Fumaric acid ester-induced renal Fanconi syndrome: evidence of mitochondrial toxicity

Elizabeth R. Wan¹, Keith Siew¹, Lauren Heptinstall¹, Stephen B. Walsh¹

¹UCL Department of Renal Medicine, Royal Free Hospital, University College London, London, UK

Correspondence to:

Elizabeth R. Wan

Email: e.wan@ucl.ac.uk
ABSTRACT

Background. Fumaric acid esters (FAEs) are used to treat chronic plaque psoriasis. Fumarate is a crucial component of the Krebs cycle, and mitochondrial function. Proximal tubule cells have high energy demands, and rely on aerobic respiration. Proximal tubular dysfunction can cause the renal Fanconi syndrome and acute kidney injury. We sought to better understand the mechanism for this in the context of FAE therapy.

Methods. We describe a case series of 10 patients with FAE-associated Fanconi syndrome. Patients were diagnosed and managed at a tertiary renal tubular disorder clinic, with examination of serum and urine biochemistry. 5 patients had a renal biopsy with examination of the specimens by electron microscopy.

Results. The median age was 36.5 years (IQR 32.25-54.25 years). The median dose of FAE was 720mg/day (IQR 390-720mg).

There was low molecular weight proteinuria: median urinary retinol binding protein (RBP) at presentation was 8385μg/ml (IQR 2793-14600μg/ml) and RBP/creatinine ratio was 710 (IQR 390-2415). All patients had hyperphosphaturia (median fractional excretion of phosphate 24.2%, IQR 20.8-26.9%, normal range <20%), as well as relative hypophosphataemia, with a median serum phosphate concentration of 0.93mmol/L (IQR 0.83-0.97mmol/L). Renal histology showed proximal tubular damage and abnormal mitochondrial morphology. Two patients had a favourable biochemical response to treatment with probenecid.

Conclusions. We document for the first time that FAE-associated renal Fanconi syndrome is associated with mitochondrial damage visible on electron microscopy.
This effect may be ameliorated by antagonism of the organic anion transporter with probenecid.

**Keywords:** acute kidney injury, chronic kidney disease, Fanconi syndrome, proteinuria, tubular
INTRODUCTION

Fumaric acid esters (FAEs) are immunomodulatory agents used to treat chronic plaque psoriasis in Germany since 1994\(^1\), although their effect on psoriasis was described in 1959\(^2\). Although they are a standard first-line psoriasis treatment in Germany\(^3\), FAEs are a third-line treatment for severe, non-responsive plaque psoriasis in the UK\(^4\). The most common commercially available formulation is Fumaderm (Biogen, Germany) consisting of dimethylfumarate and three other fumaric acid esters\(^5\). Recently dimethylfumarate has been licensed as a treatment for relapsing-remitting multiple sclerosis\(^6\). While there has been some debate about the toxicity of FAEs\(^7\); case reports and one retrospective cross-sectional study describe a risk of renal proximal tubular dysfunction\(^8\) which may manifest as the renal Fanconi syndrome\(^1\), or as acute kidney injury\(^9\). The renal Fanconi syndrome describes a pattern of reduced reabsorption of a number of solutes by the proximal convoluted tubule, causing urinary wasting of bicarbonate, phosphate, glucose, urate, amino acids, and low molecular weight proteins such as retinol binding protein (RBP). It is usually an acquired disorder, often due to drug toxicity\(^10\). As well as the risk of developing kidney injury, sufferers are predisposed to osteomalacia and pathological fractures due to hypophosphataemia.

The first component of the Krebs cycle to be identified was fumarate, securing the 1937 Nobel Prize for Physiology or Medicine for Albert Szent-Györgyi\(^11\). Fumarate is the intermediary resulting from the dehydrogenation of succinate in the Krebs cycle. Proximal tubule cells have a high ATP demand due to their role in actively transporting molecules from the filtrate, and are heavily dependent on mitochondrial aerobic respiration\(^12\). We therefore hypothesised that FAEs were likely to act as mitochondrial toxins.
Further, fumarate is a specific substrate of the organic anion transporter 1 (OAT1), a basolateral proximal tubular cell transporter. Probenecid is a prototypical OAT inhibitor, blocking both OAT 1 and 3 (two of the OAT solute carrier family which are found in the kidney). We therefore reasoned that inhibiting the organic anion transporter with probenecid should prevent delivery of the drug to the proximal tubule cells and thus renal toxicity. Such a strategy is already employed with some nephrotoxic therapies (e.g. cidofovir).

