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Transgenic swine: expression of human CD39 protects against myocardial injury

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Abstract

CD39 (Ectonucleoside Triphosphate Diphosphohydrolase-1; ENTPD-1) rapidly hydrolyzes ATP and ADP to AMP; AMP is hydrolyzed by ecto-5'-nucleotidase (CD73) to adenosine, an anti-thrombotic and cardiovascular protective mediator. While expression of human CD39 in a murine model of myocardial ischemia/reperfusion (I/R) injury confers cardiac protection, the translational therapeutic potential of these findings require further testing in a large animal model. To determine if transgenic expression of CD39 reduces infarct size in a swine model of myocardial ischemia/reperfusion injury. Transgenic pigs expressing human CD39 (hCD39) were generated via somatic cell nuclear transfer and characterized. Expression of hCD39 in cardiac tissue was confirmed by immunoblot and immunohistochemistry. Myocardial I/R injury was induced by intracoronary balloon inflation in the left anterior descending (LAD) artery for 60 min followed by three hours of reperfusion. The ischemic area was delineated by perfusion with 5% Phthalo Blue and the myocardial infarct size was determined by triphenyl tetrazolium chloride (TTC) staining. During ischemia, the rate-pressure product was significantly lower in control versus hCD39-Tg swine. Following reperfusion, compared to littermate control swine, hCD39-Tg animals displayed a significant reduction in infarct size (hCD39-Tg: 17.2 ± 4.3 % vs. Control: 44.7 ± 5.2 %, $P=0.0025$). Our findings demonstrate for the first time that the findings in transgenic mouse models translate to large animal transgenic models and validate the potential to translate CD39 into the clinical arena to attenuate human myocardial ischemia/reperfusion injury.

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Disclosures

None

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Keywords

transgenic pig; ectonucleoside triphosphate diphosphohydrolase 1; CD39; myocardial ischemia; reperfusion

1. Introduction

Myocardial infarction (MI) affects over 1 million people, resulting in over 300,000 deaths and heart failure in an additional 300,000 patients every year in the United States [1]. While numerous strategies have demonstrated efficacy in rodent models of myocardial injury, most have failed to demonstrate clinical efficacy. Indeed, the mouse cardiovascular physiology differs tremendously from human; the mouse heart beats at 600 beats-per-minute with a cardiac output of 20 mL/min while the human heart beats at 60 beats-per-minute with a cardiac output of 5 L/min [2]. Therefore how do we begin to translate findings in murine models to human?

Purine nucleotides and nucleosides influence nearly every aspect of cardiac physiology and pathophysiology including myocardial ischemia. Extracellular nucleotides (ATP and ADP) released at the site of cellular damage can induce platelet-mediated thrombosis, inflammation cell apoptosis and necrosis. These nucleotides are sequentially hydrolyzed by cell surface expressed ectonucleotidases, CD39 (ectonucleoside triphosphate diphosphohydrolase 1; ENTPDase-1) and CD73 (ecto-5'-nucleotidase), to the cellular protective molecule, adenosine [3–5]. Our laboratory recently demonstrated that human CD39 (hCD39) expression decreases infarct size following myocardial I/R injury in mice [6]. Therefore, to translate our findings to a model more physiologically comparable to humans, we utilized a unique transgenic porcine model of hCD39 expression.

Here we demonstrate for the first time that infarct size is significantly reduced following myocardial ischemia-reperfusion injury in transgenic swine expressing human CD39.

2. Methods

hCD39-expressing swine were generated using the H-2K^b promoter/hCD39 construct previously used to generate transgenic mice (Figure 1A) [7] via somatic cell nuclear transfer [8] and examined in an *in vivo* model of left anterior coronary artery myocardial ischemia-reperfusion injury. A detailed expanded Methods description is available in the Online Data Supplement.

3. Results

3.1. Expression of Human CD39 in Transgenic Swine

Expression of hCD39 in transgenic swine was confirmed on circulating blood cells (Figure 1B) and in myocardial tissue (Figure 1C–1F).

3.2. CD39 Expression Protects Against Myocardial Ischemia-reperfusion Injury in Swine

Fourteen swine were entered in the study. Two animals were excluded secondary to poor staining with Phthalo blue and an inability to accurately assess the area-at-risk (1 Control and 1 hCD39-Tg) and one animal died secondary to ventricular fibrillation at anesthesia induction (hCD39-Tg). Baseline parameters of the 11 animals completing the protocol including age, sex and weight did not differ between the groups (Supplemental Table 1).

Prior to ischemia there were no significant differences in the rate-pressure product (RPP), mean arterial pressure or heart rate between the groups (Supplemental Figure 1A–C). However, during ischemia there was a significant decrease in the RPP in WT compared to baseline and compared to CD39-Tg animals (Supplemental Figure 1A). At 3 hours following reperfusion, ejection fraction (EF) was significantly reduced in both the littermate control and hCD39-Tg animals compared to the baseline EF, but no difference was observed between the groups (Supplemental Figure 1E).

