Imaging the distribution of polymyxins in the kidney

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Objectives: Dose-limiting nephrotoxicity remains the Achilles’ heel of polymyxin B and polymyxin E (also known as colistin), which are important last-line antibiotics used against infections caused by MDR Gram-negative ‘superbugs’. An understanding of the mechanisms of nephrotoxicity, including renal tissue distribution, is crucial for the development of safer polymyxin lipopeptide antibiotics. This is the first study to visualize the kidney distribution of polymyxin B using a mouse nephrotoxicity model and in situ immunostaining of kidney sections.

Methods: Polymyxin B nephrotoxicity in mice was induced over the course of 3 days (accumulated intravenous dose 175 mg/kg) and kidneys were harvested and frozen sectioned. The sections were fixed in cold acetone, dried and treated with 1% hydrogen peroxide. Endogenous mouse immunoglobulins were blocked and the tissue sections were treated with anti-polymyxin B mouse IgM antibody. The sections were incubated with a biotinylated anti-mouse secondary antibody conjugate followed by an Alexa Fluor 647–streptavidin conjugate. Polymyxin B distribution in the kidney sections was then visualized using a fluorescence scanning microscope. Kidney sections were also subjected to haematoxylin and eosin staining to assess pathological damage from the polymyxin-induced nephrotoxicity.

Results: Immunostaining of kidney sections from a mouse with polymyxin B-induced nephrotoxicity revealed that polymyxin B distributed predominantly within the renal cortex. More specifically, polymyxin B accumulated within the proximal tubular cells.

Conclusions: The observed accumulation of polymyxin B within proximal tubular cells is consistent with the extensive renal reabsorption of polymyxins and the likely cause of the associated nephrotoxicity.

Keywords: nephrotoxicity, polymyxin B, mice

Introduction

Over the past two decades there has been a pronounced increase in the emergence of Gram-negative ‘superbugs’, which are resistant to almost all clinically available antibiotics and cause serious infections.1 This dire situation is exacerbated by a lack of novel antibiotics in the developmental pipeline of both pharmaceutical companies and research institutes, leaving the world’s population in a vulnerable state against these life-threatening infections.2 This ‘perfect storm’ has led to the revival of the polymyxin class of antibiotics [polymyxin B and polymyxin E (also known as colistin)] as a last line of defence against infections caused by MDR Gram-negative pathogens.3 However, despite their excellent antibacterial activity, the use of polymyxins has largely been limited by the associated adverse effects. In particular, nephrotoxicity is a major dose-limiting factor for the clinical use of polymyxins, occurring in up to 60% of all patients.4 Polymyxin-induced nephrotoxicity potentially arises from their significant reabsorption by renal tubular cells;4–6 for both colistin and polymyxin B, only a minor portion of the drug in the body is renally eliminated.5,6 A recent synchrotron X-ray fluorescence microscopy (XFM) single-cell study from our laboratory demonstrated that polymyxins accumulate within kidney tubular cells with intracellular concentrations ~2000–4000-fold higher than extracellular concentrations.7 At present there is a lack of information regarding the kidney tissue distribution of polymyxins. Such knowledge is critical for understanding their pharmacokinetics/pharmacodynamics and for the development of novel safer polymyxins.8,9 To the best of our knowledge, this is the first study to visualize the distribution of polymyxins in kidneys using a mouse nephrotoxicity model and in situ immunostaining of kidney sections.
Methods

Animals

The animal experiments were approved by the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee. Animal experiments were performed in accordance with the Australian National Health and Medical Research Council guidelines for the care and use of animals for scientific purposes. Female Swiss mice (6–8 weeks of age, 20–25 g) were used in this study. Mice had free access to food and water during the experiment.

