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Gene Therapy Approaches to Ventricular Tachyarrhythmias

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Abstract

Ventricular tachycardia arising from a healed myocardial infarction scar continues to be a significant cause of morbidity and mortality. Drug therapy has been inadequate to meet this challenge, and implantable devices are limited by expense and technical problems. We have proposed the use of gene therapy for cardiac arrhythmias. In this review, we present a model of post-infarct ventricular tachycardia, a method for gene delivery to this area, and results KCNH2-G628S gene transfer to manipulate cellular refractory properties in the arrhythmia model.

Despite improvements in the diagnosis and management of cardiac diseases, cardiac arrest remains the most common cause of death in developed countries. In cases where the exact cause of death can be ascertained, the most common precipitant of cardiac arrest is a ventricular tachyarrhythmia (either ventricular tachycardia (VT) or ventricular fibrillation (VF)), and the nidus for this arrhythmia is generally the border region of a healed myocardial infarction (MI) scar.¹ Currently, the only safe and reliable therapy for ventricular arrhythmias is the implantable cardioverter-defibrillator, but a variety of factors limit suitability of defibrillators as first-line therapy for cardiac arrest.²⁻⁴ We have proposed development of gene therapy as an alternative strategy to treat ventricular arrhythmias. In this review, we will discuss a new porcine model of MI scar-related VT, the mechanics of gene delivery to the porcine ventricle, and results of a study evaluating a novel gene therapeutic.

Porcine model of scar-related ventricular tachycardia

With the principle goal of creating a reliable model to evaluate VT therapies, we evaluated several experimental myocardial infarction methods: coronary endothelial damage, microsphere infusion, clot or activated thrombin infusion, and balloon occlusion. We considered the following attributes to develop an appropriate animal model of MI/VT: consistency of infarct creation, reliability of VT induction, and patency of the infarct-related artery for later gene transfer. We focused exclusively on large animal models with anatomical similarity to humans for eventual translation of the results into clinical practice. We found that balloon-occlusion of the mid-left anterior descending coronary artery (LAD) in pigs achieved all of our objectives. In a pilot study of 7 animals, we created MI by occluding the LAD immediately distal to the second diagonal branch for 150 minutes. Shorter occlusion time or more distal balloon placement did not give consistent VT inducibility. Longer occlusion did not improve the results, and more proximal balloon placement tended to cause hemodynamic instability. Prior to infarction, the animals were evaluated with echocardiography and invasive

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electrophysiology study (EPS). After the infarction, the animals were evaluated weekly by echocardiography, ECG and non-invasive EPS. After 4 weeks, the animals underwent repeat invasive EPS with basket catheter mapping during sinus rhythm and VT. Following EPS, the animals were sacrificed, and the infarcted hearts were evaluated grossly with triphenyltetrazolium chloride (TTC) staining and microscopically with Hematoxylin and eosin staining. We have published a thorough description of these methods.⁵

Prior to infarction, all animals were essentially normal. Left ventricular ejection fraction (LVEF) was $68 \pm 3\%$; LV diameter was 2.7 ± 0.1 cm, and no animal had sustained monomorphic VT induced at EPS. After infarction, The LVEF decreased progressively over the first 3 weeks before stabilizing at $42 \pm 2\%$, suggesting both an initial insult from the infarction but also short-term remodelling that further decreased LVEF. There was little dilation of the LV in the short term; LV diameter was 2.9 ± 0.2 cm 2-4 weeks after MI. VT inducibility during EP study progressively increased: 0% at week 1, 57% at week 2 and 100% at weeks 3 and 4. For each animal with inducible VT, the arrhythmia was consistent in morphology and cycle length from one week to the next (Figure 1A). Intracardiac basket catheter electrograms in all animals demonstrated fractionated, low amplitude signals in the anterior septum during sinus rhythm, and progressive activation of the same region throughout diastole during VT (Figure 1B), suggesting that the VT circuit was centered in the anterior-septum for all animals. On post-mortem examination, the VT circuit appeared to coincide with the basal anterior-septal scar border identified by TTC staining. Microscopic study of tissue sections in this area revealed surviving strands of myocardium interwoven with fibrotic scar (Figure 1C), suggesting possible pathways for conduction of VT.