MATERIALS AND METHODS

Patient population

All patients were referred to the University College London (UCL) tubular clinic for diagnosis and management. All were taking FAEs as Fumaderm. Cases were diagnosed between 2010 and 2017. Patients were investigated with serum and urine biochemistry, analysed according to standard operating procedures in the Department of Biochemistry. Five patients had a renal biopsy with examination of the specimens by light and electron microscopy.

Statistical analysis

Data was analysed with descriptive statistical methods, using R in RStudio.

Case vignettes

Case 1: A 46-year-old man was referred due to proteinuria and an incremental rise in his serum creatinine (to 140μmol/L). He had been diagnosed with chronic plaque
psoriasis 20 years previously, and had been treated over the years with a wide range of therapies (steroids, PUVA, methotrexate, Acitretin, cyclosporin, etanercept and infliximab). He started FAEs in March 2010 at a dose of 480mg with a good response in his skin disease. The renal Fanconi syndrome was diagnosed on the basis of a raised urinary fractional excretion of phosphate ($\text{FE}_{\text{PO}_4}$) and low molecular weight proteinuria (LMWP) (see Supplementary Table 1). FAEs were stopped, but his eGFR did not improve. 6 months later he underwent a renal biopsy which showed features of mild acute tubular damage with some background chronic damage. At last clinic follow-up his psoriasis was poorly controlled on topical therapy.

Case 2: A 32-year-old woman who had been taking FAEs for two years was referred after a repeated finding of blood and protein in her urine, and rise in her serum creatinine, following a dose increase to 720mg. She had a diagnosis of psoriasis and psoriatic arthritis, and had previously had Stevens-Johnson syndrome after sulfasalazine. Her serum phosphate had been as low as 0.25mmol/L (normal range 0.87-1.45mmol/L). She had an RBP of 19300 µg/L, a $\text{FE}_{\text{PO}_4}$ of 27% (normal range <20), and hypouricaemia at 82.1µmol/L (normal range 200-420µmol/L). The dose of FAEs were reduced and probenecid started to reduce proximal tubular toxicity. However, she developed a skin eruption so this was stopped. She underwent a renal biopsy; while light microscopy was unremarkable, electron microscopy showed widespread mitochondrial dysmorphology with a ‘blown-up’ appearance and disruption of the cristae. She remains on FAEs and under follow up.

Case 3: A 33-year-old woman was referred with intermittent blood and protein on urinalysis. She had been prescribed 360mg FAEs and monthly adalimumab injections for chronic plaque psoriasis; her dermatologist had measured her urinary
RBP, which was raised at 1320µg/L. She underwent a renal biopsy: electron microscopy demonstrated enlarged mitochondria with distorted cristae. She was trialled on probenecid 500mg BD, and RBP fell to nadir of <100µg/L with treatment. In fact, there was a sustained fall in the RBP/creatinine ratio down to 50, even when FAEs had to be increased to 600mg/day for a psoriasis flare. Serum urate and phosphate also rose with probenecid therapy.

Case 4: A 57-year-old man was referred from another tertiary hospital with an abnormal but stable creatinine (115µmol/L). He had been taking FAEs for 12 years, to a maximum dose of 720mg, and had experienced glycosuria and proteinuria throughout this time. A vasculitis screen, ultrasound scan of the kidney and urogenital tract, and intravenous urogram at his local centre were unremarkable. He was found to have an elevated FE_{PO4} (20.7%) and urinary RBP (10,200µg/L), with a low serum urate (190µmol/L). FAEs were stopped, and he was switched to adalimumab with good control of his psoriasis. Despite this, the proximal tubulopathy persisted. He declined a renal biopsy. Eventually, two years after stopping the drug, his serum and urine biochemistry normalised.

