

Study of skin changes in cystinosis patients under cysteamine therapy

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Background

Recently 8 cystinosis patients who were treated with relatively high doses of cysteamine were reported to develop a new type of adverse events consisting of skin striae on stretchable surfaces of extremities, bruising-like lesions on the elbows and severe muscular-skeletal pain and weakness. Side effects, and in particular skin lesions, diminished or disappeared after cysteamine dose reduction, pointing to a causal relation between cysteamine administration and the appearance of the lesions. Skin biopsies of the bruising-like lesions showed vascular proliferation also called reactive angioendotheliomatosis on light microscopy. Electron microscopy showed irregularities and variability in collagen fiber caliber, closely resembling the collagen alterations that are seen in classical Ehlers-Danlos syndrome (EDS).¹

We hypothesized that cysteamine causes skin lesions via inhibition of collagen cross-linking. This effect can be due to a direct interaction of cysteamine with collagen aldehydes that are required for stable cross-link formation, or might be due to an inhibition of the enzyme lysyl oxidase, which is responsible for the formation of these aldehydes. As copper is a co-factor of lysyl oxidase,² copper deficiency can contribute to the adverse effect of cysteamine. Furthermore, differences in cysteamine metabolism between individuals can be responsible for higher tissue cysteamine concentrations and underlie cysteamine toxicity in a subset of patients.

Specific aims

The etiology of collagen fibre abnormalities in cystinosis patients with cysteamine toxicity remains unknown. The process of collagen synthesis and degradation is under strict physiological control. Cysteamine might either directly influence the synthesis of procollagens, or interfere with collagen cross-linking, which occurs extracellular. Collagen cross-link formation is catalyzed by the enzyme lysyls oxidase, which uses copper as a co-factor.² Therefore, copper deficiency might further contribute to the adverse effect of cysteamine. With this project, we aim to study the etiology behind collagen abnormalities in cystinosis patients with cysteamine toxicity.

Key-objectives/work plan

- A. To analyze a possible effect of cysteamine on collagen synthesis in cultured skin fibroblasts.
- B. To analyze a possible effect of cysteamine on urinary deoxypyridinoline and pyridinoline levels, as a measure of bone collagen cross-linking and bone turnover.
- C. To measure plasma copper and ceruloplasmin levels in European cystinosis patients.
- D. To analysis of possible genetic polymorphisms in ATP7A gene in patients with cysteamine toxicity.
- E. To study cysteamine metabolism in a skin biopsy obtained from a cystinosis patient, treated with cysteamine.

Results

- A. We have investigated a possible effect of cysteamine on collagen synthesis in fibroblasts obtained from cystinosis patients with (n=3) and without (n=2) cysteamine toxicity. All 6 fibroblast cell lines will be cultured both with and without cysteamine 0.1 mM (which equals patients' peak plasma concentration). Different (pro)collagens (type I and type III) produced by these fibroblasts were analysed using gel electrophoresis. These include the collagen medium fraction, the collagen cellular fraction and the procollagen fraction. We found normal synthesis of all collagen fractions of all tested fibroblasts (figure 1). Also, the addition of 0.1 mM cysteamine did not influence (pro)collagen synthesis.

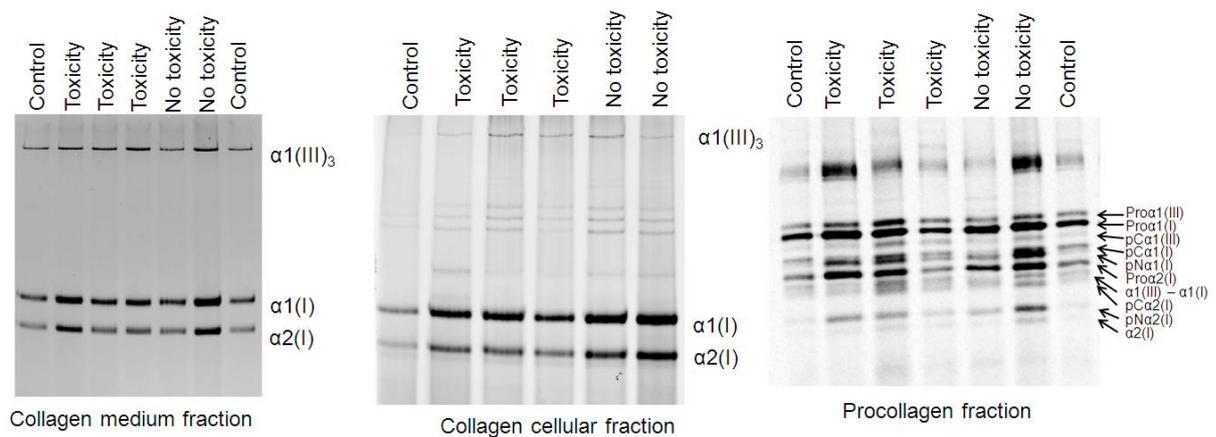


Figure 1. Collagen medium fraction, collagen cellular fraction and procollagen fraction were compared in fibroblasts obtained from patients with (n=3) and without (n=2) cysteamine toxicity. There we no differences between both groups.

