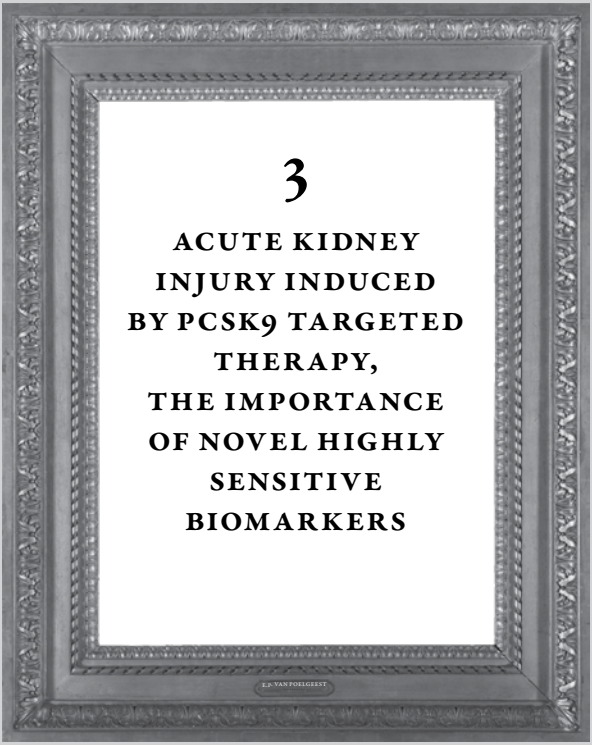
Future drugs in atherosclerotic cardiovascular disease

Poelgeest, E.P. van



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ACUTE KIDNEY INJURY INDUCED BY PCSK9 TARGETED THERAPY, THE IMPORTANCE OF NOVEL HIGHLY SENSITIVE BIOMARKERS

EP van Poelgeest, RM Swart, MG Betjes, M Moerland, JJ Weening, Y Tessier, MR Hodges, AA Levin, J Burggraaf.
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ABSTRACT

Antisense oligonucleotides are widely explored in clinical trials, and generally considered non-toxic for the kidney, even at high concentrations. Here, we report a case of toxic acute tubular injury in a healthy 56 year old female volunteer after administration of a pharmacologically active dose locked nucleic acid antisense oligonucleotide. The patient received 3 weekly subcutaneous doses of experimental drug SPC5001, a Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) inhibiting antisense oligonucleotide to lower LDL-Cholesterol. Five days after the last dose serum creatinine had increased from 0.81 mg/dL (eGFR: 78 mL/min/1.73 m^2) at baseline to 2.67 mg/dL (eGFR: 20 mL/min/1.73 m^2) and this coincided with the presence of white blood cells, granular casts and minimal hematuria on urine microscopy. Serum creatinine peaked at 3.81 mg/dL (eGFR: 13 mL/min/1.73 m^2) 1 week after the last oligonucleotide dose. Kidney biopsy showed multifocal tubular necrosis and signs of oligonucleotide accumulation. Upon conservative treatment serum creatinine decreased gradually to baseline at 44 days after last oligonucleotide administration. The patient recovered fully and kidney function was normal at every follow-up visit.

INTRODUCTION

Oligonucleotide-based therapies such as antisense-, silencing-, interfering- and micro-RNAs are widely investigated and considered a viable treatment option for a wide variety of clinical conditions [1-3]. Antisense oligonucleotide (ASO) based therapies are most advanced and considered safe [1]. Although clinical experience is limited, oligonucleotides share common features such as their ability to prolong a PTT, elicit injection site skin reactions, and accumulate in kidney proximal tubular cells.

SPC5001 is a short synthetic ASO containing DNA flanked at both ends by locked nucleic acids (LNA), binding to the mRNA of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9). This protease is expressed primarily in liver, intestine, and kidneys [4-7] and involved in various cellular functions [6;8;9]. The role of PCSK9 in LDL-Receptor degradation renders it a potential target for the treatment of hypercholesterolemia [10;11]. Here, we report on a case of acute tubular injury in a healthy female participating in a first-in-human trial with SPC5001. Other participants treated with at an equal SPC5001 dosage also showed signs of transient tubular dysfunction, suggesting a causal relationship. We aim to increase awareness for the toxic effects of ASOs on the kidney.

