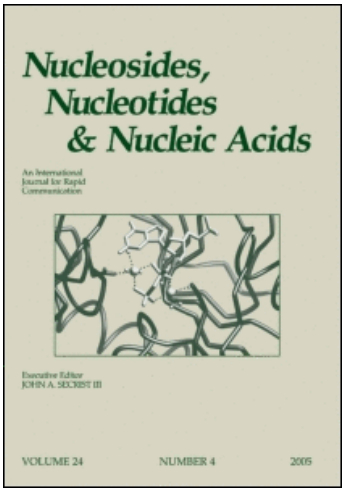

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EXPOSURE TO HUMAN BLOOD INACTIVATES SWINE ENDOTHELIAL ECTO-5′-NUCLEOTIDASE

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▫ *Ecto-5′-nucleotidase (E5′N) is an extracellular enzyme forming anti-inflammatory and immunosuppressive adenosine. We evaluated whether confrontation of pig heart and endothelial cells with human blood changes the activity of E5′N. Pig hearts were perfused ex vivo with fresh human blood for 4 h. Pig aortic endothelial cells (PAEC) were incubated in vitro with human plasma for 3 h. Ex vivo perfusion of pig heart with fresh human blood resulted in a decrease in E5′N activity to 62% and 61% of initial in wild-type and transgenic pig hearts, respectively. PAEC activity of E5′N decreased to 71% and 50% of initial after 3 h exposure to heat-inactivated and active complement human plasma, respectively, while it remained constant in controls. Pig heart activity of E5′N decreased following exposure to human blood, which may affect adenosine production and exacerbate hyperacute and vascular rejection.*

Keywords Ecto-5′-Nucleotidease, Xenotransplantation, Nucleotides, Endothelial Cells, Complement

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INTRODUCTION

Hyperacute ejection in pig to primate xenografts can be overcome using genetically modified pigs; for example, over-expression of human complement regulatory proteins (e.g., human decay accelerating factor [hDAF]), increases survival of pig to primate cardiac xenografts.[1] However, grafts are subsequently rejected by a process known as delayed xenograft rejection or acute vascular rejection.[2] Nucleotides usually trigger inflammatory responses, cytotoxic effects, and platelet aggregation, whereas adenosine attenuates these mechanisms. The final step of extracellular nucleotide breakdown is catalyzed by ecto-5′-nucleotidase. Changes in the activity of ecto-5′-nucleotidase (E5′N) may play an important role in xenotransplant rejection. Decreased E5′N activity may reduce the capacity of cells to produce adenosine and increase cytotoxic lymphocyte effects, neutrophil infiltration, free-radical injury, and thrombus formation.[3–5] In the present study, we investigated the activity of E5′N in pig hearts or endothelium when exposed to human blood or plasma on the activity of ecto-5′-nucleotidase. We found a selective decrease in ecto-5′-nucleotidase activity. These results provide evidence that changes in surface enzymes such as ecto-5′-nucleotidase may play a significant role in xenograft rejection.

MATERIALS AND METHODS

1. *Heart perfusion:* Perfusion was performed at (37°C) using retrograde constant flow perfusion system at 37°C for 4 h. Hearts were collected from transgenic (T, $n = 5$) and control (C, $n = 6$) pigs of either sex weighing 70–120 kg.
2. *Cell culture:* Pig aortic endothelial cells (PAEC) were prepared from porcine aortas as described in details previously.[6]
3. *Exposure of endothelial cells to human plasma:* Confluent pig aortic endothelial cells (PAEC) were incubated in M199 medium with 50% fresh human plasma (active complement) or 50% heat-inactivated (56°C for 1 h). PAECs were incubated with human plasma for 0, 60, and 180 min at 37°C.
4. *Enzyme assays:* The adenosine metabolizing enzymes were assayed in heart specimens and endothelial cell as described in detail previously[7] using reversed-phase HPLC.[8]
5. *Statistical analysis:* All values are presented as the mean ± standard error of the mean (S.E.M.), calculated using paired Students *t*-test or one-way analysis of variance.

