Gene transfer of arginine kinase to skeletal muscle using adeno-associated virus

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Introduction

Creatine kinase (CK) catalyzes the rapid conversion of ADP to ATP in vertebrates at the cost of phosphocreatine (PCr), and arginine kinase (AK) is the functionally equivalent enzyme in invertebrates with phosphoarginine (PArg) as the phosphate donor. Viral-mediated gene transfer of AK into mammalian muscle results in the production of PArg, which can be detected using ^{31}P-MRS (Walter et al. 2000). The gene delivery of AK is ideally suited to act as a reporter gene as it provides a unique signal against a mammalian background, its coding sequence is small enough to be expressed with therapeutic genes, and it does not interfere with normal tissue function. In this study we tested the feasibility of noninvasively measuring the effect of a commonly used nonpathogenic adeno-associated virus (AAV) delivery system to transfer the AK gene to the mouse hindlimb. This was achieved by evaluating the time course and regional distribution of PArg with ^{31}P-MRS.

Methods

Replication deficient adeno-associated virus encoding for the AK gene were injected into the gastrocnemius of the left hindlimb of control C57Bl10 mice (age 5wk, male) using self-complementary AAV, type 2/8 with desmin promoter. ^{31}P-MRS data were acquired (TR 2 s, 256 NSA, 2048 data points, 8k sweep width) using a 11.1 T 40 cm horizontal bore Bruker Avance spectrometer (Paravision V3.02) with an oblong transmit/receive surface coil (6 mm X 12 mm) centered on the posterior region of the lower hindlimb. ^{31}P data were collected at weekly intervals for the initial four weeks after gene delivery, then at 2-4 week intervals up to 16 weeks. In addition, at 16 weeks after gene transfer, ^{31}P 2-D chemical shift imaging (CSI) was acquired (8X8 matrix, 10-15 mm FOV, 3 mm slice thickness, TR 1 s, 32 NSA) to examine the spatial distribution of PArg. The relative concentration of Pi, PCr, PArg, and γ-, α-, and β-ATP were estimated using the AMARES fitting algorithm of jMRUI (version 4.0). Localized ^{31}P spectra were zero-filled to 16X16 and analyzed using 3DiCSI software (Version 1.9.7) (Zhao et al. 2005).

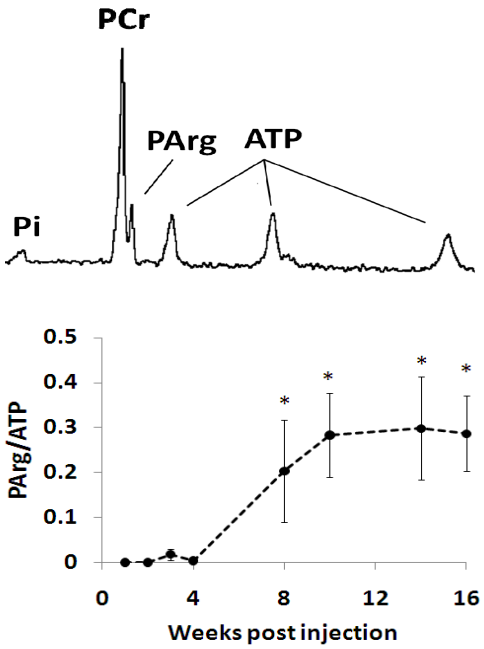

Fig. 1 Example non-localized ^{31}P spectrum after 8 weeks of injection (top) and time course of PArg changes in hindlimb (bottom).*Significantly different than baseline.

Results

The presence of PArg was evident as a distinct peak in non-localized spectra in each injected mouse hindlimb after 8 weeks of gene delivery (PArg/ATP range: 0.1-0.5; Fig 1), and remained elevated for at least 16 weeks (Fig.1). Furthermore, using localized ^{31}P-MRS, PArg was primarily localized to the injected posterior hindimb region, and was not evident in deeper regions of the lower leg (Fig. 2) or in the contralateral limb.

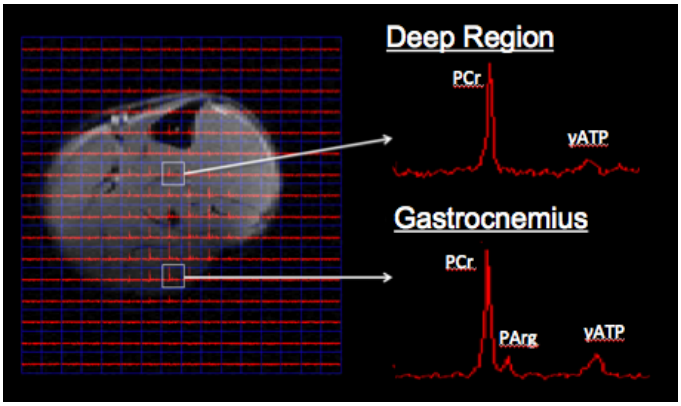

Fig. 2 PArg was evident in the gastrocnemius muscle (bottom right), but not in other regions of the leg, such as in the deeper leg muscles (top right).

Conclusions

The results of this study showed the viability of AAV gene transfer of AK gene to skeletal muscle of mice. This method may be effective in providing a gene reporter to noninvasively monitor the delivery of genes for therapeutic interventions.

References

Walter G, Barton ER & Sweeney HL. Proc Natl Acad Sci USA 97 (10), 5151-5155, 2000.
Zhao Q, Patriotis P, Arias-Mendoza F, Stoyanova R & Brown TR. Proc Int Soc Mag Reson Med 13, 2465, 2005.