Adefovir- and Tenofovir-related renal changes from 28-day oral investigation on Sprague-Dawley rat:

histopathology, electronic microscopy, genomics and urinary kidney biomarkers end-points

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INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) are the basis of clinically successful anti-retroviral therapy to control HIV-1 infections. Despite this distinct benefit, NTRI-based therapies may have limitations due to potential organ toxicity such as kidney toxicity.

Adefovir (ADF) and Tenofovir disoproxil fumarate (TDF) are two related NTRI drugs.

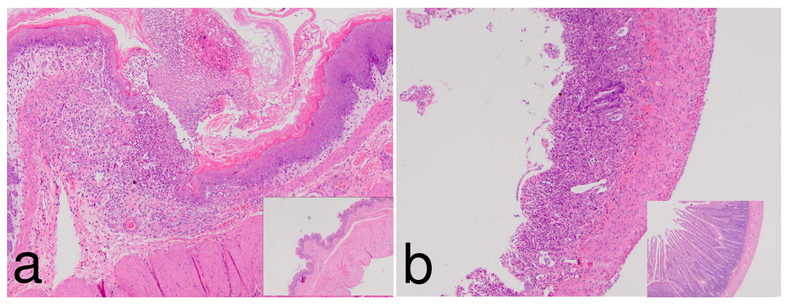
- ADF is no longer used for HIV-1 infection because of the high incidence of renal toxicity,
- TDF has been occasionally linked to cases of proximal tubular dysfunction, Fanconi syndrome and acute tubular injury.

ADF and TDF were tested in a 4-week oral study in Sprague-Dawley rats, to compare the nephrotoxic potential of the two compounds in a rodent model.

Doses (as base) were 11 and 28 mg/kg/day for ADF and 300 and 600 or 1000 mg/kg/day for TDF (each dose selected according to ~5 or ~20X human exposure). Renal function was assessed by a panel of urinary kidney biomarkers; renal lesions were characterized at histopathology, electron microscopy (EM) and gene expression profiling.

Tenofovir-induced gastrointestinal toxicity

Tenofovir at 1000 or 600 mg/kg/day was not tolerated (animals prematurely sacrificed at day 6 or 7, respectively) due to severe gastrointestinal toxicity: forestomach (a) and/or duodenal (b) ulcerations (10x)
Control mucosa in the inlets



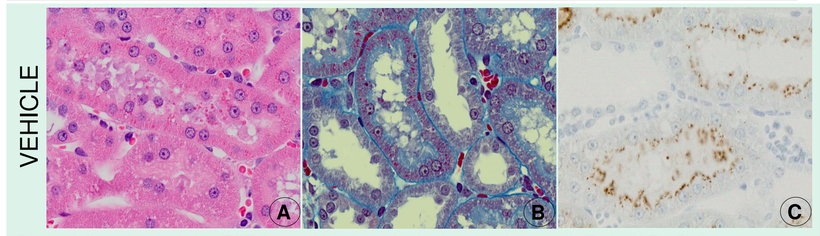
Hyaline droplet accumulation in the kidneys

KIDNEY	vehicle	TDF	ADF	
Dose (mg/kg/day)	0	300	11	28
Hyaline droplets, PCT	8	8	8	6
minimal	6	•	•	3
slight	2	2	8	3
moderate	•	6	•	•

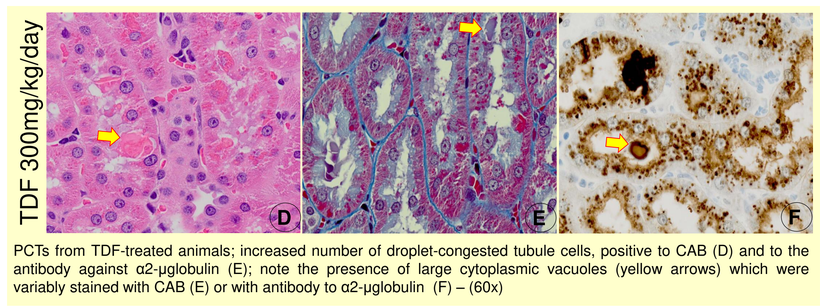
Tenofovir at 300 mg/kg/day resulted in:

1) an increase in severity of bright eosinophilic droplets, present mainly in the cytoplasm of PCTs, stained in red with the CAB staining (consistent with the protein nature of the contents), and confirmed to be composed of α2-μglobulin with the specific antibody at IHC.

