Cyclodextrin Improves Renal Function in Experimental Alport Syndrome

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Abstract

Alport syndrome is an inherited progressive form of glomerular disease that account for 2% of end stage renal disease (ESRD) prevalent cases and that affects primarily young adults. No treatments strategies are currently available for Alport syndrome (1). 2-hydroxy propyl-β-cyclodextrin (CD) is a cholesterol depleting agent that is now in clinical trials for the treatment of Niemann-Pick disease Type C. We have recently reported that CD protects podocytes in experimental diabetic kidney disease (DKD) by reducing cholesterol dependent damage in podocytes (2). We now hypothesize that treatment with CD improves renal function in the experimental model of Alport syndrome.

Four-week-old collagen Col4a3 knockout (KO) and wild type (WT) female mice were injected subcutaneously with CD (4000 mg/kg) or vehicle (0.9% Saline solution) 3 times per week for 3 weeks. Four experimental groups were analyzed: WT+vehicle (n=4), WT+CD (n=5), KO+vehicle (n=4), and KO+CD (n=4). Measurement of body weight and urine collections for ACR (albumin/creatinine ratio) determinations were performed weekly. Serum creatinine and blood urine nitrogen (BUN) were determined by mass spectroscopy and ELISA respectively at sacrifice. Perfused kidneys and skin samples at the site of injection were collected for histological analysis with Periodic Acid Shiff (PAS), Picrus Sirius Red (PSR) and Oil Red O Staining (ORO).

H&E staining of skin samples showed no toxicity at the site of CD injections. No weight changes were reported during the time of treatment. CD administration prevented the development of mesangial expansion and of Oil Red O staining in KO+CD group compared to KO+Vehicle. A significant reduction in the ACR ($p<0.001$) and in the BUN ($p<0.05$) was observed after 3 weeks of CD treatment in KO+CD when compare to KO+Vehicle mice. This was accompanied by a trend to a reduction in serum creatinine. CD treatment did not affect ACR, renal function or mesangial expansion in WT mice.

Based on these results, we concluded that 2-hydroxy propyl-β-cyclodextrin improves renal function in experimental Alport syndrome and could become a new therapeutic strategy for patients affected by Alport syndrome.

Hypothesis

Mice affected by Alport syndrome accumulate lipid droplets in kidney cortex. Cyclodextrin treatment improves renal function in the animal model of Alport syndrome.

Methods

Collagen Col4a3 knockout (KO) mice were used as a model for AS. Four-week-old Col4a3 (KO) and wild type (WT) female mice were injected subcutaneously with CD (4000 mg/kg) or vehicle (0.9% Saline solution), 3 times per week for 3 weeks. Four groups were analyzed: WT+vehicle (n=4), WT+CD (n=5), KO+vehicle (n=6), and KO+CD (n=4). Body weight and ACR (albumin/creatinine ratio) from urine were determined weekly. Serum creatinine and blood urea nitrogen (BUN) were analyzed by mass spectroscopy and ELISA respectively at treatment initiation and at sacrifice. Perfused kidneys and skin samples at the site of injection were collected for histological analysis (H&E, PAS, PSR) and for Oil Red O (ORO) Staining. Quantification of lipid droplets and fibrosis was performed by Imahe J analysis.