Gene delivery to the porcine ventricular myocardium

A prerequisite to gene therapy for scar-related VT is the development of a successful gene delivery method that targets ventricular myocardium. This issue can be interpreted in 2 ways: what is the best vector, and what is the best delivery method. For the time being, virus vectors appear to be the most efficient at transferring genetic material to mature, post-mitotic cells. A full review of virus vectors is not possible here. Myocardial gene transfer has been shown effective in a few reports with lentivirus vectors, and in single reports with a variety of other viruses. None have reached the level of success found with adenovirus (AD) and adeno-associated virus (AAV) vectors. AD are easy to create and amplify, so they are used most commonly for short-term applications. The principle weaknesses of AD vectors are that they incite strong immune responses, and they only induce gene expression for 2-6 weeks after gene transfer. As such, they are not useful for long-term study. AAV are more cumbersome to amplify than AD. They are usually reserved for situations where short-term efficacy is established and long-term results are needed. Several reports have confirmed that AAV vectors are capable of inducing long-term gene expression in multiple organ systems and animal models.⁶⁻⁸

For delivery of the gene transfer vector to the ventricular myocardium, currently available methods include injection directly into the myocardium or perfusion through the coronary arteries. Injection methods are capable of dense, very localized gene transfer,⁹ but the area of gene expression typically does not extend more than 1-2 mm beyond the path of the injecting needle.¹⁰ In situations where the target location is small and precisely known, injection methods are very effective, although there is some suggestion that damage from the injection can cause localized inflammation and fibrosis. In situations where the target is not exactly known, efficacy of gene transfer (or lack thereof) may not become apparent until gene expression occurs, days to weeks after the delivery procedure. Since the size and location of the VT circuit is often not definable with an accuracy of 1-2 mm using clinical tools, we favor coronary perfusion methods for gene delivery (please see references on efficacy of VT ablation

to understand the precision in clinical localization of VT circuits).^{11;12} Perfusion is a much less efficient gene delivery method than injection, but perfusion allows gene transfer over a broader area.

Development of intracoronary perfusion gene transfer methods has been a focus of our group for an extended period of time. We originally began these studies in isolated ventricular myocytes and *ex vivo*-perfused intact rabbit hearts in order to develop an understanding of the basic physiological variables relevant to the gene transfer process. From these studies we developed a general picture that gene transfer by intracoronary perfusion required overcoming three barriers: broad distribution at the local capillary-myocardial layer, efficient transit across the microvascular endothelial barrier, and occupation of the interstitial space long enough to allow attachment of viruses to receptors on the myocytes.¹³⁻¹⁵ For study of gene delivery methods, we and others typically use recombinant adenoviruses encoding reporter genes to allow quantification of the location and extent of gene transfer. The virus that we most commonly use for this purpose is Ad β gal, an E1, E3-deleted recombinant adenovirus encoding the *Escherichia coli lac Z* gene. The distribution and percentage of myocytes expressing *lac Z* can be quantified by X-gal staining, which colors *lac Z*-expressing cells blue. The amount of *lac Z* expressed within tissues can be quantified using a variety of biochemical or immunological methods.

Our most recent work in the area of gene delivery has focused on testing our *in vitro* and *ex vivo* results in the *in vivo* environment, using intracoronary perfusion of the LAD in pigs.¹⁶ Our starting point for these experiments was chosen to have a mid-range level of gene transfer, so that any increase or decrease in gene transfer efficiency could be easily distinguished from the baseline. To achieve this, we chose mid-range levels of the factors we had previously found to improve *ex vivo* gene transfer. Pretreatment included oral administration of a phosphodiesterate-5 inhibitor and intracoronary infusion of vascular endothelial growth factor (VEGF), nitroglycerin (TNG), and adenosine in 1 mM calcium Krebs's solution. After pretreatment, 5×10^9 pfu/ml AD was delivered in Krebs's solution containing the same concentrations of adenosine, calcium, and TNG as the pretreatment. We compared animals receiving Ad β gal to animals receiving control AD, and quantified gene transfer using conventional *lac Z* detection with X-gal staining. With these methods, our starting conditions led to a gene transfer level of $34 \pm 3\%$ of cardiac myocytes in the anterior septum and anterior right and left ventricles.