Case 5: A 70-year-old man was referred for assessment from a tertiary dermatology centre due to proteinuria. He had psoriasis for 45 years, and had been on FAEs for the last 24 years. He had a raised urinary RBP and raised FE_{PO4} of 26%. FAEs were stopped; unfortunately, the patient developed severe pustular psoriasis requiring admission to the intensive care unit. He is now well and controlled on adalimumab.

Case 6: A 28-year-old woman was referred with heavy proteinuria (++++) on urinalysis and previous hypophosphatemia. At the point of referral, she had been taking 720mg FAEs in addition to etanercept for 3 years. She had a renal biopsy which appeared
normal by light microscopy, but enlarged mitochondria with disrupted cristae were
demonstrated by electron microscopy. The renal Fanconi syndrome was managed
by reducing the dose of FAEs to 360mg, and psoriasis control was optimised by
switching etanercept to adalimumab. The renal Fanconi syndrome resolved with this
approach.

Case 7: A 39-year-old man was referred for assessment due to urinalysis showing
glycosuria, microscopic haematuria and proteinuria. He had recently increased the
dose of FAEs to 720mg from 600mg/day. At clinic review his creatinine was raised at
114µmol/L, the FE_{PO4} was 23.2% and urinary RBP was 59900µg/L. He went on to
have a renal biopsy; light microscopy showed features of acute tubular damage
particularly to proximal tubules. Electron microscopy showed a ‘blown up’
appearance of the mitochondria. FAEs were stopped with a plan to start biologics.
This was delayed for 3 months while he was treated for latent tuberculosis. After this
treatment he was started on adalimumab with a good skin response.

Case 8: A 34-year-old man with chronic plaque psoriasis was referred to the UCL
tubular clinic with a one-year history of dipstick proteinuria and microscopic
haematuria. He was taking FAEs, having previously failed Acitretin and
methotrexate therapy. He was started on probenecid 500mg BD, which was well
tolerated, and continued on the same dose of FAEs. His urinary RBP fell from
939µg/L to 570µg/L. He remains under clinic surveillance.

Case 9: A 29-year-old man was referred with an abnormal urine dip (blood and
protein) and a deterioration in his serum creatinine from 70µmol/L to 91µmol/L. He
had been taking 720mg FAEs for the previous year, having been unable to tolerate a
lower dose due to psoriasis flares, and having experience liver toxicity from
methotrexate. He was found to have a raised urinary RBP of 13100µg/L, with a low-normal serum phosphate of 0.89mmol/L. The same dose of FAE was continued with close monitoring.

Case 10: An 81-year-old man was referred to the nephrology service with chronic renal impairment and glucose, blood and protein on urinalysis. He had lived for 40 years with psoriasis, at times with up to 97% skin coverage. He had been treated with a wide range of therapies previously, most recently stopping cyclosporine due to nephrotoxicity. He had been treated with FAEs for the last 9 years. He declined a renal biopsy, and a diagnosis of renal Fanconi syndrome was made on serum and urinary biochemistry. He continued to take FAEs at a much-reduced dose of 60mg OD. He had a normal serum and urine phosphate at follow-up.

RESULTS

We describe 10 patients, all of whom were diagnosed at our unit. 70% of the cases were men; the median age at diagnosis of renal Fanconi syndrome was 36.5 years (IQR 32.25-54.25). The most frequent ethnic group was white British (50%). The median dose of FAEs was 720mg (IQR 390-720mg), with the median dose by weight being 8.71mg/kg (IQR 5.90-10.29mg/kg). Biochemistry is summarised in Table 1, and individualized data are available in Supplementary Table 1. Low molecular weight proteinuria was present: the median urinary retinol binding protein (RBP) at presentation was 8385µg/ml (IQR 2793-14600µg/ml) and RBP/creatinine ratio was 710 (IQR 390-2415). All patients had relative hypophosphataemia, with a median serum phosphate concentration of 0.93mmol/L (IQR 0.83-0.97mmol/L). All patients had an elevated FEPO$_4$ (median 24.2%, IQR 20.8-26.9%, normal range <20%).
Hypouricaemia was also prevalent (median serum urate concentration 150μg/L, IQR 145-190μg/L).

