LETTER

REPLY TO NIKAIDO:

The pyruvate cycle provides respiratory energy and potentiates aminoglycosides to kill multidrug-resistant bacteria

Hui Li[a,b], Tian-tuo Zhang[c], Zhuang-gui Chen[d], Jin-zhou Ye[a], Song Zhang[a], Jun Yang[a], Xuan-xian Peng[a,1], and Bo Peng[a,b,1]

Nikaido (1) claims, contrary to our work (2), that the P cycle is not a general mechanism for energy production and that it does not sensitize bacteria toward aminoglycosides. However, the argumentation for both points is not convincing to us.

The P cycle is proposed from the stable-isotope–based nontargeted isotope fate detection analysis, in which we found labeled glutamate flux to glucogenesis and identified three additional steps—transforming oxaloacetate (OAA) to citrate through phosphoenolpyruvate (PEP), pyruvate, and acetyl-CoA—to finish the metabolic process. The finding was further supported by ^{13}C-labeled glucose in figure 7G of ref. 2. The PEP-pyruvate-OAA node, also referred to as the anaplerotic node, is a main way by which OAA is generated from glycolysis, whereby PEP and pyruvate are transformed to OAA through PEP carboxylase and OAA decarboxylase, respectively (3). The OAA-generating process is regulated by citrate synthase (4), a pacemaking enzyme (5). We further support, by enzyme kinetics (2) measured using recombinant proteins [but by crude extracts in the previous report (6)], that OAA prefers the P cycle to the TCA cycle. More importantly, we never exclude the critical role of citrate synthase in the P cycle, as shown in figures 1, 2, 5, 7, and 9 of ref. 2. However, the metabolic flow from OAA to citrate is strictly regulated by substrates; for example, the aminoglycoside-mediated killing efficacy is dependent on PEP and citrate concentrations (2). Lastly, we thank the author for pointing out the errors in the number of molecules of CO_2 in the equation and figure, and we will make the corrections accordingly.

The uptake of aminoglycoside is dependent on proton motive force (PMF) (7, 8). The P cycle plays a central role in providing PMF to revert aminoglycoside resistance. We performed the metabolite-enabled killing by antibiotics according to an established protocol, in which glucose, a metabolite being actively transported across the membrane, like these acidic compounds, was used (7, 9). We agree that the rates of metabolism and PMF are affected by the rate of transportation of the metabolites. That is why we used different concentrations of metabolites to exclude this possibility, which was indicated in the discussion (2). In corroborating our results of the P cycle in fighting clinic isolates, we have demonstrated that the inhibition of the P cycle would abrogate the synergistic effects of gentamicin or kanamycin and glutamate or fructose in killing *Escherichia coli* strains tgc6, mcc31, Y1, and Y17, as well as *Vibrio* isolates (figure 8C of ref. 2). In addition, the synergistic effect of glutamate and kanamycin was detected in killing *Edwardsiella tarda* EIB202 with a 43,703-bp conjugative plasmid harboring multidrug-resistant determinants (10) (figure S1 of ref. 2). We also provided evidence in figure S6G of ref. 2 that glutamate potentiates the gentamicin-mediated killing in vivo, which should be related to the synergistic effect of glutamate and glucose.

We thus do not agree with Nikaido's (1) claim regarding the role of the P cycle in providing energy and in reverting aminoglycoside resistance.

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[a]Center for Proteomics and Metabolomics, State Key Laboratory of Biocontrol, School of Life Sciences, Guangdong Province Key Laboratory for Pharmaceutical Functional Genes, School of Life Sciences, Sun Yat-sen University, University City, Guangzhou 510006, People's Republic of China; [b]Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, People's Republic of China; [c]Department of Respiratory Medicine, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, People's Republic of China; and [d]Department of Pediatrics, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, People's Republic of China

Author contributions: H.L., X.-x.P., and B.P. designed research; H.L., T.-t.Z., Z.-g.C., J.-z.Y., S.Z., and J.Y. analyzed data; and X.-x.P. and B.P. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. Email: pengb26@sysu.edu.cn or pxuanx@sysu.edu.cn.

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