We found that microvascular permeability manipulations were essentially maximized with the baseline conditions. Reduction of an individual component in the pretreatment cocktail (adenosine, VEGF or TNG) reduced the gene transfer rate, but increasing any of these factors did not significantly improve gene transfer. Decreasing perfusate calcium concentration had an additive effect similar to the one observed *ex vivo*. Compared to the baseline calcium concentration of 1 mM, a significant improvement in gene transfer was achieved by reducing calcium concentration to 0.05 mM ($46 \pm 3\%$, $p=0.02$).

Manipulation of physical factors appeared to have the greatest effect for the *in vivo* environment. In particular, participation of the venous system in virus delivery dramatically increased transgene efficiency. Virus infusion through the great cardiac vein (GCV) achieved significantly higher levels of gene transfer than LAD infusion ($64 \pm 4\%$, $p<0.001$). Delivery through LAD with occlusion of the GCV showed a trend toward increased transgene expression ($45 \pm 4\%$, $p=0.13$). Simultaneous infusion through LAD and GCV exhibited additive effects. The transgene efficiency was $78 \pm 6\%$ ($p<0.001$), achieving our goal of high density, homogeneous gene transfer to the target area. Currently, we find that the best conditions for intracoronary perfusion include pretreatment with Cialis 5 mg 2-10 hours before gene transfer. Infusion of adenosine 5 mg/ml, TNG 250 μ g/ml and VEGF 0.5 μ g/ml in 0.05 mM calcium-

Krebs' solution simultaneously through the coronary artery and vein for 3 minutes and then infusion of the gene transfer vector in the same solution (without VEGF) for an additional 2 minutes.

Using the LAD/GCV simultaneous perfusion technique, we evaluated gene transfer in the MI/VT model. In 5 animals we created an infarct in the mid-LAD territory using the above protocol. Three weeks after infarction, we simultaneously perfused the mid-LAD and the GCV with Ad β gal after pre-treatment with adenosine, VEGF and TNG. The mid-LAD positioning of the catheter approximated the position of the balloon catheter that created the MI. Quantification of gene transfer showed that $45 \pm 7\%$ of cardiac myocytes in the target region expressed the *lac Z* reporter gene. For all of these studies (baseline and MI model), there was no *lac Z* expression in the hearts of control animals and no *lac Z* expression outside the target region in the Ad β gal animals.

Gene therapy for scar-related ventricular tachycardia

Using the LAD/GCV gene delivery method, we evaluated VT gene therapy in 15 animals with MI and reproducibly inducible VT. Five animals receiving AD encoding KCNH2-G628S (G628S animals) were compared to 5 animals receiving Ad β gal for infected controls (β gal animals) and 5 animals without gene transfer for uninfected controls (No GTx animals). KCNH2-G628S is a dominant negative mutation in the α subunit for I_{Kr} ,¹⁷ so expression of this gene should reduce or eliminate I_{Kr} in affected cells. One week after gene transfer, all of the G628S animals no longer had any sustained ventricular arrhythmias inducible on EP study. All of the *lacZ* and uninfected control animals continued to have reproducibly inducible VT on follow-up study (Figure 2). To verify gene transfer, we measured total KCNH2 mRNA in all animals. The G628S animals had a 2.7 ± 0.4 -fold ($p = 0.05$) increase in KCNH2 mRNA in the mid-anterior septum and a 2.0 ± 0.7 fold ($p = 0.1$) increase in the distal anterior septum relative to the levels in the control animals. KCNH2 expression outside the target region was not increased in the G628S animals relative to controls.

We evaluated functional effects of gene transfer using basket catheter, monophasic action potential (MAP) catheter and patch clamp analysis. Basket catheter recordings were unchanged in comparison to pre-gene transfer recordings: all animals had continued low amplitude, fractionated electrical activation of the infarct border zone during sinus rhythm, suggesting that gene transfer did not disrupt conduction at sinus heart rates. MAP catheter recordings changed significantly for the G628S animals. Both action potential duration (APD) and effective refractory period (ERP) increased in the anterior septum but not in other regions of the heart relative to pre-gene transfer measurements (increase in APD: mid-anterior septum 34 ± 7 ms, $p = 0.04$, distal anterior septum 44 ± 8 ms, $p = 0.04$; increase in ERP: mid-anterior septum 36 ± 9 , $p = 0.04$, distal anterior septum 44 ± 6 , $p = 0.02$). The control animals had no significant changes in either APD or ERP relative to pre-gene transfer measurements. Patch clamping analysis of cardiac myocytes isolated from the gene transfer region corroborated the MAP data; APD was significantly longer in the G628S-expressing cells (G628S: 937 ± 117 ms, control: 592 ± 69 ms, $p < 001$).