2) Increased number of large vacuoles was also seen in TDF treated animals



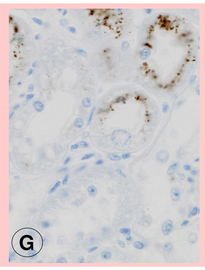
Proximal Convoluted tubules (PCT) from vehicle treated animals at (A) Hematoxylin/Eosin (H/E); (B) Chromotrope Aniline Blue staining (CAB); or (C) immunohistochemistry for α2-microglobulin (IHC for α2-μglobulin) – (60x)

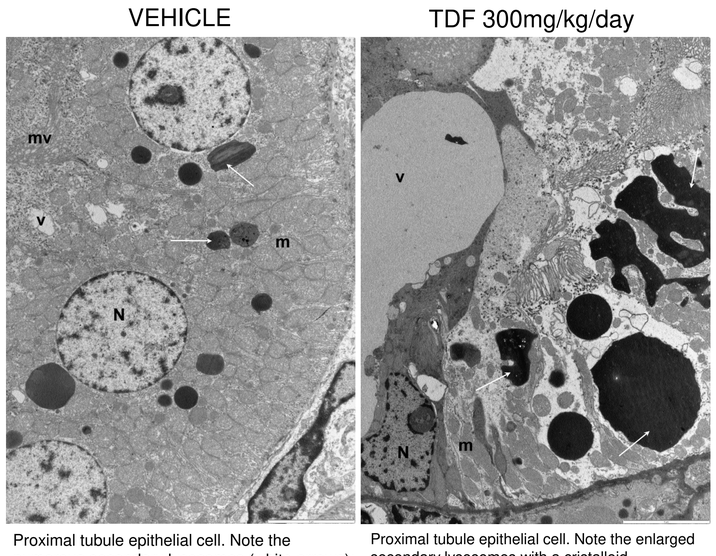


PCTs from TDF-treated animals; increased number of droplet-congested tubule cells, positive to CAB (D) and to the antibody against α2-μglobulin (E); note the presence of large cytoplasmic vacuoles (yellow arrows) which were variably stained with CAB (E) or with antibody to α2-μglobulin (F) – (60x)

ADF 28mg/kg/day

Although Adefovir at 11mg/kg/day showed a slight tendency towards a hyaline droplets accumulation, this was not see at 28mg/kg/day, where hyaline droplets were reduced to almost missing from some PCT tubules. (G) – (100x)





Proximal tubule epithelial cell. Note the numerous secondary lysosomes (white arrows); N=nucleus; m=mitochondria, v=apical vacuole, mv=microvilli; Magnification 4'800x

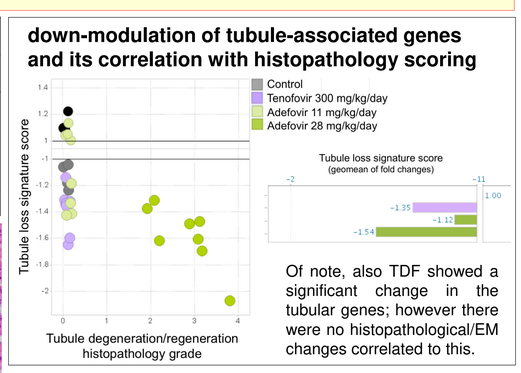
Proximal tubule epithelial cell. Note the enlarged secondary lysosomes with a cristalloid appearance (white arrows) and the enlarged vacuole (v); m=mitocholdion, N=nucleus; Magnification 4'800x

At electron microscopy (EM) investigation, increased number of secondary lysosomes were seen in PCTs, appearing as variably enlarged, polygonal to irregular dark structures with a condensed, fibrillar to crystalloid morphology, suggestive of aggregated proteins in pure form (α2-μglobulin). Beside this, the cellular organelles had the same appearance and distribution as seen in the control animals, with the exception of the large, single-membrane bounded apical vacuoles, filled with a homogenous amorphous material, possibly representing exaggerated re-absorption vacuoles