CASE REPORT

A 56-year-old caucasian female participated in a first-in-human trial with SPC5001. The patient was healthy as shown by a complete medical screening and did not smoke or use medication. Blood pressure was 126/70 mmHg and ECG and routine laboratory parameters were all normal.

She received 3 sc injections of SPC5001 (5 mg/kg; days 1, 8 and 15). Apart from developing injection site reactions (4x4 cm erythematous spots), there were no remarkable findings up to 24h after the last dose (day 16). At a scheduled assessment at day 18 the patient was feeling well but mentioned she had been nauseous on day 16/17 and had had limited intake of food and fluids. Urine production had been normal and physical examination was unremarkable. Serum creatinine had increased from 0.81 mg/dL at baseline (estimated glomerular filtration rate (eGFR): 78 mL/min/1.73 m^2), to 1.33 mg/dL (eGFR: 44 mL/min/1.73 m^2). At day 20, the patient was still well clinically, but serum creatinine had increased to 2.76 mg/dL (eGFR: 19 mL/min/1.73 m^2) and urine microscopy showed white blood cells, granular casts and minimal hematuria. The patient was admitted and treatment with iv isotonic saline was started. On day 21, serum creatinine

had increased to 3.59 mg/dL (eGFR: 14 mL/min/1.73 m^2), and kidney biopsy was obtained. Histopathology showed several foci of severe tubular injury with total denudation, nuclear apoptosis, and eosinophilic epithelial degeneration with shedding into tubular lumen which also contained debris with granular material (Figure 1). The tubular basal membrane was mostly intact. Intact tubules showed quite high epithelium and a brush border. Glomeruli and vessels were normal; the interstitium showed focal edema and lymphocytic infiltrates. Immunofluorescent staining ruled out immunoglobulin or complement deposition. Electron microscopy showed cell necrosis, vacuolization, and collapse of the cytoskeleton, a picture commonly seen in toxic tubular damage. Electron microscopy also showed abundance of phagolysosomes and endosomes in the affected tubular epithelial cells, reflecting antisense accumulation [13;14]. Without therapeutic intervention, serum creatinine decreased from its peak at 3.81 mg/dL (eGFR: 13 mL/min/1.73 m^2) at day 22, and the patient was discharged at day 24 with 2-weekly return visits scheduled. Serum creatinine decreased further and baseline value 0.88 mg/dL (eGFR: 71 mL/min/1.73 m^2) was reached at day 59. Urine dipstick and microscopy was normal during this follow-up period, except for a single observation of a trace (0-0.3 g/L) of protein at dipstick analysis on day 44. At the final visit at 8 months after the event the patient was well and routine laboratory parameters were normal.

Post-hoc analysis of biobanked spot urine samples, collected before each administration of study medication, was performed for assessment of kidney injury markers β2-microglobulin, α-Glutathione S-Transferase (αGST), Kidney Injury Molecule-1 (KIM1), and N-acetyl-β-D- glucosaminidase (NAG). NAG levels remained unchanged, but urinary β2-microglobulin increased 4-fold, αGST increased 24-fold, and KIM1 increased 60-fold upon administration of SPC5001 (Figure 2). Importantly, these markers preceded the rise in serum creatinine, increasing already after the first SPC5001 administration. These observations suggest that SPC5001 adversely affects proximal tubular function [15;16].

DISCUSSION

We report a case of acute tubular injury observed in a healthy female exposed to the LNA oligonucleotide SPC5001. Kidney toxicity became apparent within a week after the third and final dose, suggesting causal relationship. The toxicity was first noted by increases in serum creatinine, but was in hindsight preceded by increases in αGST, KIM1, and β2-microglobulin after the first dose implying that proximal tubular dysfunction was already present before the clinical manifestation

or routine laboratory measures increased. The causal relationship with the drug is supported by observations in other healthy volunteers treated with the same SPC5001 dosage in whom in the week after the final dose, transient but less pronounced (~15%) increases in serum creatinine, urinary damage markers and casts (3 out of 5 subjects) were noted.