The primary safety concern for any new arrhythmia treatment is the possibility of proarrhythmic effects. In particular, for this gene transfer strategy, we looked for evidence of proarrhythmia from heterogeneous expression in the target region, from triggered activity caused by AP prolongation in the transduced cells, or from global myocardial expression essentially causing the acquired Long QT Syndrome. We implanted defibrillators to monitor and potentially treat any spontaneous arrhythmias but found none in any group. We looked for ventricular ectopic activity during daily evaluation of the animals, with the idea that frequent premature ventricular contractions might be a sign of triggered activity from the transduced

cells. There were only rare premature ventricular contractions, which did not differ in frequency between groups. We compared 12 lead ECG measurements of QT duration in G628S to control animals and found no change with gene transfer (G628S pre- 397 ± 5 ms, post- 409 ± 3 ms, $p = \text{NS}$; controls pre- 399 ± 6 ms, post- 402 ± 12 ms, $p = \text{NS}$). During EPS, we looked for arrhythmias inducible with burst ventricular pacing (which can elicit triggered activity), and we found none in any group. We also looked for evidence of afterdepolarizations during MAP recording *in vivo* and during current clamp of isolated cells. We did not observe any afterdepolarizations in either situation. Finally, we looked for non-target gene expression using quantitative polymerase chain reaction analysis as noted above, and we did not see any evidence of transgene expression outside the anterior septal peri-infarct target region.

Conclusions

The development of new therapies for ventricular arrhythmias has stagnated under the weight of studies showing proarrhythmia and increased mortality from antiarrhythmic drug use and improved mortality after defibrillator implantation for virtually anyone with a LVEF less than 35%.¹⁸⁻²² The need for new options has been amplified by recent defibrillator malfunctions and recalls and by the realization of the staggering costs accompanying the decision to implant defibrillators in everyone with reduced LVEF.²⁻⁴ Our data suggest an alternative. We show complete elimination of ventricular arrhythmia inducibility with focally targeted KCNH2-G628S gene transfer. The number and time course of observations is small, but these data motivate further study. In addition to previous studies of gene transfer for AV node modification and biological pacing, these results firmly establish the concept that gene transfer has utility for treatment of cardiac arrhythmias.²³⁻²⁵ The current results suggest that malignant ventricular arrhythmias can be effectively treated with this technology.

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Figure 1.

Ventricular tachycardia in the porcine myocardial infarction model. **A.** ECG example showing identical VT morphology and cycle length from one week to the next in a representative animal.

B. Intracardiac electrograms during sinus rhythm (SR) show low amplitude, fractionated signals in the anterior septal region (splines D and E). The ventricular tachycardia recording demonstrates slow, progressive activation of this region through diastole. **C.** Hematoxylin-Eosin stained microsection of the infarct border zone reveals surviving strands of myocardium surrounded by fibrotic scar in the anterior septum. (with permission from Sasano et al. *Nat Med*)⁵

a **b** **Figure 2.**

KCNH2-G628S gene transfer in the MI-VT model. **A.** Prior to gene transfer, VT was repeatedly inducible in all animals. After gene transfer, no arrhythmias could be induced in any **KCNH2-G628S** infected animal, but all control animals remained inducible. **B.** Example of triple extra stimuli in a representative G628S animal. The top tracing shows VT induced with triple extra stimuli immediately before gene transfer. The bottom two panels show triple extra stimuli one week after gene transfer. The middle panel shows the tightest possible coupling of S2-4 where all 3 extra stimuli capture the ventricles (which is tighter than the intervals that consistently produced VT before gene transfer). The bottom panel shows refractory stimulation. After progressive tightening of S2-4 coupling intervals, extra stimuli are now so tightly coupled that the ventricles are refractory to stimulation. (with permission from Sasano et al. *Nat Med*)⁵