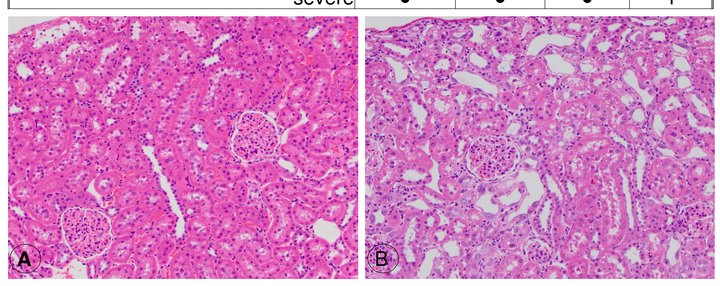
Urine analysis revealed creatinine-normalized increases in Ca (+86%) and P (+203%) vs controls. In genomics there were no toxicologically relevant changes attributable to TDF with the exception of minimal down-regulation of tubule-associated genes, but this gene modulation was considered not significant (see next) .

Adenofovir-induced nephropathy

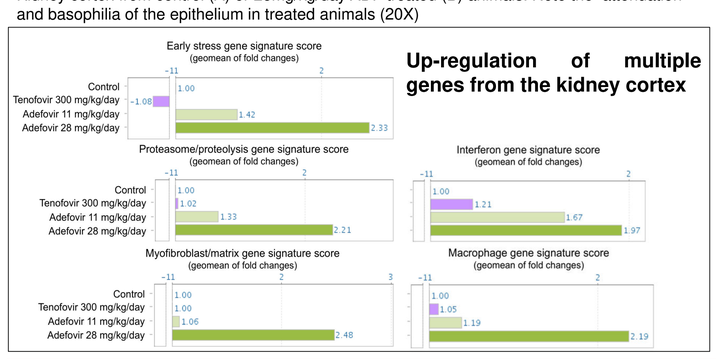
KIDNEY	vehicle	TDF	ADF	
Dose (mg/kg/day)	0	300	11	28
"Nephropathy", PCT	0	0	0	8
mild	•	•	•	3
moderate	•	•	•	4
severe	•	•	•	1



Of note, also TDF showed a significant change in the tubular genes; however there were no histopathological/EM changes correlated to this.

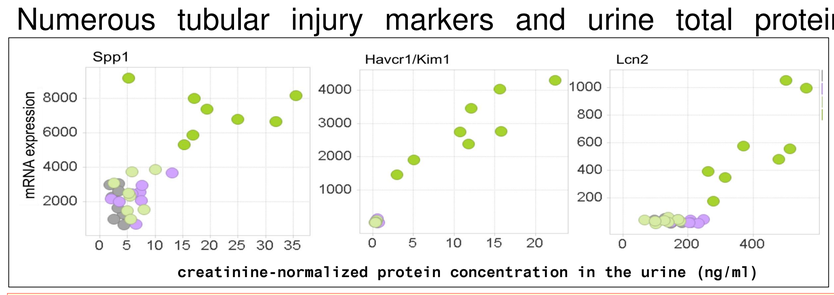


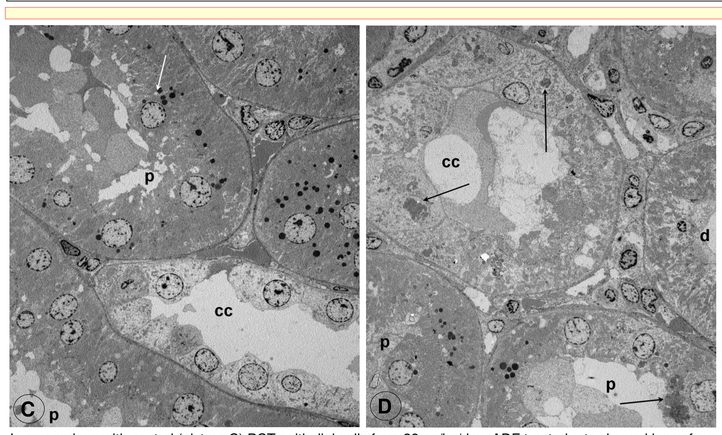
Kidney cortex from control (A) or 28mg/kg/day ADF treated (B) animals. Note the attenuation and basophilia of the epithelium in treated animals (20X)