Antisense compounds generally behave similarly with regard to kidney accumulation. Among the different chemistries of ASOs, 2'-O methoxyethyl modified phosphorothioate moieties (MOE-ASO) and 2'-4' O methylene LNAs are most researched and have been shown to accumulate without causing functional impairment even at dosages above those required for pharmacologic activity and during dehydration in animals [17]. Indeed, SPC5001- uptake, as with other oligonucleotides [18], is most prominent in the proximal tubule, but no adverse biochemical or histological kidney effects were noted in non-human primates after a loading dose of 20 mg/kg followed by 4 weekly doses of 5 mg/kg [19]. Dosages of this magnitude with other oligonucleotides did not induce cell toxicity and loss of renal function [20;21].

However, it has been reported that ASOs may concentration-dependently affect tubular cells [17], and that (repeated) exposure to high ASO doses may result in kidney toxicity [20;22-24]. This could reflect lysosomal stress and downstream apoptosis due to oligonucleotide accumulation in proximal tubule cells that are highly metabolically active to maintain integrity/function [25].

ATN associated with long term (74 doses of 10mg/kg weekly) MOE-ASO treatment has been described to occur in a cancer patient [26], but we now show that also 3 weekly doses of an LNA-ASO in healthy subjects may have caused acute renal injury. The MOE-ASO and LNA-ASO clinical case have in common that the target of the oligonucleotide is expressed in the kidney. Differences include the general condition of the patients (healthy vs. metastatic melanoma), the cumulative dose (1050 vs. 55500 milligram), the time course of recovery (1.5 vs. 4 months), and the time of the biopsy (1 wk vs. 4 months after final dose).

The precise mechanism by which SPC5001 caused kidney injury could have been target-related as PCSK9 is expressed in the kidney [27;28] and upregulation of renal PCSK9 mRNA occurs during inflammation [29], probably as a mechanism by which kidney injury is mitigated in a mouse model of drug-induced renal toxicity [30]. However, a direct effect of PCSK9-inhibition on the kidney seems at odds with the data showing that a novel interfering RNA inhibited PCSK9 by 60% without any effect on renal function [31], and loss of function mutations seem unassociated with impaired kidney function [32]. The oligonucleotide exposure in our SPC5001-treated patient is in line with exposure levels of previously studied

ASOs [33;34], and the urinary excretion was comparable to the other volunteers making renal toxicity due to excessively high local SPC5001 exposure unlikely. All observations point into the direction that renal oligonucleotide accumulation may have untoward effects in susceptible subjects. It is conceivable that susceptibility to develop toxic kidney disease varies between subjects. It may have been that our patient was predisposed to develop overt tubulotoxicity, as genetic differences affecting intracellular transport proteins and drug efflux transporters have been described [35]. Although the exact cause of the observed renal toxicity remains uncertain, and there may be differences between ASOs based on their chemistry, on- or off-target pharmacology and possibly other factors [1], we advocate careful monitoring of kidney function in the clinical development and utilization of ASOs. This should include not only serum creatinine or blood urea nitrogen (BUN), but also urine microscopy [36] and specific renal damage markers. With this approach, earlier detection and cessation of treatment is possible without relying solely on traditional markers for renal function such as creatinine, BUN, and electrolytes, particularly in subjects with uncompromised kidney function in whom early signs of toxic kidney disease might be masked [37]. These markers may also provide important mechanistic insight into the nature of potential renal damage.