Five patients underwent renal biopsy. In two of the five biopsies there were features of acute damage to the proximal tubules visible on light microscopy (Figure 1), with irregular flattening of the tubules, and occasional small cytoplasmic vacuolations visible on simple haemotoxylin and eosin staining. Periodic Acid-Schiff staining highlighted patchy loss of the brush border (Figure 2). Glomeruli were normal. The remaining three biopsies were superficially normal with light microscopy. However, electron microscopy revealed abnormal mitochondria, always with abnormal enlargement (‘blown up’ appearance) and distortion of the cristae (for example, Figure 3). There did not appear to be any correlation between the severity of the biopsy appearances and the severity of the low molecular weight proteinuria, which is generally considered the most sensitive measure of proximal tubular dysfunction in the renal Fanconi syndrome.

While most patients stopped the drug or were managed at lower doses, 3 patients were treated with probenecid. In one of these cases, the patient terminated treatment after a severe skin reaction. However, in the remaining two we saw a biochemical response, with a reduction in the urinary retinol-binding protein (Table 2).

DISCUSSION

As proximal tubular toxins, FAEs may cause both the renal Fanconi syndrome\(^1\) and even acute kidney injury (AKI)\(^9\). As previous authors have noted, development of Fanconi syndrome may herald AKI\(^18\). However, stopping FAE treatment is not
without risk. FAEs are used as a third-line agent in the UK\(^4\), so patients have resistant or severe disease. Indeed, in one of our cases, cessation of FAEs resulted in the patient developing life-threatening pustular psoriasis.

Our data support the mechanism of proximal tubular injury by FAEs being mitochondrial toxicity. This is plausible; proximal tubule cells are involved in the active transport of many solutes to and from the tubule lumen and are metabolically very active. They possess a dense population of mitochondria for the production of ATP\(^{19}\) and as they have little glycolytic ability, they are heavily dependent on aerobic respiration\(^{20}\). Mitochondria are dynamic organelles which can undergo both fission (in an ATP-depleted environment) and fusion (for example damaged mitochondria might fuse to create enlarged, healthy, organelles).

Malate, another Krebs cycle component, can cause the renal Fanconi syndrome. In fact, malate-induced renal Fanconi syndrome in mice is a well-established model of both the renal Fanconi syndrome and acute renal failure\(^{21,22}\). Malate results from the hydration of fumarate catalysed by fumarase. Malic acid is also a specific substrate of OAT\(^{13}\), present on proximal tubular cells. Malic acid depresses the Na-K-ATPase activity in the proximal convoluted tubule\(^{23}\), consistent with ATP depletion. Na-K-ATPase activity in distal renal segments is unaffected by the drug\(^{24}\), possibly due to reduced OAT expression there\(^{25}\) or greater glycolytic capability of that nephron segment. The closely related malonic acid was recognised to cause morphological changes in mitochondria by disrupting the Krebs cycle as early as 1972\(^{26}\). There may be a single common pathway of injury which is shared between malate-induced renal Fanconi syndrome and hypoxic-ischaemic acute kidney injury\(^{21}\). Indeed, fumarase itself may be a biomarker of acute kidney injury\(^{27}\).
Therefore, there may be some parallels in the mechanism of toxicity induced by maleate and fumaric acid esters. However, it has recently been demonstrated in rat kidneys that malate causes small, fragmented mitochondria\textsuperscript{28}, whereas in our study the mitochondria were observed to be enlarged. An alternative mechanism of fumarate-induced nephrotoxicity might be that of ‘fumarate overflow’, as demonstrated by Chouchani \textit{et al} in a model of ischaemia reperfusion injury in the heart\textsuperscript{29}. Here, partial reversal of the malate/aspartate shuttle results in fumarate overflow, reversal of succinate dehydrogenase and accumulation of succinate. Succinate accumulation results in reverse electron transport in respiratory complex 1 and production of toxic reactive oxygen species.

