Characterization of a Two-Phase Drug Delivery System to Treat Perthes Disease

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Introduction: Legg-Calvé-Perthes disease is an idiopathic osteonecrosis of the immature capital femoral epiphysis that affects about 1 out of 1200 children aged between 4 and 10 years [AAOS and NONF, 1996]. This disease not only causes clinical symptoms in childhood, but it also has lifelong implications, such as early osteoarthritis because of joint distortion and incongruity [*J. Bone Joint Surg.* 66:860, 1984]. The primary goal of treatment is to help the femoral head recover and grow to a normal. Current treatments include both nonsurgical and surgical approaches, which need either long recovery or secondary surgery.

In this study, an injectable drug delivery system was developed to expedite recovery and eliminate the need of surgery for Perthes patients. This system can be introduced directly into the femoral head and then sequentially release two drugs to prevent hip deformity and stimulate local bone formation.

Materials and Methods: Clodronate was chosen as the antiresorptive agent to halt collapse of the femoral head, and simvastatin was used as an osteogenic agent. Simvastatin was first hydrolyzed to the more soluble and active β-hydroxyacid form. Carboxymethyl cellulose (CMC) was used to control the flowability of whole system and deliver clodronate, while gelatin microspheres were used to delay release of simvastatin.

Gelatin microspheres were prepared using an emulsion process, crosslinked with glutaraldehyde, and lyophilized. Simvastatin was loaded by absorption into lyophilized microspheres. To alter the burst release, loaded microspheres were pre-washed by immersion in 10 ml phosphate-buffered saline, pH 7.4 (PBS) with mild agitation for 1 hour to remove freely absorbed simvastatin and lyophilized again.

Gelatin microspheres and clodronate solution were homogeneously mixed with 1-5% CMC solution, and 3 ml of mixture was injected into dialysis tubing (8 kDa cut-off). The 3ml volume contained 24mg of clodronate and 5mg of simvastatin. The tubing was immersed in 40 ml of PBS and incubated at 37°C with shaking. Supernatants were collected and replaced with fresh PBS every 24 hours for 4 weeks, except for 1 hour intervals during the first 4 hours. The concentration of simvastatin was determined based on its absorbance at 240 nm. Clodronate was assayed by mixing release supernatant with copper sulfate under acidic conditions to a form clodronate-copper complex, which also exhibited absorbance at 240 nm. Net absorbance of complex was determined after the contribution of simvastatin was deducted.

Results and Discussion: The effect of pre-washing on the burst release of simvastatin was evaluated as shown in Figure 1. The result showed less than 40% simvastatin was burst-released from the pre-washed gelatin microspheres during first 24 hours, compared to the 56% burst release from the unwashed particles.

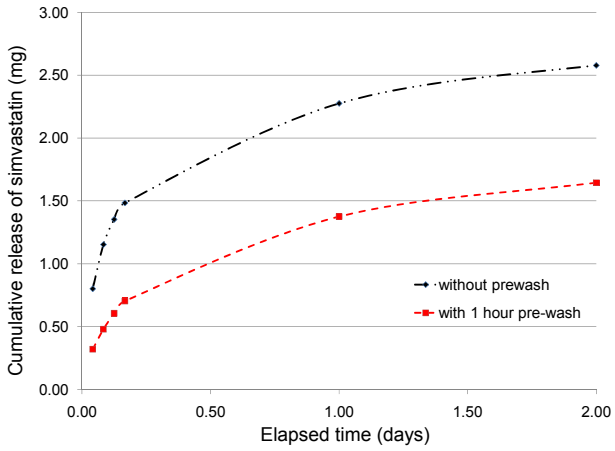

Figure 1. Effect of 1 hr prewash on release of simvastatin.

The release profiles for both clodronate and simvastatin from 10mM crosslinked gelatin microspheres were obtained as shown in Figure 2. Clodronate was quickly released within the first 24 hours, while 45% of simvastatin was burst-released during first 24 hours. After the burst release, a steady release of simvastatin was observed up to 2 weeks.

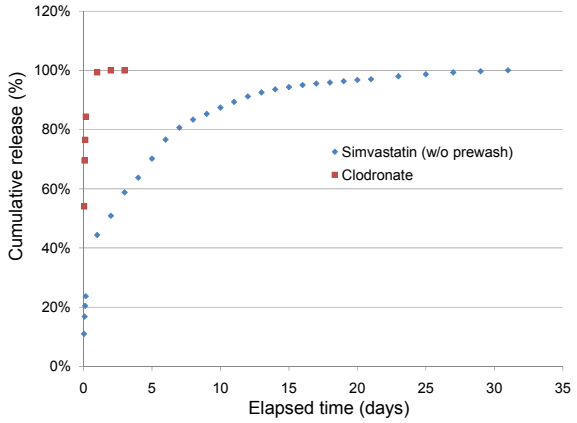

Figure 2. Release profiles of the two component system.

Conclusions: An injectable drug delivery system for treating Perthes disease was developed, and release profiles for two drugs were characterized. A sequential release pattern was observed. Such an approach will be useful if non-overlapping release of two drugs is desired. Based on these findings, bioactivity of the system is being investigated.

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