Evaluation of a phenylboronic acid-decorated, sialic acid-targeting novel BNCT agent against B16 melanoma-bearing mouse model

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Introduction: The most fascinating advantage of the boron neutron capture therapy (BNCT) might be a target-specific tumoricidal effect on small-range nuclear fission, initiated by selective interactions between boronic agents and thermal neutrons. Nonetheless, most of the boronic agents currently available are not always reliable, mainly because of rapid systemic clearance and unwanted diffusion to non-target tissues, which are unavoidable drawbacks of using low molecular weight compounds such as boronophenylalanine (BPA) and sodium borocaptate (BSH). Because of these problems, the BPA and BSH should be given to the patients by hours of intravenous infusion even during the irradiation. Accordingly, boron concentration in the systemic circulation is unnecessary elevated during irradiation, narrowing down the therapeutic window of boronic agent. To this end, we report an actively targeted, boron-loaded polymeric nanoparticle as a novel boronic agent.

Materials and Methods: A phenylboronic acid (PBA)-installed diblock copolymer, PBA-polyethylene glycol (PEG)-b- poly(lactic acid) (PLA), was synthesized. The PBA end-group of this polymer was further protected by pinacol ester, resulted in PBA pinacol ester-PEG-b-PLA. These two polymers were separately dissolved in dimethylformamide, diluted by pure water, followed by purification by dialysis against pure water. Accordingly, two different PBA-decorated nanoparticles, either with or without pinacol protection (Pina-NP and PBA-NP, respectively), could be prepared. These nanoparticles were assessed by a series of in vitro and in vivo analyses. Briefly, B16-F10 mouse melanoma cells were subcutaneously inoculated to the C57BL6/j mice. When the average tumor volumes were reached around 50 mm3, the mice were randomly divided into four groups, subcutaneously injected with Pina-NP, PBA-NP, BPA-fructose, and vehicle (n = 6), followed by thermal neutron irradiation. Because of attenuated metabolism and gradual tumor accumulation of the nanoparticles, Pina-NP and PBA-NP injected groups were irradiated on 48 h post-injection, while the BPA-fructose and vehicle-injected group were irradiated on 2 h post-injection.

Results: The Pina-NP and PBA-NP were highly stable in physiological condition, as their average hydrodynamic diameters were sustained between 70-80 nm for at least 24 h in physiological condition. In vitro incubation of the hypersialyated cell lines with the fluorescently labeled nanoparticles clearly showed rapid localization of PBA-decorated nanoparticles on cellular

membranes, demonstrated cancer cell targeting effect. In vivo irradiation experiments validated the feasibility of Pina-NP, as it showed significant anti-tumor effects, which were comparable to the BPA-fructose. It is noteworthy that both of the nanoparticles were administered at a 100-folds lower effective dose than that of BPA-fructose. Contrary to the Pina-NP, the PBA-NP exhibited almost no effect, as the tumor growth profile was almost identical to the vehicle, and the tumor accumulation was significantly lower than Pina-NP and BPA-fructose. This is presumably because of non-specific associations with erythrocytes and circulating glycoproteins, which may accelerate the clearance through reticuloendothelial system.

Conclusion: We developed a tumor-targeting, PBA-decorated nanoparticle as a novel BNCT agent. Pinacol ester protection of the PBA groups may abrogate non-specific interactions between nanoparticles and serum sialic acids, gradually deprotected on prolonged circulation and efficiently reaching the tumors, resulted in highly potent antitumor effect on a mouse melanoma model.