In our cohort there was a slight preponderance of men, although the number of cases is small. It has been previously asserted that FAE-associated renal Fanconi syndrome is more common in women than men\textsuperscript{30}. It is widely reported that there are sex differences in the expression of the OATs, although OAT1 is actually expressed more in males than females, at least in rats (see review by Sekine \textit{et al}\textsuperscript{14}).

This data also suggests the potential utility of treatment with probenecid in permitting continued FAE treatment. Probenecid treatment resulted in improvement in urine biochemistry in two of the three patients treated with this strategy. Unfortunately, the third developed a severe skin reaction and had to terminate the probenecid. Use of this drug can cause erythroderma\textsuperscript{15}. For those patients not able, or not willing, to convert from FAEs to another medication (the main alternative being biologic agents), probenecid may be considered as a rescue strategy.
CONCLUSIONS

Here we demonstrate for the first time that human FAE-associated renal Fanconi syndrome is associated with mitochondrial damage visible on electron microscopy, and we hypothesize two possible molecular mechanisms. This could be better characterised by further animal models (for example, direct comparison with the well-established malate model), and by exploring unbiased approaches in the tissue/urine, for example with metabolomics.

PATIENT CONSENT

Patient consent has been obtained for this article.

CONFLICT OF INTEREST STATEMENT

None declared.
REFERENCES

1. Pathirana D, Ormerod A, Saiag P, et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. *J Eur Acad Dermatol Venereol*. 2009;23(Suppl 2):1-70.

2. Schweckendiek W. Treatment of psoriasis vulgaris. *Med Monatsschr*. 1959;13:103-104.

3. Nast A, Kopp I, Augustin M. German evidence-based guidelines for the treatment of Psoriasis vulgaris. *Arch Dermatological Res*. 2007;299(May 2014):111-138. doi:10.1007/s00403-007-0744-y

4. National Institute of Clinical Excellence (NICE). Psoriasis: assessment and management [CG153]. https://www.nice.org.uk/guidance/cg153. Published 2017. Accessed July 1, 2019.

5. Mrowietz U, Altmeyer P, Bieber T, Röcken M, Schopf RE, Sterry W. Treatment of psoriasis with fumaric acid esters (Fumaderm®). *JDDG - J Ger Soc Dermatology*. 2007;5(8):716-717. doi:10.1111/j.1610-0387.2007.06346.x

6. Linker RA, Haghiakia A. Dimethyl fumarate in multiple sclerosis: latest developments, evidence and place in therapy. *Ther Adv Chronic Dis*. 2016;7(4):198-207. doi:10.1177/2040622316653307

7. Reich K, Thaci D, Mrowietz U, Kamps A, Neureither M, Luger T. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis - A retrospective study (FUTURE). *JDDG - J Ger Soc Dermatology*. 2009;7(7):603-611. doi:https://doi.org/10.1111/j.1610-0387.2009.07120_supp.x

8. Menzies S, Ismail N, Abdalla A, et al. Renal dysfunction in patients taking fumaric acid esters – a retrospective cross-sectional study. *J Eur Acad Dermatology Venereol*. 2017;31(4):686-691. doi:10.1111/jdv.14025

9. Roodnat JI, Christiaans MH, Nugteren-Huying WM, et al. Acute kidney insufficiency in the treatment of psoriasis using fumaric esters. *Schweiz Med Wochenschr*. 1989;119(23):826-830.

10. Kashoor I, Batlle D. Proximal renal tubular acidosis with and without Fanconi syndrome. *Kidney Res Clin Pract*. 2019;38(3):267-281.

11. Szent-Györgyi A. Nobel Lecture: Oxidation, energy transfer, and vitamins. Presented at the: 1937.

12. Bagnasco S, Good D, Balaban R. Lactate production of the rat nephron in isolated segments. *Am J Physiol*. 2019;248(4):F522-526.

