Boosting Therapeutic Potential of *p*-Boronophenylalanine Using Biocompatible Polymers

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p-Boronophenylalanine (BPA) is the most powerful drug in the current clinical neutron capture therapy, because it can accumulate selectively within target tumor cells by internalizing into cells via LAT1 transporters, which are overexpressed on many tumor cells. However, therapeutic potential of BPA is sometimes compromised by rapid clearance from the tumor possibly due to the untoward exchange of extracellular amino acids and intracellular BPA through antiport mechanism of LAT1. To improve the tumor retention, in this study, we synthesized two kinds of biocompatible polymers: poly(vinyl alcohol) (PVA) and poly(ethylene glycol)-poly(L-lysine) conjugated with fructose (PEG-P[Lys/Lys(fructose)]). Because these polymers have polyol structures that can form boronate esters with boronic acid in BPA molecules, simple mixing of the polymers with BPA results in the polymer-BPA complex formation, maintaining phenylalanine structure that is critical for the recognition by LAT1. Thus, the polymer-BPA complex formation is expected to induce cellular internalization of BPA through LAT1-mediated endocytosis. The PVA-BPA and PEG-P[Lys/Lys(fructose)]-BPA complexes were indeed taken up into tumor cells through LAT1-mediated endocytosis and localized in the endo-/lysosomes while conventional fructose-BPA complexes accumulated within cytosol. Importantly the polymer-BPA complexes slowed the untoward efflux of intracellular boron, compared with fructose-BPA complexes. This prolonged intracellular retention might be explained by the altered subcellular localization of BPA. That is, BPA in the cytosol should be subject to the antiport of LAT1; however, the localization in the endo-/lysosomes may avoid this antiport mechanism. Even in *in vivo* experiment, both of PVA-BPA and PEG-P[Lys/Lys(fructose)]-BPA exhibited efficient tumor accumulation and longer tumor retention than fructose-BPA in subcutaneous CT26 and BxPC3 tumor models. Consistent with the improved tumor accumulation and retention, our polymer-BPA complexes accomplished significantly enhanced antitumor activity upon irradiation of thermal/epi-thermal neutrons. Although PVA-BPA exhibited the highest therapeutic efficacy, PEG-P[Lys/Lys(fructose)]-BPA showed the higher tumor/blood boron concentration ratio. The slightly cationic charge of PEG-P[Lys/Lys(fructose)]-BPA might facilitate the clearance from the bloodstream through glomerular filtration. These results indicate that the physicochemical properties of the polymers should critically affect the biodistribution and therapeutic potential of BPA.

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