- B. Study is currently in progress.
- C. Thirty-five cystinosis patients were included: 21 with renal Fanconi syndrome (including 6 with cysteamine toxicity), 12 after renal transplantation, 1 on hemodialysis and 1 with ocular cystinosis. Serum copper, ceruloplasmin and urinary copper/creatinine ratio were determined. Results are shown in figure 2. All patients with renal Fanconi syndrome had increased urinary copper excretion. In the transplanted patients, only 3/12 patients showed increased urinary copper/creatinine ratio. This difference was statistically significant ($p < 0.01$). The patient on hemodialysis was anuric, the patient with ocular cystinosis had a normal urinary copper excretion. Serum copper and ceruloplasmin levels were decreased in 8 patients, including all 6 patients with cysteamine toxicity. Thus, patients with cysteamine toxicity has significantly lower serum copper ($p < 0.01$) and serum ceruloplasmin ($p < 0.01$) levels. The patient on hemodialysis and the patient with ocular cystinosis both had normal serum copper and ceruloplasmin levels.

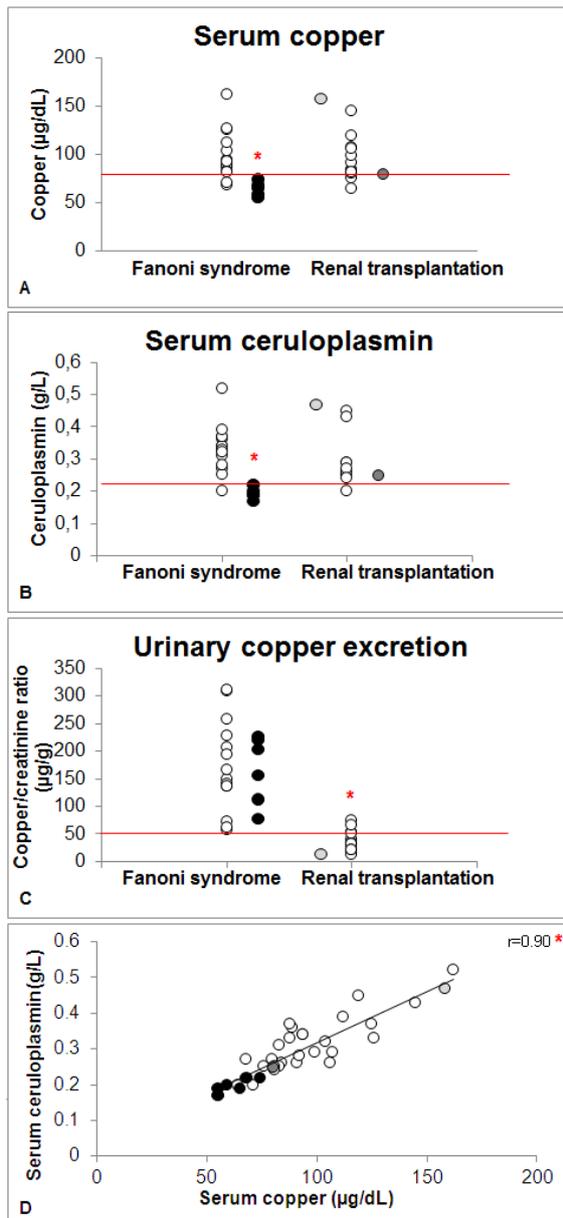


Figure 2. Comparison of serum copper levels (A), serum ceruloplasmin levels (B) and urinary copper/creatinine ratio (C) between patients with renal Fanconi syndrome and after renal transplantation, and correlation between serum copper and serum ceruloplasmin (D). Patients with cysteamine toxicity are indicated in black, the patient with ocular cystinosis is indicated in light grey, the anuric patient on hemodialysis is indicated in dark grey. The red asterisk indicates a statistically significant difference ($p<0.01$), red bar indicates lower limits of normal levels. Normal levels: serum copper 80-120 µg/dL, serum ceruloplasmin 0.22-0.58 g/L, urinary copper/creatinine ratio <50 µg/g.

- D. The possibility of underlying genetic causes for genetic susceptibility for the development of cysteamine toxicity will be further studied.
- E. Since cysteamine is produced from pantethine by vanin under physiological conditions, we will examine whether there is a difference in vanin expression of a cystinosis patient receiving exogenous cysteamine, when compared to healthy control subjects. After obtaining informed consent from both the patient and parents, a skin biopsy will be performed at the moment when the patient is having surgery for a reason not related to skin problems. This study is currently in progress.

Reference List

1. Besouw MT et al. Cysteamine toxicity in patients with cystinosis. *J Pediatr* (2011);159;1004-1011.
2. Siegel RC, Pinnell SR, Martin GR. Cross-linking of collagen and elastin. Properties of lysyl oxidase. *Biochemistry* (1970);9;4486-4492.