Liver targeted Primaquine drugamers for the treatment of malaria in high risk settings

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Statement of purpose: Malaria still exists as a serious global health issues despite extensive measures to eradicate the disease and prevent the spread. This infection is caused by Plasmodium parasites. There were an estimated 216 million infections and a death toll of 445, 000 in 2016 (WHO). Primaquine (PQ), an 8-aminoquinoline antimalarial is widely used to treat both liver stage infection and blood parasites, and is the only FDA approved drug for the prevention of hypnozoite stage of *P. vivax* and *P. ovale* and stage V gametocyte of *P. falciparum*. However, the downside is that the 14-day dosing regimen necessitated by its short elimination half-life (3~6 h) poses a high risk for hemolytic anemia in individuals with genetic deficiency in glucose-6-phosphate dehydrogenase (G6PD), and this limits the therapeutic index of PQ in public health settings. To reduce the mass drug administration and associated toxicity, we have designed long acting controlled delivery materials (drugamers) to specifically deliver PQ to the liver hepatocytes and minimize blood PQ exposure levels.
Methods: RAFT polymerization was employed to prepare all the drugamers. *In vitro* release kinetics were monitored with HPLC. *In vivo* druagmer and PQ pharmacokinetic studies were performed using intravenous (I.V.) retro orbital injection at 5 mg/kg PQ dose, and blood and liver tissue were collected at 0.08, 0.5, 1, 4, 8, 24 and 48 h time points. Biodistribution of tritium labeled drugamer were measured with scintillation counter. PQ pharmacokinetics were evaluated by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Hemolytic toxicity evaluation was conducted with the G6PD deficiency humanized mouse model.
Results: Three different architectures linear copolymer, dioblock copolymer and radiant star nanoparticles (RSN) were synthesized from polymerizable PQ and GalNAc methacrylate monomers. The drug is covalently connected to the drugamer backbone via a hydrolyzable 4-hydroxybenzyl carbamate linkage that triggers drug release by esterase mediated hydrolysis followed by rapid 1,6-elimination. GalNAc moiety for receptor targeting is connected via a stable alkyl linkage. I*n vitro* hydrolysis profile in human serum at 37 ºC showed morphology dependent variations, 70 % PQ release for RSN over 48 h and a similar release for copolymer over 120 h. The diblock polymer where the hydrophobic PQ units are assembled in discreet hydrophobic blocks exhibited a much slower release profile, 14 % PQ over 120 h. Drugamer biodistribution profile showed drugamer accumulation in the liver out to 24 hours post injection and rapid blood clearance. PQ PK profiles evaluated with LC-MS/MS showed extended exposure time of PQ in the liver for both copolymer and RSN compared to free PQ. PQ exposure to the blood was very low for all drugamers. Hemolytic toxicity (figure 1) evaluated with G6PD deficiency humanized mouse model, which uses actual blood from G6PD deficient patients, showed that the copolymer didn't cause any toxicity at 5 mg/kg or 12.5 mg/kg in the Mediterranean variant model, even though similar PQ doses are usually toxic to human RBCs. In the African variant model, copolymer wasn't toxic at 12.5 or 25 mg/kg/day for three days, the RSN was also tested at 12.5 mg/kg/day for three days and found to be non-hemolytic. Both IV (10 mg/kg/day) and an equivalent oral (20 mg/kg/day) dosing of free PQ for three days resulted in hemolytic toxicity. The absence of toxicity is due to the fast clearance of drugamer form the blood to the liver before the drug is released, resulting in very low levels of PQ in the blood.

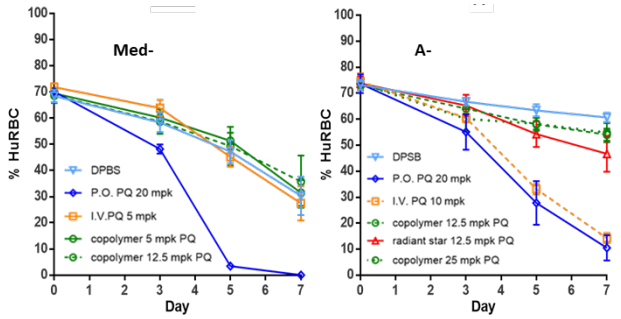

Figure 1. Hemolytic toxicity evaluation of copolymer and RSN in Med- and A- G6PD deficient mouse models.

Conclusions: Polymerizable prodrug PQ monomer carrying a hydrolytic linker and a liver targeting GalNAc monomer were successfully made and incorporated into three structurally different macromolecular drugamer constructs. Two materials - copolymer and RSN were selected and taken forward due to their drug release profiles fitting the project. *In vivo* PK profiles showed that the copolymer and RSN were quickly cleared from the blood and accumulated in the liver providing sustained delivery of PQ, a desirable property aimed to overcome the hemolytic toxicity. Toxicity studies conducted with two G6PD deficiency humanized mouse models – Mediterranean and African variants exhibited no hemolytic toxicity, often noted with free PQ administration. Absence of hemolytic toxicity combined with sustained release of PQ at the target liver site strongly demonstrate the GalNAc targeted PQ drugamer platform as a safe promising system with better therapeutic efficacy for treating malaria, particularly in high-risk G6PD deficient patients.