Post-mortem analysis of cardiac tissue revealed no difference in the area-at-risk for myocardial infarction between groups (Control: $46.5 \pm 1.3\%$ vs. hCD39-Tg: $44.8 \pm 1.9\%$, $P > 0.05$ Figure 2A). However, infarct size, expressed as a percent of the area-at-risk, was significantly reduced in hCD39-Tg compared to the littermate control animals (hCD39-Tg: $17.2 \pm 4.3\%$ vs. Control: $44.7 \pm 5.2\%$, $P = 0.0025$; Figure 2B).

4. Discussion

The current study demonstrates that transgenic hCD39 expression in swine protects against myocardial ischemia/reperfusion injury, providing new rationale for human therapies based on this pathway. While one prior published study examined the effect of intracoronary infusion of apyrase (a soluble CD39-like molecule) on porcine myocardial ischemia-reperfusion injury, no benefit was found [9]. However, in our model CD39 is an intact membrane-expressed molecule in the vasculature and heart where it can exert a beneficial effect immediately during ischemia. Whether delivery of recombinant soluble hCD39 or short-term increases in CD39 activity can convey cardiac protection is the focus of ongoing work.

Our lab previously published that hCD39 expression in mice conveys marked cardiac protection against myocardial ischemia-reperfusion injury. This protective mechanism in mice involves increased adenosine generation, adenosine A_{2B} receptor engagement, and activation of the cellular protective signaling pathways [6]. Ongoing work will examine if similar or alternative signaling pathways mediate the protection observed in hCD39-Tg mice and swine is the focus of ongoing studies.

CD39 expression is increased by exposure to hypoxia [10] or ischemic preconditioning [3]. Molecular studies demonstrate that activating transcription factor 2 and Sp1-dependent mechanisms regulate CD39 transcription [11, 12]. Therefore therapies targeting modulation of CD39 expression may provide therapeutic benefit in groups at high risk for myocardial injury. Indeed, prior studies also have identified particular single nucleotide polymorphisms that correlate with the level of CD39 RNA expression and disease state [13]. Whether innate differences in CD39 expression levels affect cardiovascular disease processes require further evaluation.

While the current study was conducted in a blinded design, there are limitations. The ischemic insult did not involve thrombus formation, the hallmark of acute myocardial infarction in humans. The animals were sedated, intubated and ventilated, therefore secondary hemodynamic changes were present. The assessment of LV function by ventriculography is rudimentary but validated clinically. The limited period of reperfusion does not exclude the possibility that myocardial necrosis is delayed in hCD39 animals. Despite these caveats the use of swine as a model for cardiovascular pathophysiology is well validated [14]; indeed, the porcine model was chosen by the NIH consortium for preclinical Assessment of CARDioprotective therapies (CAESAR) to assess potential cardioprotective drug candidates [15]. Our future studies will examine the effect of hCD39 expression in conscious animals that are followed long-term for infarct size and post-myocardial infarction left ventricular remodeling.

In summary, the current work suggests that observations from transgenic murine cardiovascular models translate to large animal transgenic models; hCD39 transgene expression reduces myocardial injury in a porcine model of myocardial acute ischemia-reperfusion injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
MI	myocardial infarction
ENTPD-1; CD39	ectonucleoside triphosphate diphosphohydrolase 1
CD73	ecto-5'-nucleotidase
I/R	ischemia/reperfusion
LAD	left anterior descending
TTC	triphenyl tetrazolium chloride
RPP	Rate-pressure product
WBC	white blood cells
FACS	Fluorescence Activated Cell Sorter

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Figure 1. Expression of hCD39 in swine

A. Schematic of CD39 transgene. **B.** FACS analysis of hCD39 expression on porcine white blood cells (WBC): column i.) upper panel: FSC/SSC profile; lower panel: Control IgG staining of hCD39-Tg WBC, column ii.) upper panel: FSC/SSC profile; lower panel: Control WBC stained with anti-hCD39, column iii.) upper panel: FSC/SSC profile; lower panel: hCD39-Tg WBC stained with anti-hCD39. **C.** Expression of porcine CD39 in porcine myocardium. **D.** Expression of human CD39 in porcine myocardium. Brown precipitate indicates hCD39 expression. **E.** Representative immunoblot of CD39 using anti-human CD39 antibody and β -actin expression in myocardial lysates from control and hCD39-Tg hearts and **F.** CD39 expression normalized to β -actin levels. P -value shown.

Figure 2. Assessment of myocardial injury transgenic swine

A. Area-at-risk for infarction as a percent of the total left ventricle; *NS*= non-significant, B. Myocardial infarct size as a percent of the area-at-risk. (Control: N=5; hCD39-Tg: N=6). *P*-value shown.