Comment on: Development and validation of a reversed-phase high-performance liquid chromatography assay for polymyxin B in human plasma

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Sir,

We read with great interest the article by Cao et al.¹ on the development of a reversed-phase HPLC assay for polymyxin B in human plasma. The research group from the Facility for Anti-Infective Drug Development and Innovation (FADDI) should be commended for their sustained contribution to understanding the pharmacology of polymyxins over the years. However, before this assay can be adopted widely for pharmacokinetic studies, several issues need to be discussed.

First, we corroborate with Cao et al.¹ that the prevalence of multidrug-resistant Gram-negative bacteria is on the rise and polymyxin B is increasingly used as the last viable therapeutic option. The pharmacokinetics of polymyxin B remains poorly understood; the most commonly used dosing regimens are often based on convention rather than being supported by pharmacokinetics and pharmacodynamics. Commercial formulations of polymyxin B contain multiple components. Current quality control procedures focus on overall antimicrobial activity, rather than on the proportion of each component. Therefore, manufacturing lot-to-lot variation in the composition of the polymyxin B components may exist. In addition, patients with multidrug-resistant bacterial infections are often given other antimicrobial agents concurrently. Consequently, we agree that using a micro-biological assay to detect polymyxin B in clinical samples may not be very satisfactory.

Our group has also been working on the pharmacokinetics and pharmacodynamics of polymyxin B over the years.²,³ The complete assay is still under development, but several key points have been learnt. Polymyxin B1 was found to be the major component among more than 30 other components (e.g. polymyxin B2, B3 etc.) present in the complex.⁴ Using our HPLC-MS assay, a typical chromatogram of polymyxin B (USP) detecting several of these components is shown in Figure 1. In contrast, the chromatography used by Cao et al.¹ (Figure 2 of the article) may not have the desired resolution to distinguish these closely related polymyxin B components. Without an optimal chromatographic separation, using mass-charge ratios to confirm the identity of various polymyxin B components may also be misleading. As shown in Figure 1, polymyxin B1 and B5 have an identical mass-charge ratio, as well as polymyxin B2 and B3.

Second, as analytical grade forms of each of these components are presently not commercially available, a relative method of quantification was used. In view of the possible lot-to-lot variations in the relative composition of various polymyxin B components, for the assay to be useful to quantify polymyxin B concentration in a patient, the same mixture of known total quantity (formulation batch) should be used as the reference standard. We are unsure if this is practical as: (i) different patients may be given different manufacturing lots of polymyxin B; and (ii) the same patient may also be given different manufacturing lots of polymyxin B during a course of therapy. As a result, multiple lots of reference standards may have to be used in a study.

Finally, it is unclear to us if each polymyxin B component has different antimicrobial activity, pharmacokinetic property and propensity for toxicity. Consequently, the validity of correlating the sums of polymyxin B1 and B2 peaks to ‘total polymyxin B’ may be questionable. By characterizing the pharmacokinetics of total polymyxin B concentrations over time, a more rapid clearance of one component could have been compensated by a more gradual clearance of its counterpart. Until the antimicrobial activity and pharmacokinetics of polymyxin B1 and B2 can be proved to be identical, it may be prudent to characterize them as separate entities.

With a better understanding of its pharmacokinetics, we are hopeful that the dosing regimens of polymyxin B could be designed more rationally in the future to improve patient outcomes and avoid toxicity. There is still much to be learnt about polymyxin B. It is our expectation that this communication will encourage clinicians and other investigators to examine these