ADF at 28 mg/kg/day, resulted in tubular degeneration/ regeneration, single cell necrosis and interstitial fibrosis/inflammation (nephropathy), mainly affecting proximal convoluted tubules (PCTs) underneath the cortex. Consistently, genomic investigation revealed up-regulation of classical kidney toxicity markers, stress-response induced genes, proteasome associated genes, among others, and down-modulation of tubule-associated genes.

Numerous tubular injury markers and urine total protein were increased in urine with some markers [e.g. osteopontin/SPP1, KIM-1, Lcn2 (neutrophil gelatinase associated lipocalin)] having a strong correlation with mRNA expression in the kidney

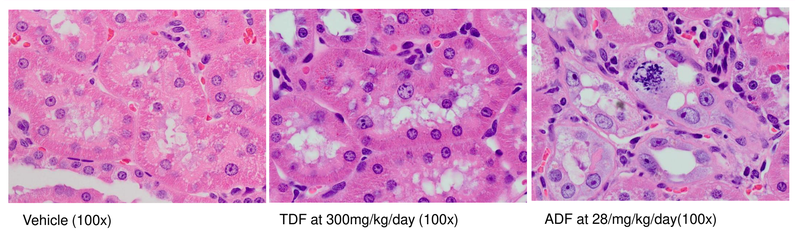


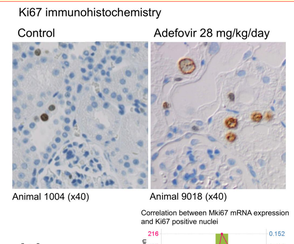


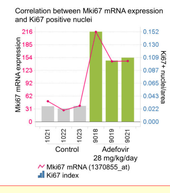
At EM large cytolysosomes (autophagic vacuoles) filled with mitochondria at different stages of degradation, loss of cytoplasmic mitochondria and pronounced alteration in the appearance and distribution of other cellular organelles were noted .

In comparison with control (picture C) PCT epithelial cells from 28mg/kg/day ADF treated rats showed loss of superficial microvilli, resulting in lower cell height, associated with mildly larger nuclei and pronounced alteration in the appearance and distribution of cellular organelles, including loss of mitochondria. PCT and cortical colleting tubules showed also presence of enlarged cytolysosomes (autophagic vacuoles) filled with mitochondria at different stages of degradation (picture D). In detail in picture E degenerating mitochondria within an autophagic vacuole (mitophagy). Enlarged cytolysosomes (black arrows); secondary lysosomes (white arrows) p= PCT, cc=cortical collecting tubule, d=distal tubule, m=mitochondria, v=apical vacuole, mv=microvilli. Magnification 1'200x for C and D, 11'000x for E.

TDF and ADF nuclear changes







ADF resulted also in prominent nuclear enlargement, correlating with up-regulation of cell division-associated genes (not shown). C-Kit examination revealed an increased expression in ADF-treated animals Subtle nuclear enlargement was also seen with TDF at 300mg/kg/day.

Conclusions

Adefovir and Tenofovir revealed two different toxicity profiles in Sprague-Dawley rats after 4 weeks of treatment in this study:

- **Treatment with TDF** caused minor kidney effects (mainly nuclear enlargement of the tubular epithelium and hyaline-droplet accumulation) at the 300 mg/kg/day. With higher doses, moribundity after 1 week of treatment due to gastrointestinal toxicity limited further investigations on the kidney. The cause of the increased hyaline droplets with TDF was not clear in this study.
- **Treatment with ADF** caused dose-dependent nephrotoxic effects mainly centered in the PCTs and suggested a mitochondrial-degeneration/depletion mechanism of toxicity.
- **Nuclear enlargement** of the tubular epithelium was the only common finding observed in the kidney of ADF- and TDF-treated rats.

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