Mouse polymyxin nephrotoxicity model and immunostaining

A mouse nephrotoxicity model was established by our group. On the day of the experiment, polymyxin B or sterile saline was administered subcutaneously to mice (n = 3) over the course of 3 days (accumulated dose 175 mg/kg, with 35, 10, 10 and 20 mg/kg administered at 2 h intervals on day 1; 35, 10, 10 and 15 mg/kg administered at 2 h intervals on day 2; and a final dose of 30 mg/kg on day 3). On the third day, mice were euthanized via an isoflurane overdose 2 h after the last dose and kidneys were collected immediately and sectioned frozen at 12 μm. The sections were fixed in cold acetone, dried and treated with 1% hydrogen peroxide for 5 min. Blocking of endogenous mouse IgG was carried out using the Vector M.O.M. kit (Vector Laboratories, CA, USA) in accordance with the manufacturer’s instructions. Anti-polymyxin B mouse IgM antibody (Thermo Fisher Scientific, Rockford, USA) was diluted 1:500 and incubated with the tissue section overnight at 4°C. The sections were washed and incubated with M.O.M. biotinylated anti-mouse secondary antibody (Vector Laboratories, CA, USA) for 10 min, followed by incubation with an Alexa Fluor 647–streptavidin conjugate at 1:500 dilution (Life Technologies, VIC, Australia). Kidney sections were also subjected to haematoxylin and eosin staining. Sections were mounted with Dako fluorescence mounting medium (Dako, NSW, Australia) and imaged using a Metasystems VSlide scanner.

Results and discussion

Currently, dose-limiting nephrotoxicity remains a major problem for the clinical usefulness of polymyxins. Understanding the mechanisms of polymyxin-induced nephrotoxicity is not only very important for optimizing their clinical use, but also for guiding the development of novel, safer polymyxin lipopeptides. Polymyxin B was employed in this study as recent pharmacological studies demonstrated that it has significantly better pharmacokinetic/pharmacodynamic characteristics than colistin. A mouse polymyxin nephrotoxicity model established in our novel polymyxin discovery programme was employed in the present study.

In this study we have examined the distribution of polymyxins in the kidney tissue of mice with polymyxin-induced nephrotoxicity (Figure 1, see the haematoxylin and eosin panel showing histopathological damage). Histological abnormalities were observed in the kidneys of the polymyxin B-treated mice, with marked tubular dilatation and degeneration. Tubular casts were identified in the cortex and the kidneys appeared encapsulated by fibrotic tissue. The kidneys of the mice that received the saline control appeared normal. Immunostaining of the kidney sections revealed that polymyxin B predominantly accumulated in the

Figure 1. Distribution of polymyxin B (PMB) within the mouse kidney visualized by in situ fluorescence imaging. The inset shows the cortex and medulla regions of the PMB-treated kidney image magnified ×10.
Distribution of polymyxins in the kidney

Renal cortex (Figure 1, see the polymyxin B treatment panel). More specifically, substantial accumulation was observed in the renal proximal tubular cells (Figure 1, see the polymyxin B treatment panel, magnified cortex region). Polymyxin B does not appear to accumulate in the distal tubular cells, as indicated by the low staining intensity of these cells (Figure 1, see the polymyxin B treatment panel, magnified cortex region). This finding is consistent with our preclinical and clinical pharmacokinetic data showing that polymyxins undergo significant renal reabsorption after filtration by glomeruli.\textsuperscript{5,13,14} The observed distribution of polymyxin B is also in line with our animal and cell culture studies, which suggested substantial accumulation of colistin and polymyxin B within renal proximal tubular cells and induction of apoptosis by activation of the death receptor, mitochondrial and endoplasmic reticulum pathways.\textsuperscript{13,15,16} Accumulation of polymyxins within renal tubular cells is the probable cause of the associated nephrotoxicity.\textsuperscript{5–6} Further studies on the distribution of polymyxins in proximal and distal tubular cells are being conducted.

In conclusion, this study provides valuable insights into the renal tissue distribution and nephrotoxicity of polymyxins, and also serves as a discovery platform for novel polymyxin lipopeptide development programmes.

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Transparency declarations
None to declare.

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