Figure 1. A typical chromatogram of polymyxin B (USP) 100 µg/mL. Chromatographic separation was performed using an Alliance Waters 2695 separation module. Reversed-phase chromatography was carried out at ambient temperature using a Waters Symmetry C₁₈ column (2.1 mm × 75 mm, 3.5 µm) equipped with a Symmetry C₁₈ guard column (2.1 mm × 5 mm, 3.5 µm). A gradient elution procedure with a varying proportion of acetonitrile and 1% formic acid was used as the mobile phase. The flow rate of the mobile phase was sustained at 400 µL/min. The analytes were detected using a Waters EMD 1000 mass spectrometer (Milford, MA, USA) equipped with a Waters ESI multi-mode ionization source in positive ion mode. Optimal MS parameters are as follow: capillary voltage, 3.9 kV; cone voltage, 40 V; source temperature, 100°C; desolvation temperature, 400°C; desolvation gas flow rate, 300 L/h; cone gas flow rate, 25 L/h. The m/z of various polymyxin B components were monitored using SIM mode at 602.5 ([M + 2H]⁺⁺), 595.4 ([M + 2H]⁺⁺) and 610 ([M + 2H]⁺⁺), respectively.
Letters to the Editor

Development and validation of a reversed-phase high-performance liquid chromatography assay for polymyxin B in human plasma—authors’ response

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Sir,

We thank Tam et al.1 for their comments. This provides an opportunity to further clarify several important issues relating to the analysis of polymyxin B for pharmacokinetic and pharmacodynamic studies.

At the outset, we would make the general observation that polymyxin B is not alone in being a multi-component antibiotic. Indeed, a number of antibiotics comprise multiple components. For gentamicin, the analytical methods that have been used in the vast majority of studies to define the clinical pharmacokinetics and pharmacodynamics allowing optimization of dosing regimens, and also used for routine therapeutic drug monitoring, are assays that do not discriminate between the various components. Notwithstanding, it is important to consider the implications of the multi-component nature of an antibiotic when determining drug concentrations in biological fluids.

We do not believe that the pharmacokinetics of polymyxin B based on polymyxin B1 and B2 are misleading. It is clear that there are multiple components in polymyxin B, with polymyxin B1 and B2 as the two major components.1-4 Polymyxin B1 and B2 generally account for >85% of total polymyxin B,2,4 and the ratio of polymyxin B1 to B2 can vary from ~1.5 to 4 from batch to batch and product to product.2,5,6 Our recent unpublished data have demonstrated that colistin A (polymyxin E1) and B (polymyxin E2) have comparable MICs. Because there is only one amino acid difference between colistin and polymyxin B,7 it is very likely that polymyxin B1 and B2 have similar MICs (work in progress). Importantly, polymyxin B MICs in clinical laboratories are measured using a mixture, rather than polymyxin B1 alone. Therefore, if only polymyxin B1 concentration is measured without knowing the ratio of polymyxin B1 to B2 and the total proportion of these two major components in the product/batch administered, derived pharmacokinetic data based on quantification of polymyxin B1 alone7 can be very misleading in terms of pharmacokinetics/pharmacodynamics, particularly for a product/batch with a low ratio of polymyxin B1 to B2 (e.g. ratio of ~1.5). Thus, in chromatographic assays, we believe that it is a better and more accurate approach to use the summed peak area of polymyxin B1 and B2 for the quantification of polymyxin B in biological samples.

Tam et al.1 allude to the possible difference in pharmacokinetics between polymyxin B1 and B2 and suggest that it may be prudent to characterize them as separate entities (although in their recently reported clinical pharmacokinetic study, polymyxin B1 only was quantified following administration of polymyxin B1).1 In our clinical pharmacokinetic study,1 the ratios of chromatographic peak areas of polymyxin B1 to B2 in plasma were 3.97 ± 0.44 (unpublished results), and there was no apparent change over time during the dosing interval. Therefore, it is very likely that no major differences exist in key pharmacokinetic parameters between polymyxin B1 and B2, as suggested previously.7 The currently available data, therefore, would not support the comment by Tam et al.1 that a more rapid clearance of one component could have been compensated by a more gradual clearance of its counterpart. Further evaluation of the pharmacokinetics and pharmacodynamics of polymyxin B1 and B2 is currently being undertaken in our laboratory.

It should be noted that it is uncommon in pharmacokinetic studies that the product administered to patients is used as the analytical standard in the method for quantifying concentrations in biological fluids; indeed, it would be unwise to use this approach. There is no pure standard provided by the USP of each component of polymyxin B. In their clinical pharmacokinetic study in which polymyxin B1 was quantified in serum following administration of polymyxin B, Kwa et al.7 employed

References


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