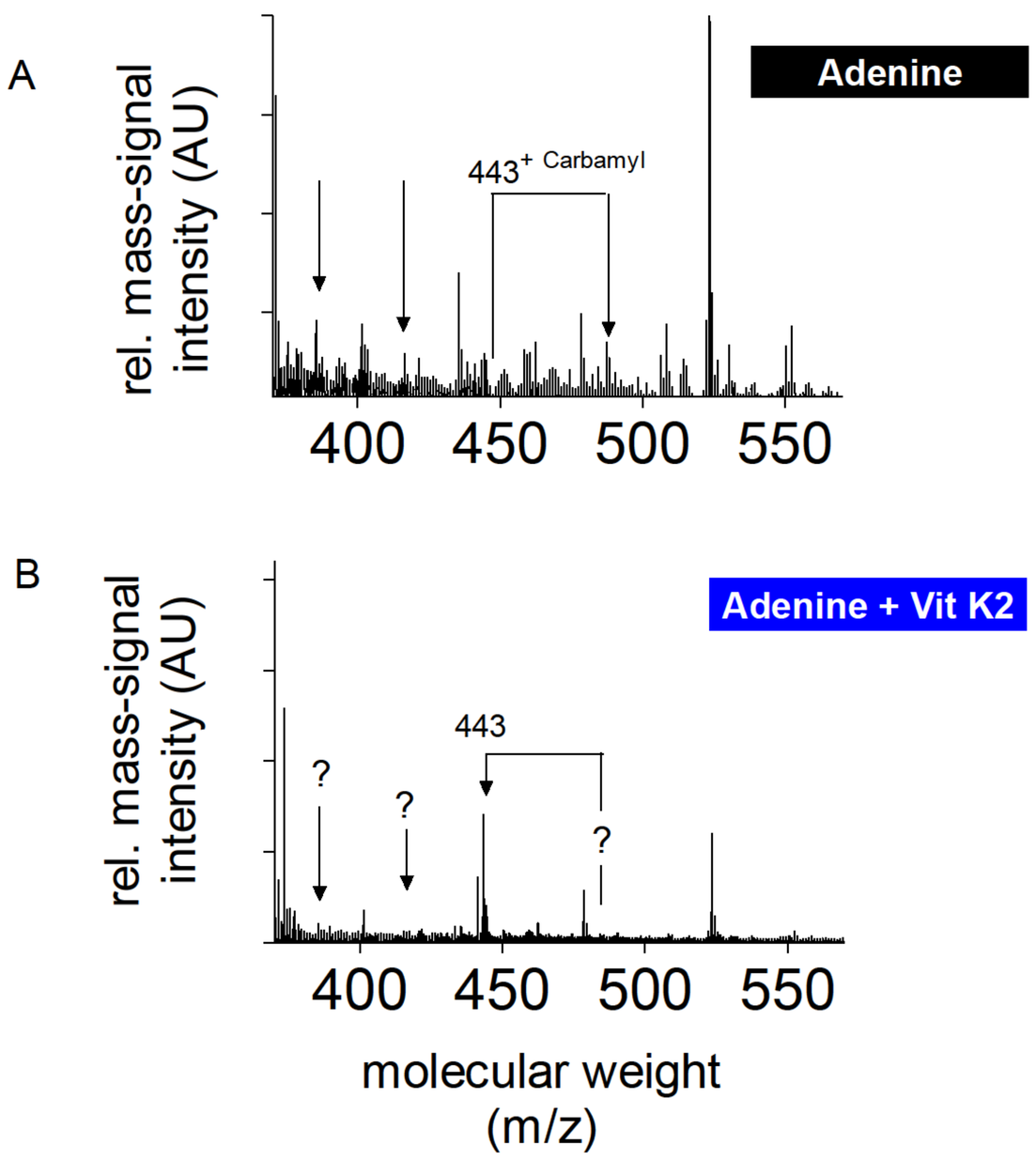

Figure. 5: Characteristic mass fingerprint-spectrum of tryptic digests of GGCX from adenine nephropathy rats. The arrows indicate the molecular mass of the peptide fragments after tryptic digests of GGCX modified by carbamyl. **A**: Adenine group with carbamylation resulting in a molecular mass of 487; **B**: Adenine + vitamin K2 diet lacks carbamylation with a molecular mass of 443.

No posttranslational modifications were found in healthy animals.

GGCX was carbamylated in all uremic animals.

Carbamylations were present in adenine rats at Arg 9, 463, 673 and 687 and in 5/6 Nx mice at Arg 426, 480 and 672.

Guanidinylation was only present in uremic rats at Lys 351 and 520.

No posttranslational modification of GGCX was found in uremic animals after vitamin K2 diet.

A homology matching with the human GGCX proposes Lys 351 as potential guanidinylation site and Arg 347, 436. 673 and 687 for carbamylation.

SUMMARY

Posttranslational modification of GGCX by the uremic milieu might explain the recently found reduced GGCX activity in adenine nephropathy. The modifications can be reversed by high vitamin K intake.

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