Figure 1A&B. Light micrograph of the renal cortex at original magnification (A) x40 and (B) x80. Approximately 1 cm of kidney cortex with 21 glomeruli was viewed by light microscopy; ~20% of the tubules showed severe tubular necrosis (**A-B**). One glomerulus in the renal cortex is displayed showing several foci of severe tubular necrosis (*) with total denudation, vacuolization with loss of cytoplasm, nuclear apoptosis, and eosinophilic epithelial degeneration with shedding into tubular lumina, which contained debris with granular material (arrow). The tubular basal membrane was mostly intact (**A**). Glomeruli and vascular architecture showed no abnormalities, and interstitial tissue showed some edema and patchy lymphocytic infiltrates. (**B**) *(See figure 1C&D on next page).*

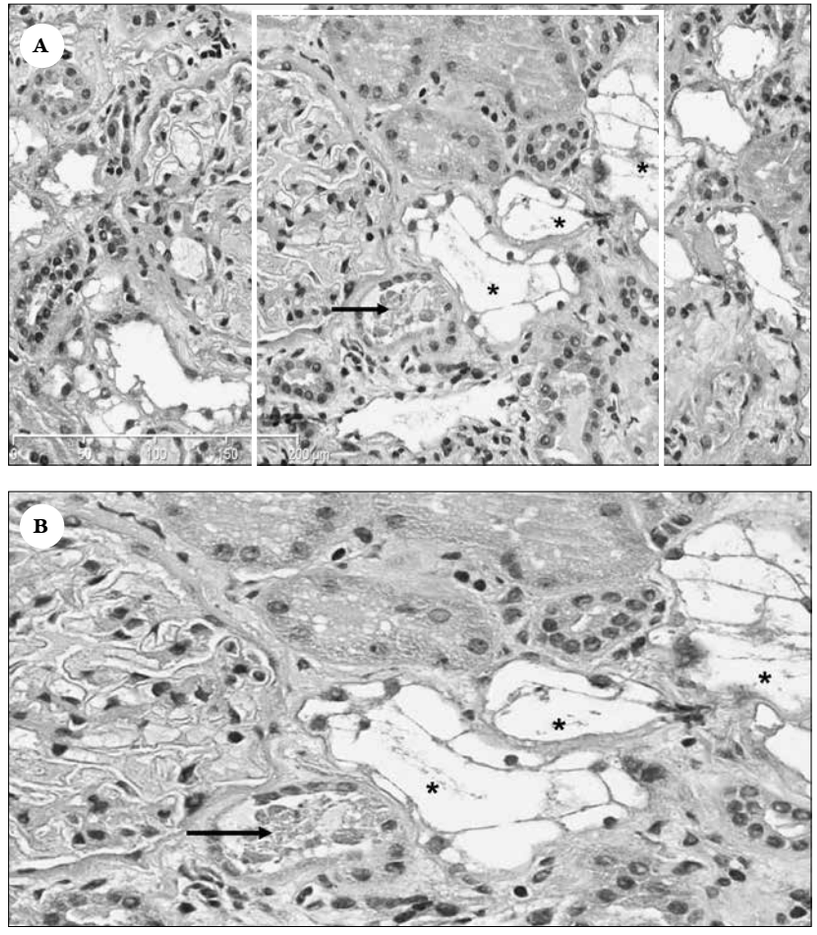
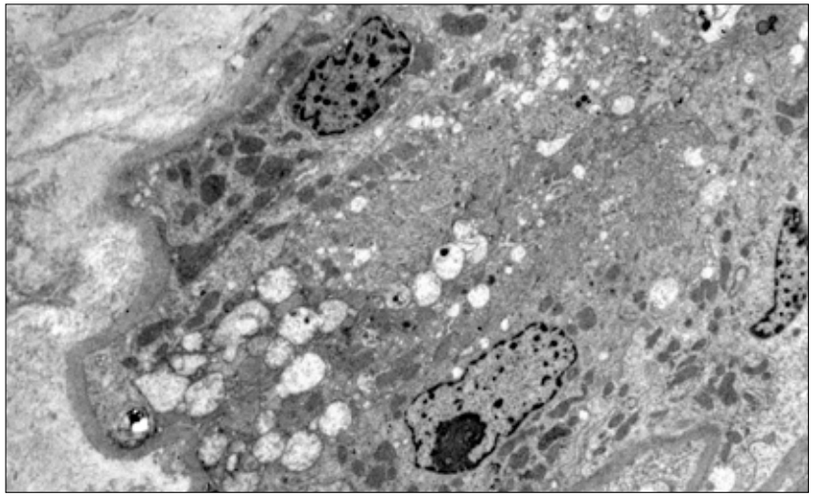