13. Kaler G, Truong DM, Khandelwal A, et al. Structural variation governs substrate specificity for Organic Anion Transporter (OAT) homologs: Potential remote sensing by OAT family members. *J Biol Chem*. 2007;282(33):23841-23853. doi:10.1074/jbc.M703467200

14. Sekine T, Miyazaki H, Endou H. Molecular physiology of renal organic anion transporters. *Am J Physiol Physiol*. 2006;290(2):F251-F261. doi:10.1152/ajprenal.00439.2004
15. Polis MA, Spooner KM, Baird BF, et al. Anticytomegaloviral Activity and Safety of Cidofovir in Patients with Human Immunodeficiency Virus Infection and Cytomegalovirus Viruria. *Antimicrob Agents Chemother*. 1995;39(4):882-886.

16. Team RC. R: A Language and Environment for Statistical Computing. 2017. https://www.r-project.org.

17. Team Rs. RStudio: Integrated Development Environment for R. 2016. http://www.rstudio.com.

18. Häring N, Mähr HS, Mündle M, Strohal R, Lhotta K. Early detection of renal damage caused by fumaric acid ester therapy by determination of urinary β2-microglobulin. *Br J Dermatol*. 2011;164(3):648-651. doi:10.1111/j.1365-2133.2010.10171.x

19. Schmidt U, Guder WG. Sites of enzyme activity along the nephron. *Kidney Int*. 1976;9(3):233-242. doi:10.1038/ki.1976.26

20. Ruegg CE, Mandel LJ. Bulk isolation of renal PCT and PST: Glucose-dependent metabolic differences. *Am J Physiol - Ren Fluid Electrolyte Physiol*. 1990;259(1 28-1). doi:10.1152/ajprenal.1990.259.1.f164

21. Zager RA, Johnson ACM, Naito M, Bomsztyk K. Maleate nephrotoxicity: mechanisms of injury and correlates with ischemic/hypoxic tubular cell death. *Am J Physiol Physiol*. 2008;294(1):F187-F197. doi:10.1152/ajprenal.00434.2007

22. Sawas-Dimopoulou C, Sigalas I, Margaritis L. Induction of an experimental Fanconi syndrome in mice: Its effect on the glomerular filtration function studied by 99mTc-DTPA. *Nucl Med Biol*. 1996;23(6):807-812. doi:10.1016/0969-8051(96)00077-7

23. Kramer KM, Brock JA, Bloom K, Moore JK, Haber JE. Two different types of double-strand breaks in Saccharomyces cerevisiae are repaired by similar RAD52-independent, nonhomologous recombination events. *MolCellBiol*. 1994;14:1293-1301.

24. Eiam-Ong S, Spohn M, Kurtzman NA, Sabatini S. Insights into the biochemical mechanism of maleic acid-induced Fanconi syndrome. *Kidney Int*. 1995;48(5):1542-1548. doi:10.1038/ki.1995.444

25. Kellerman PS, Norenberg S, Guse N. Exogenous adenosine triphosphate (ATP) preserves proximal tubule microfilament structure and function in vivo in a maleic acid model of ATP depletion. *J Clin Invest*. 1993;92(4):1940-1949. doi:10.1172/JCI116787

26. McDowell EM. Light and electron microscopic studies of rat kidney after administration of inhibitors of the citric acid cycle in vivo - A morphological and histochemical study of the pars recta during malonate poisoning. *Am J Pathol*. 1972;15(1):187-208. doi:10.1007/BF02889336

27. Nielsen PM, Eldirdiri A, Bertelsen LB, Joergensen HS, Ardenkaer- Larsen JH, Laustsen C. Fumarase activity: An in vivo & in vitro biomarker for acute kidney injury. *Sci Rep*. 2017;7(January):1-10. doi:10.1038/srep40812

28. Molina-Jijón E, Aparicio-Trejo OE, Rodríguez-Muñoz R, et al. The
nephroprotection exerted by curcumin in maleate-induced renal damage is associated with decreased mitochondrial fission and autophagy. *BioFactors*. 2016;42(6):686-702. doi:10.1002/biof.1313

29. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*. 2014;515(7527):431-435. doi:10.1038/nature13909

30. Balak DMW, Nico J, Bavinck B, et al. Drug-induced Fanconi syndrome associated with fumaric acid esters treatment for psoriasis: a case series. *Clin Kidney J*. 2016;9(1):82-89. doi:10.1093/ckj/sfv114
Table 1. Summary data for case series

| Units                  | Normal Range | Median | IQR       |
|------------------------|--------------|--------|-----------|
| Age at diagnosis       | Years        | 36.5   | 32.25-54.25 |
| Weight                 | kg           | 70.58  | 62.5-74.78  |
| Dose FAEs in 24hrs     | mg           | 720    | 390-720   |
| Dose FAEs by weight    | mg/kg        | 8.71   | 5.90-10.29 |

**Serum results**

| Parameter             | Units            | Normal Range          | Median | IQR        |
|-----------------------|------------------|-----------------------|--------|------------|
| Creatinine            | µmol/L           | 0.66-112              | 81     | 65-113     |
| eGFR                  | (mL/min/1.73 m²)  | 98.5                  |        | 61.75-107.75 |
| Sodium                | mmol/L           | 135-145               | 142    | 141-143.5  |
| Potassium             | mmol/L           | 3.5-5.1               | 4.05   | 3.0-4.43   |
| Uric acid             | µmol/L           | 200-420               | 150    | 145-190    |
| Inorganic phosphate   | mmol/L           | 0.87-1.45             | 0.86   | 0.80-1.01  |

**Urine results**

| Parameter                                         | µg/L                 |                      |         |
|---------------------------------------------------|----------------------|----------------------|---------|
| Urine retinol binding protein (RBP)               |                      | 8385                 | 2793-14600 |
| Urine RBP: creatinine ratio*                      | µmol/L/ µg/L         | <14                  | 709.5   | 390.25-2415.25 |
| Urine creatinine                                  | µmol/L               | 8500                 | 4440-11600 |
| Urine inorganic phosphate                         | mg/dL                | 14.2                 | 11.50-20.0 |
| Fractional excretion phosphate                    | %                    | <20                  | 24.2    | 20.8-26.9   |

*Calculated by creatinine (µmol/L)/ RBP (µg/L)

Table 2. Probenecid treatment

| Case   | Baseline urine RBP* (µg/L) | Baseline RBP: creatinine ratio** | Urine RBP after treatment (µg/L) (% reduction) | RBP: creatinine ratio after treatment (% reduction) |
|--------|---------------------------|---------------------------------|-----------------------------------------------|--------------------------------------------------|
| Patient 4 | 1320                     | 228                             | 181 (86%)                                    | 43 (81%)                                         |
| Patient 9 | 6570                     | 939                             | 570 (91%)                                    | 31 (97%)                                         |

*RBP = Retinol-binding protein

**Calculated by creatinine (µmol/L)/ RBP (µg/L)
Legends to figures:

**FIGURE 1:** Haematoxylin and eosin staining, original magnification x400. Tubules show acute damage indicated by irregular flattening (solid arrows) and occasional small cytoplasmic vacuolations (dashed arrow).

**FIGURE 2:** Periodic Acid Schiff staining, original magnification x400, highlighting patchy loss of the brush border (solid arrows).

**FIGURE 3:** Electronic microscopy image, visualised at 6000x, showing the proximal tubules with focal mitochondrial changes, including swollen forms (solid arrow) and distorted cristae (dashed arrow).
Haematoxylin and eosin staining, original magnification x400. Tubules show acute damage indicated by irregular flattening (solid arrows) and occasional small cytoplasmic vacuolations (dashed arrow).

551x492mm (96 x 96 DPI)
Periodic Acid Schiff staining, original magnification x400, highlighting patchy loss of the brush border (solid arrows).

525x472mm (96 x 96 DPI)
Electronic microscopy image, visualised at 6000x, showing the proximal tubules with focal mitochondrial changes, including swollen forms (solid arrow) and distorted cristae (dashed arrow).

705x541mm (96 x 96 DPI)