Decellularized Skeletal Muscle Extracellular Matrix as an *in vitro* Model for Intramuscular Drug Development

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Statement of Purpose: For certain therapeutics, intramuscular (IM) injection as a delivery route may be advantageous; it eliminates the first-pass effect of oral medications, is considered less dangerous and easier to use compared to intravenous (IV) administration, and absorbs faster than subcutaneous injections. However, the translation of drug candidates from *in vitro* affinity data to *in vivo* efficacy involves additional factors including absorption from the muscle mass into circulation. Delay in absorption could be affected by a variety of reasons including poor solubility, inability to cross biological membranes, and cellular interactions at the injection site. While many of these aspects can be examined *in vitro*, so far no model exists to investigate possible interactions of drug candidates with the extracellular matrix (ECM). New cyanide antidotes are being developed that can be given by IM injection, since this mode of administration would be preferable over IV injection for treating a large number of cyanide-poisoned patients. A promising candidate is cobinamide, a vitamin B_{12} analog. IV infusion of aquohydroxocobinamide (AH-Cbi) yields rapid and complete reversal of cyanide poisoning in animal models[1]. However, IM injection of AH-Cbi had little effect due to poor absorption. Adding sodium nitrite to AH-Cbi yielded dinitrocobinamide (NN-Cbi), which was rapidly absorbed, reaching 90% of peak concentration at 7.6 min post IM injection [manuscript in preparation]. The difference in absorption is hypothesized to be due to binding of AH-Cbi to skeletal muscle ECM. We therefore developed an *in vitro* model for IM drug development using decellularized skeletal muscle. With cobinamide as a proof of concept, we have demonstrated that decellularized skeletal muscle ECM can be used as an *in vitro* tool to assess differences in the absorption of IM delivered therapeutics.

Methods: Porcine skeletal muscle ECM was prepared as previously described[2]. After decellularization, 0.5 g of ECM pieces were placed in 24-well plates, and cobinamide solutions of known concentration were added (Fig 1). Dicyanocobinamide (CN-Cbi) was hypothesized not to interact with the ECM, and thus was used as a negative control. At various time points, samples of the solution were taken to measure cobinamide concentration using a NanoDrop 2000c Spectrophotometer (Thermo Scientific). Peak absorbance for AH-Cbi, NN-Cbi, and CN-Cbi were 346 nm, 349 nm, and 364 nm respectively.

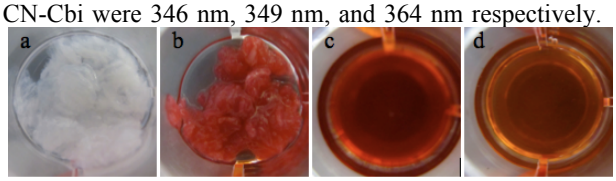

Figure 1. a) decellularized skeletal muscle ECM pieces, b) ECM pieces removed after 8 hours in c) 1 mM AH-Cbi solution, d) AH-Cbi solution after ECM is removed

ECM water content was determined by lyophilizing ECM pieces and calculating the change in weight attributed to water loss. Triton (0.5%), NaCl (1 kEq), and $NaNO_2$ (2 Eq) were added to determine the nature of the cobinamide-ECM interaction.

Results: After 4-8 h, less AH-Cbi remained in solution compared to NN-Cbi (Fig 2), indicating a greater degree of sequestration by skeletal muscle ECM. We found the CN-Cbi concentration to be 60-65% of the initial concentration, and hypothesize that loss of cobinamide is due to its distribution into the interstitial fluid of the highly hydrated ECM pieces. To account for this additional fluid, we determined the water content of ECM pieces to be 94%. After correcting for this effect, CN-Cbi is shown to interact minimally with ECM, and the difference between AH-Cbi and NN-Cbi is greater (Fig 2). Adding Triton or NaCl to the solution did not affect AH-Cbi or NN-Cbi binding to ECM, suggesting the interaction was neither hydrophobic nor ionic. However, adding $NaNO_2$ increased the concentration of NN-Cbi remaining in solution significantly (25.6% to 50.0%), indicating that NN-Cbi improves IM absorption by competition of the nitrite group with an ECM moiety.

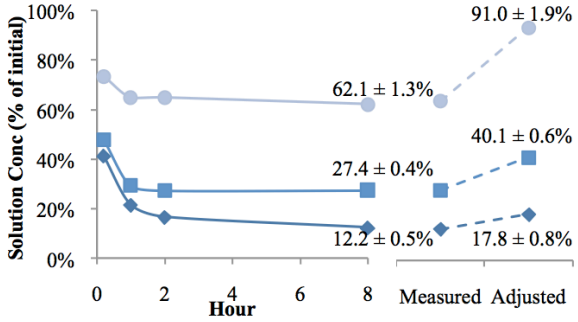

Figure 2. Concentration of AH-Cbi (◆), NN-Cbi (■), and CN-Cbi (●) when 1mL of 1mM solution is added to 0.5 g of skeletal muscle ECM after 12 min, 1 h, 2 h, and 8 h. Values are reported as a percentage of the initial concentration, where a lower percentage suggest stronger ECM interaction. Dashed lines show adjustments for ECM water content.

Conclusions: We have demonstrated that decellularized skeletal muscle ECM can be used as an *in vitro* model for interactions between a drug and ECM. Furthermore, we show that the nature of the interaction can be elucidated using compounds that alter binding, and our data indicate that cobinamide is binding to the ECM via the cobalt ion. We are currently studying the specific ECM component to which the binding occurs. This model could allow for improved formulation of drugs intended for IM injection.

References:

[1] Dequach JA. PloS ONE 2010;5(9):e13039.
[2] Brenner M. J Biomed Opt. 2010;15(1):017001.