Results

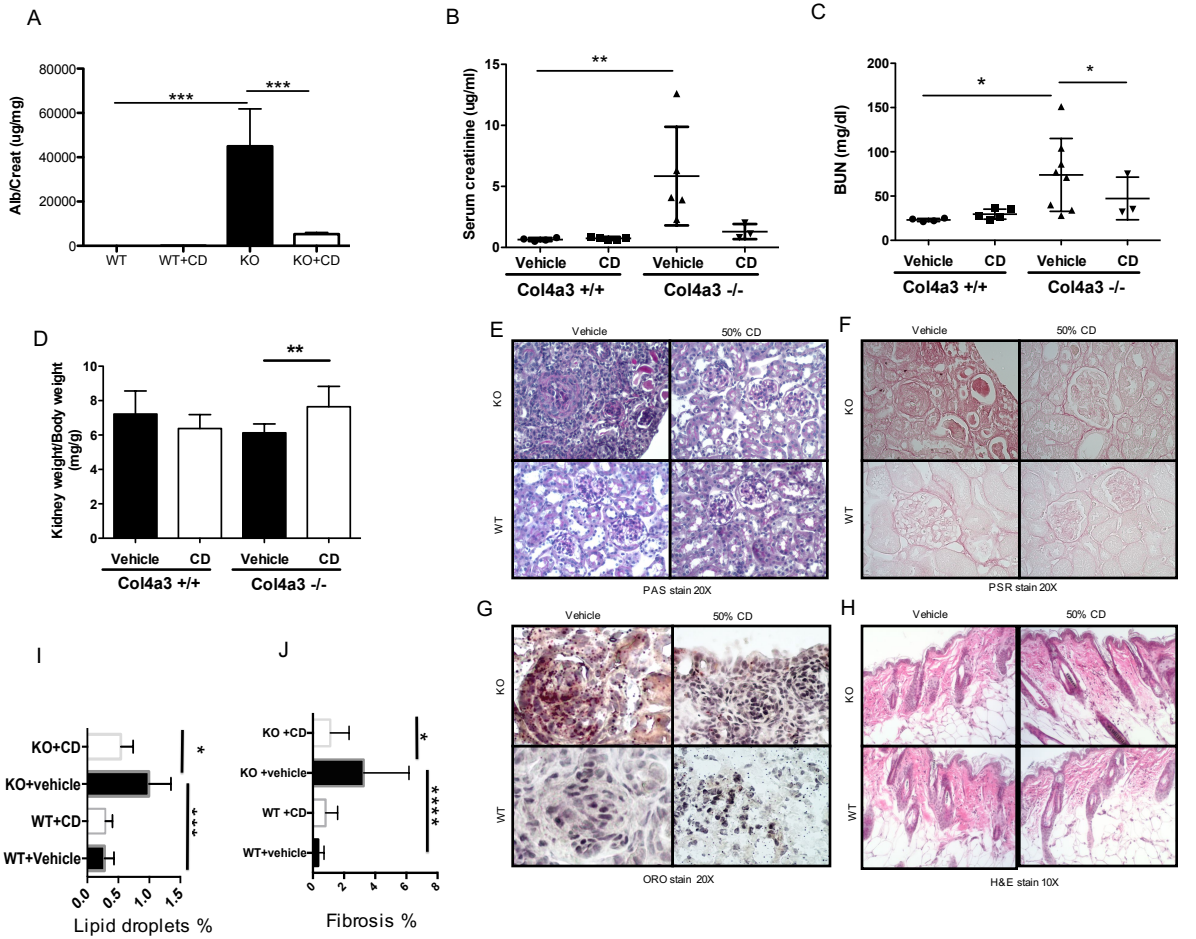

Figure 3. A. Cyclodextrin improves renal function in vivo. A: CD administered to knockout and wild type mice subcutaneously three time a week (n: 4-5 per group) resulted in a reduction in albumin/creatinine ratios (mean+SD) after the initiation of the treatment. ***P < 0.001. **B and C**:Serum creatinine and BUN concentration were preserved in knockout mice after CD treatment *P<0.01, **P<0.01. **D**: CD treatment preserved the kidney/body weight in knockout mice **P <0.01. **E,F and G**: Representative PAS stain, PSR stain and ORO stain of kidney sections from WT and knockout mice after one month of treatment with either CD or vehicle. **H**: Representative H&E staining of skin showed no toxicity at the site of injection of 50% CD solution. **I**: Bar graph analysis of ORO quantification using imaging **J** program, demonstrating increased ORO staining in knockout mice when compared to wild type and demonstrating CD protection from lipid droplets accumulation in kidney cortex. ***P < 0.0001 and p<0.05. J: Fibrosis measured by PSR stain was significantly increased in knockout mice when compared to wild type as expected, and CD treatment significantly reduced reduced renal fibrosis. ****P < 0.0001 and *p<0.05

Summary

- Col4a3 -/- mice demonstrated increased lipid droplets content in kidney cortex.
- When compared to wild type mice, CD treatment of Col4a3 -/- mice resulted in:
  a. decrease in the number of lipid droplets in kidney cortex.
  b. reduced urine albumin/creatinine ratios.
  c. Prevention of fibrosis and mesangial expansion.
  d. Preservation of kidney weight/body weight ratios.
  e. Preservation of BUN and creatinine
- Administration of 50% solution of CD subcutaneously did not cause any skin toxicity.

Conclusions

Our results suggest that CD is an effective and safe treatment strategy to protect from the renal manifestation of experimental Alport syndrome, and it may therefore be tested as a new therapeutic strategy for patients affected by Alport syndrome

References

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CONFLICTS of INTEREST

A.F is Vice-President and CSO of L&F Health LLC

L&F Health LLC and affiliated companies have a patent estate covering some of the topics being presented

L&F Health LLC has consulting agreements with and/or has received honoraria from Hoffman La Roche, Genentech, Mesoblast, Bristol Myers Squibb, Abbvie, Jenssen, Boehringer Ingelheim, Astra Zeneca, Pfizer.

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