Figure 1C&D. Electron microscopy detail of proximal tubule cell. Electron microscopy of tubular cells shows increased endosomes (C) and clear nuclear condensation with increased lysosomes between swollen mitochondria (D). *(See figure 1A&B on previous page).*



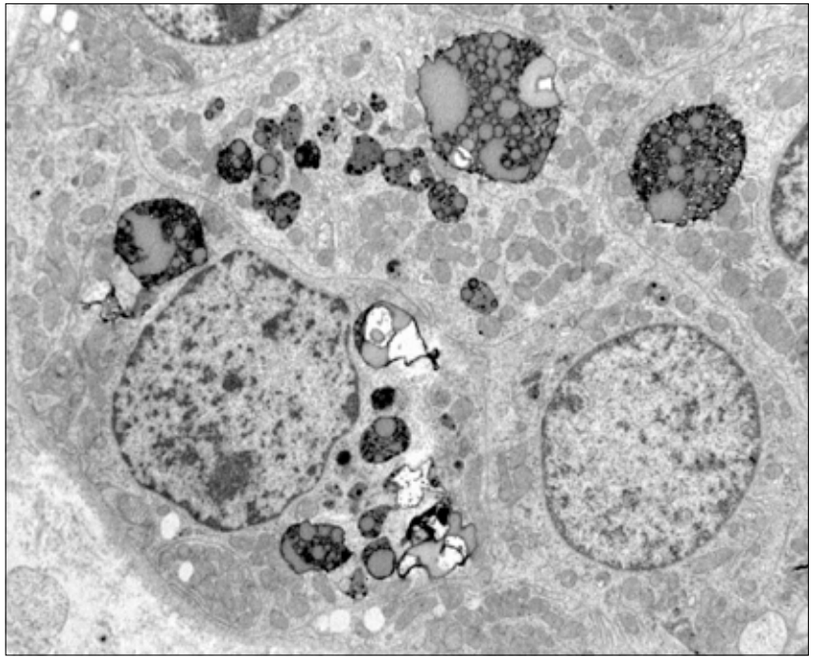
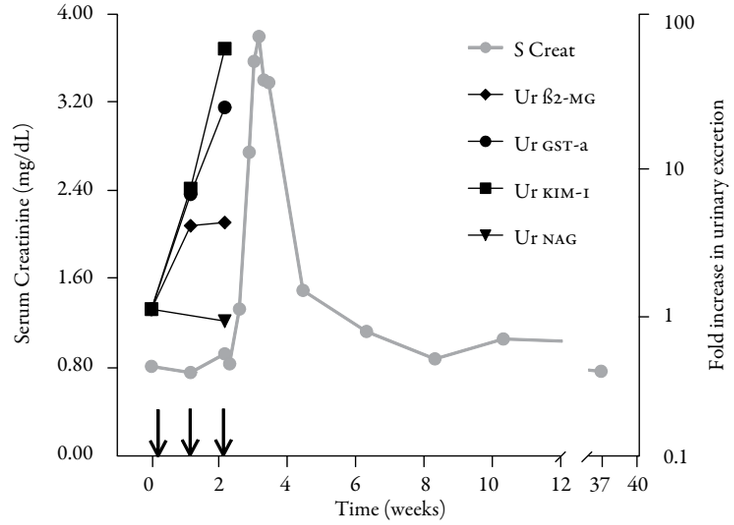


Figure 2. Time course of serum creatinine (S Creat) and urinary kidney damage marker levels. Arrows denote administration of SPC5001 on study days 1, 8, and 15. Conversion factor for S Creat in mg/dL to µmol/L, x88.4.



Abbreviations: Ur β2MG, urinary β2-microglobulin; Ur GSTα, urinary α-glutathione *S* transferase; Ur KIM1, urinary kidney injury molecule 1; Ur NAG, urinary *N*-acetyl-β-D-glucosaminidase.

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