RESULTS AND DISCUSSION

Activity of E5′N was 6.60 ± 0.33 nmol/min/mg protein in normal pig hearts and 8.54 ± 2.10 nmol/min/mg protein in transgenic pigs over-expressing hDAF. Ex vivo perfusion of pig heart with fresh human blood for 4 h results in a decrease in E5′N activity to 62 and 61% of initial in normal and transgenic pig hearts, respectively (Figure 1). No significant changes in the adenosine deaminase (ADA), adenosine kinase (AK), or AMP deaminase activities were observed while purine nucleoside

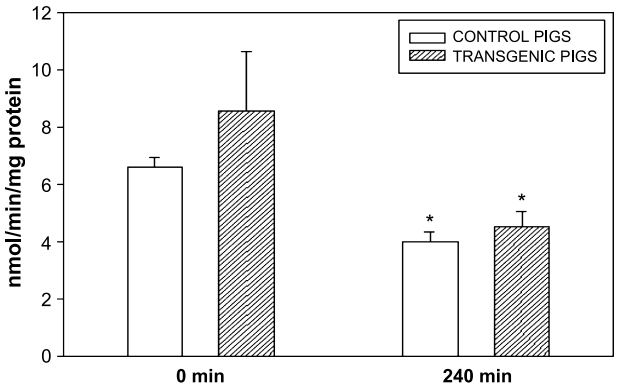

FIGURE 1 Effects of 4 h perfusion of transgenic or normal pig hearts with human blood on ecto-5′-nucleotidase activity. Values represent the means ± S.E.M. $n = 5–6$. *$p < 0.05$ vs. start of perfusion.

phosphorylase (PNP) activity increased significantly (×3) in control non-transgenic hearts after 4 h of perfusion. Initial pig aortic endothelial activity of E5′N was 9.10 ± 1.40, 9.62 ± 1.56, and 9.15 ± 1.87 nmol/min/mg protein in control, heat-inactivated, and active-plasma groups (Figure 2). Activity decreased to 71 and 50% of initial after 3 h exposure to heat-inactivated and active-complement human plasma, respectively. The activity of LDH was 23.5 ± 3.6 nmol/min/mg protein initially and was unchanged in all groups. There were no significant changes in AK, ADA, or PNP activities during incubation in any groups.

The results of the present study demonstrate a novel potential mechanism of xenograft rejection, which may involve a reduction in the hydrolytic capacity of the endothelium to convert extracellular nucleotides into adenosine due to decrease in

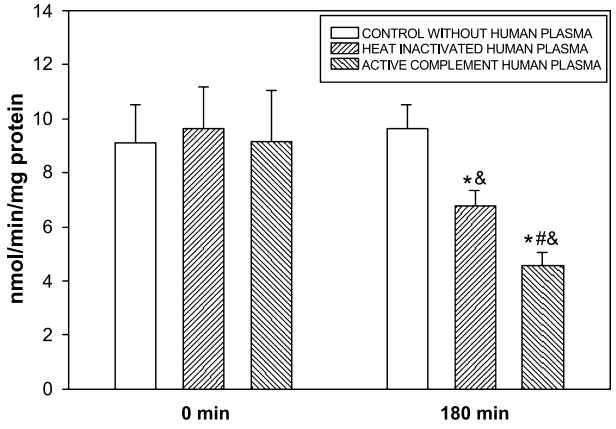

FIGURE 2 Activity of ecto-5′-nucleotidase in lysates of pig endothelial cell surviving after incubation for 3 h with active-complement human plasma, heat-inactivated human plasma, or in controls (medium without plasma). Values represent the means ± S.E.M. $n = 3–5$ *$p < 0.05$ vs. activity at the start of incubation, &$p < 0.05$ vs. control, #$p < 0.05$ vs. heat-inactivated plasma.

the ecto-5′-nucleotidase activity. This change in activity occurred rapidly following contact with human blood or plasma; heat inactivation of plasma partially prevents ecto-5′-nucleotidase activity decrease, and over-expression of hDAF in transgenic hearts was unable to block this effect. Understanding the role of nucleotide metabolism and purinergic signaling in xenograft rejection may lead to the development of new therapies to prolong discordant xenograft survival.

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