Quantification of Mucoadhesive Micelles Retention in Nasal Applications

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Statement of Purpose: Rapid clearance of therapeutics is a problem in many applications, including ophthalmic eye drops and nasal sprays, as it limits the bioavailability of the drug at the target tissues. Phenylboronic acid (PBA) has a strong affinity for mucin and has been used in biomedical applications. We have previously shown that novel, mucoadhesive self-assembling micelles could improve treatment efficacy by binding to mucin to extend the residence time of the therapeutics [1]. Additionally, radiolabeling a protein, molecule or polymer can provide accurate and precise quantification. The radioiodine labeling method has been well established for protein quantification through iodination of phenol groups of tyrosine within the protein structure [2]. Using a novel method to ^{125}I label the polymer, it was possible to quantify the increased residence time of the mucoadhesive polymer micelles in a nasal application *in vivo*.

Methods: Copolymers were synthesized by RAFT polymerization with and without PBA while incorporating a phenol monomer, N-(4-hydroxyphenethyl)acrylamide, whose synthesis is shown in Figure 1. Copolymer composition and molecular weight were determined using proton nuclear magnetic resonance. Micelles were formed by precipitation into PBS buffer from acetone. ICl method labeling (Figure 1) was conducted using PBS buffer, with the unreacted iodide removed by dialysis.

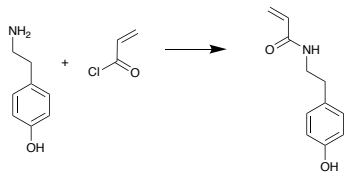

Figure 1: Synthesis of N-(4-hydroxyphenethyl)acrylamide.

^{125}I micelle formulations with and without PBA incorporated into the polymer were, dosed via intranasal administration to Brown Norway rats (n=6 per treatment arm). Doses were administered three times per day for 3 days. Animals were sacrificed either immediately after their last dose (0h) or 4 hours later (4h). The nose and most of the maxillary region of the rat were dissected for quantification. Gamma counting (Wizard 3 1480 Automatic Gamma Counter, Perkin-Elmer) was used to accurately quantify the amount of ^{125}I labeled polymer chains.

Results: Successful radiolabeling is determined by good radioactivity obtained with the materials, low free iodide content and high material recovery. This was achieved for the mucoadhesive polymer labeling. While ICI labeling is typically done in aqueous conditions, the low water solubility of the polymer necessitated a modified buffer of PBS with 10% (v/v) 1,4-dioxane to be used as a solvent. Acetone was added during dialysis to ensure polymer solubility to facilitate free iodide removal, which resulted in only 0.29-0.51% free iodide in the final product.

As seen in Figure 2, the radioactivity, and thus the amount of micelles, were approximately four times higher ($p=0.005$ and $p=0.068$ for 0h and 4h respectively) for the PBA-containing micelles. The radioactive counts in the lungs were negligible, indicating the micelles applied to the nostril via liquid suspension did not migrate deeply into the respiratory tract. This suggests greater binding to higher mucosal surfaces. These results support the hypothesis that micelles with mucin binding regions do in fact bind to the nasopharyngeal mucosa and remain at higher concentrations than traditional formulations.

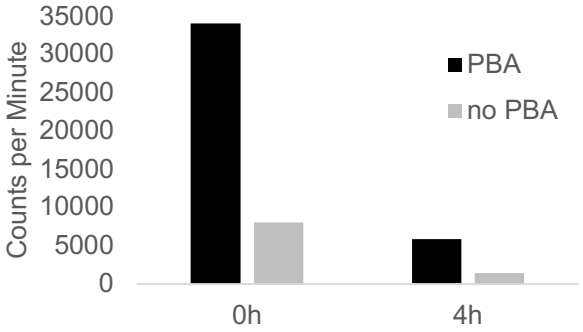

Figure 2: The relative counts per minute in nasal tissues after 10µl of I-125 labelled micelles with or without PBA was applied to each nostril of a Brown Norway rat.

References:

[1] Prosperi-Porta, G.. Biomacromolecules. 2016; 17: 1449-1457.
[2] Gopal B. Saha, Radiopharmaceuticals and methods of radiolabeling, Fundamentals of nuclear pharmacy, pp80-108, Springer-Verlag, New York, 1992