INTRODUCTION:
Delivery of gene vectors to local muscle or heart tissue has been achieved by direct intramuscular (i.m.) injection or by local blood vessel perfusion of viral vectors, particularly the AAV vectors. However, a major challenge is how to deliver the therapeutic genes into most, if not all, of the diseased muscles. Previous efforts to deliver genes into multiple muscles have relied on isolated vessel perfusion or pharmacological interventions to enforce broad vector spread. In the current study, we have compared a number of AAV serotype vectors (AAV1, 2, 5, 6, 7, and 8) for their ability to deliver genes systemically into muscle and heart without additional interventions. We showed that AAV8 is the most efficient vector for crossing the blood vessel barrier to attain systemic gene delivery to skeletal muscles and cardiac muscles.

MATERIALS AND METHODS:
AAV vectors were generated by triple plasmid transfection of 293 cells. Viruses were purified with CsCl gradient ultracentrifugation. The titers of vector-genome (v.g.) particles were determined by a standard dot-blot assay. All of the AAV vectors contained an identical, double-stranded vector DNA cassette that harbored a green fluorescent protein (GFP) gene driven by a constitutive CB promoter. The GFP expression in mice was examined by whole-body imaging under the illumination with a handheld long wavelength UV lamp. Cryo-sections of various tissues were then prepared for microscopic examination. Analysis of viral genomes in tissues was performed by southern blotting.

RESULTS:
1. Intraperitoneal and intravenous injection of various AAVs in neonatal mice reveals diverse efficiencies in systemic muscle gene transfer. By injecting various serotypes of AAV-GFP vectors into the peritoneal cavity of neonatal mice, we surprisingly observed that AAV1, 6, 7, and 8 led to efficient muscle transduction beyond the abdominal wall and yielded widespread and strong GFP expression in the abdominal wall, chest, and hind leg muscle (Figure 1). In addition, AAV8-treated mice showed widespread GFP expression in muscles of remote sites (facial muscles, the forelimb and shoulder muscles, and cardiac muscle). AAV7 had a similar, but slightly weaker, profile. On the other hand, lower levels of fluorescence were observed in the AAV5-treated mice, and the AAV2-treated mice displayed fluorescence mainly in the abdominal muscles and localized on the vector injection side. These results suggest that AAV8 is the most efficient vector in disseminating into the vast majority of the muscles, both skeletal and cardiac. Time course experiments on the AAV8 vector showed that significant GFP gene expression started as early as 3 days post-lp. injection, and increased and persisted to adulthood in the vast majority of the muscles throughout the body (Figure 2). We next investigated intravenous delivery in neonatal mice. Again, AAV8 demonstrated the most efficient and persistent systemic transduction in both skeletal and cardiac muscles in all the AAV serotypes tested. Interestingly, examination of non-muscle tissues showed that GFP-positive cells were essentially undetectable in brain, spleen, gonad glands (testes and ovary), or smooth muscle of the intestine and blood vessels at 2 months after vector delivery into neonatal mice both i.v. and i.p. route) with all six serotypes. Only a few sporadic positive green cells were detected in the lungs and kidneys of some mice. Less than 1% of the hepatocytes remained GFP-positive in the liver even after AAV8 treatment, which was reportedly the most efficient in the liver. Southern blot analysis of viral genomes in tissues revealed that this phenomenon was mainly due to vector instability in rapidly dividing cells, the vector DNA was rapidly degraded during cell growth and division. On the other hand, in tissues that undergo minimal cell division, such as heart and muscle, the vector DNA persisted (Figure 3).

2. Systemic muscle gene transfer via intravenous route in adult mice. We then investigated if intravenous injection of AAV8 in adult mice could also achieve systemic gene delivery to muscle and heart. Fluorescence microscopy demonstrated efficient GFP expression in cross sections of various skeletal muscles and heart, although the efficiency was lower in adult mice than in the neonatal mice. However, a striking difference between adult and neonatal delivery of AAV8 was the detection of GFP expression in non-muscle tissues, such as in the liver, pancreas, testes, interstitial tissues, and kidney, indicating that muscle-specific promoter is necessary to avoid unwanted transgene expression in nonmuscle tissues.

3. Superior permeability to vasculature by AAV8 vector over other serotypes might contribute to the systemic transductions to skeletal and cardiac muscle fibers. The above results strongly suggest AAV8 is highly effective at crossing the blood vessel barrier in muscle tissues. To directly test this hypothesis, we perfused AAV vectors into isolated hind limbs without high pressure via blood vessels in adult mice. As expected, AAV8-perfused mice demonstrated much stronger GFP expression than mice perfused with other AAV serotypes.

DISCUSSION:
Here we demonstrated that AAV8 could efficiently overcome the physical and biological barrier imposed by the capillary blood vessels and achieved systemic muscle gene delivery. We attribute the superiority of systemic gene delivery by the AAV8 to its capability to cross the blood vessel barrier. But potential mechanisms such as trans-cytosis across the endothelial cells remain to be further investigated. Another interesting mechanism observed in this study is the selective retention of vector DNA and transgene expression in muscle and heart cells after systemic gene delivery in neonatal mice. These findings suggest that, systemic gene delivery in neonates may be a viable therapeutic option, particularly for childhood diseases with high mortality and morbidity that require early intervention.

References: