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# Reply to "Measuring Polymyxin Uptake by Renal Tubular Cells: Is BODIPY-Polymyxin B an Appropriate Probe?"

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We read with interest the comments by Azad et al. (1) on our recently published article (2). They have raised a concern about the use of a BODIPY-polymyxin B (BODIPY-PMB) fluorescent conjugate for examining the uptake of polymyxin B into renal epithelial cells and questioned the suitability of this probe for our study. We agree with Azad et al. that some domains of BODIPY-PMB might be altered from those of the unconjugated polymyxin B molecule. However, we have no reasons to believe that these alterations would compromise our experimental design or affect the overall conclusion of our study.

It is well established that the binding of polymyxins with the lipopolysaccharide (LPS) of the bacterial outer membrane involves the interaction between polymyxin's free diaminobutyric acid (Dab) residues and the lipid A moiety of the LPS. However, this interaction pathway is exclusive to Gram-negative bacteria. Generally speaking, the mechanisms of drug uptake are not comparable between bacterial and renal epithelial cells (3). We agree with Azad et al. that conjugation of polymyxin B with a BODIPY fluorophore through the Dab residues might hinder its interaction with the bacterial lipopolysaccharide. Thus, BODIPY-PMB might not be suitable for conducting efficacy studies or for testing the penetration of polymyxin B into bacterial cells, which is, however, beyond the scope of our paper.

Our rationale for using BODIPY-PMB conjugate in our study was based on a careful review of the literature. BODIPY fluorophores are commonly used to generate various fluorescent drug conjugates to examine the uptake and transport dynamics of their native drug molecules into mammalian cells (4–6), including megalin-mediated uptake (7). Thus, we think that the use of BODIPY-PMB in our study was justifiable.

The overall objective of our study was to study the mechanism of polymyxin B uptake into renal cells. BODIPY-PMB was initially used as a screening tool in view of logistic convenience. Key experimental findings were further validated using unconjugated polymyxin B (USP); intracellular polymyxin B1 concentrations were assayed using a validated UPLC-MS/MS method (8). The results obtained using unconjugated polymyxin B were consistent with our findings from the experiments performed using BOPIPY-PMB. This enhanced our confidence in the appropriateness of the use of this probe in our study. Furthermore, our study conclusion was consistent with that of a recently published study examining the uptake of colistin in renal cells (9).

In conclusion, we believe that BODIPY-PMB was a reasonable screening probe for our experimental design. We also believe that the findings from this study have enhanced our knowledge of the mechanism of polymyxin B uptake into renal cells, which is crucial for understanding its mechanism of nephrotoxicity.

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