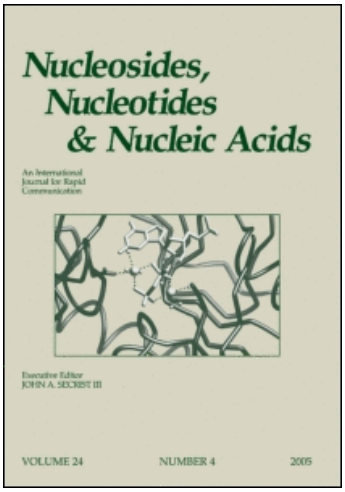

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DIFFERENCES IN ACTIVITIES OF THE ENZYMES OF NUCLEOTIDE METABOLISM AND ITS IMPLICATIONS FOR CARDIAC XENOTRANSPLANTATION

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□ *Xenotransplantation is one be possible solution for a severe shortage of human organs available for transplantation. However, only a few studies addressed metabolic compatibility of transplanted animal organs. Our aim was to compare activities of adenosine metabolizing enzymes in the heart of different species that are relevant to clinical or experimental xenotransplantation. We noted fundamental differences: ecto-5′nucleotidease (E5′N) activity was 4-fold lower in pig and baboon hearts compared to the human hearts while mouse activity was compatible with human and rat activity was three times higher than human. There also were significant differences in AMP-deaminase (AMPD), adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) activities. We conclude that differences in nucleotide metabolism may contribute to organ dysfunction after xenotransplantation.*

Keywords Xenotransplantation; Ecto-5′-nucleotidase; Purine metabolism

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INTRODUCTION

Despite efforts to develop pharmacological therapy to treat cardiac dysfunction, transplantation still seems to be the only option for the end stage heart failure. There is a serious shortage of human organs available for allotransplantation and xenotransplantation seems to be a potential solution.[1,2] Pig organs are considered to be the best substitution for human organs, not just because of the similarity of anatomy and cardiovascular function but also for economical and ethical reasons.[3] While extensive studies were undertaken to identify and overcome immunological barriers,[4] only very few addressed metabolic incompatibility. Mouse-to-rat cardiac transplant is the most commonly used experimental model of xenotransplantation while advanced studies to mimic pig-to human xenotransplantation have been done using pig-to-baboon model. The aim of this study was to compare the activities of selected enzymes of adenosine and nucleotide metabolism in human, pig, baboon, rat, and mouse hearts.

MATERIALS AND METHODS

Enzyme activity analysis in heart homogenates was described in detail previously.[5] Heart biopsies from human, pig, baboon, rat, and mouse were homogenised in homogenisation buffer (150 mmol/l KCl, 20 mmol/l TRIS/HCl, 1 mmol/l EDTA, 1mmol/l dithiothreitol, and 0.1% triton, pH 7.0) at 4°C. Ecto-5′nucleotidease (E5′N) assay was carried out with the crude homogenate using method specific for extracellular but not cytosolic isoform of 5′-nucleotidase. The remaining homogenate was spin at 3700 rpm for 30 minutes at 4°C and assay for AMP-deaminase (AMPD), adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) at 37°C under optimal conditions for each of these enzymes. After incubation, substrates and products were separated using HPLC[6] and the rate of product formation was used to express enzyme activity. All results are expressed as mean ± standard error of mean (SEM).

RESULTS

Activity of E5′N in human, pig, baboon, rat, and mouse heart is presented on Figure 1. E5′N activity in rat heart was significantly higher than in the heart of the other species. Pig and baboon have a very low activity while human and mouse was intermediate. Figure 2 presents AMPD activity. Pig and baboon AMPD activity was significantly lower than human, rat or mouse. Baboon has the highest ADA activity (4.34 ± 0.07 nmol/min/mg wet weight), followed by rat (1.62 ± 0.23 nmol/min/mg wet weight). Human, pig, and mouse have the lowest activity in ADA (0.73 ± 0.06, 0.91 ± 0.03,

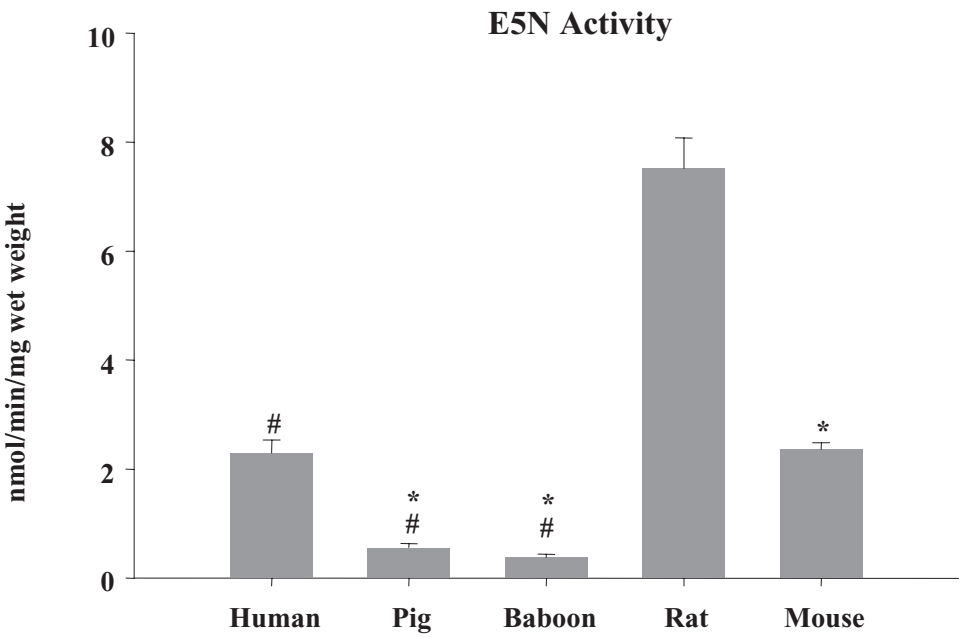

FIGURE 1 Ecto-5′-nucleotidase activity in the heart of different species ($^{*}p < 0.05$ in comparison with human; $^{\#}p < 0.05$ in comparison with rat).

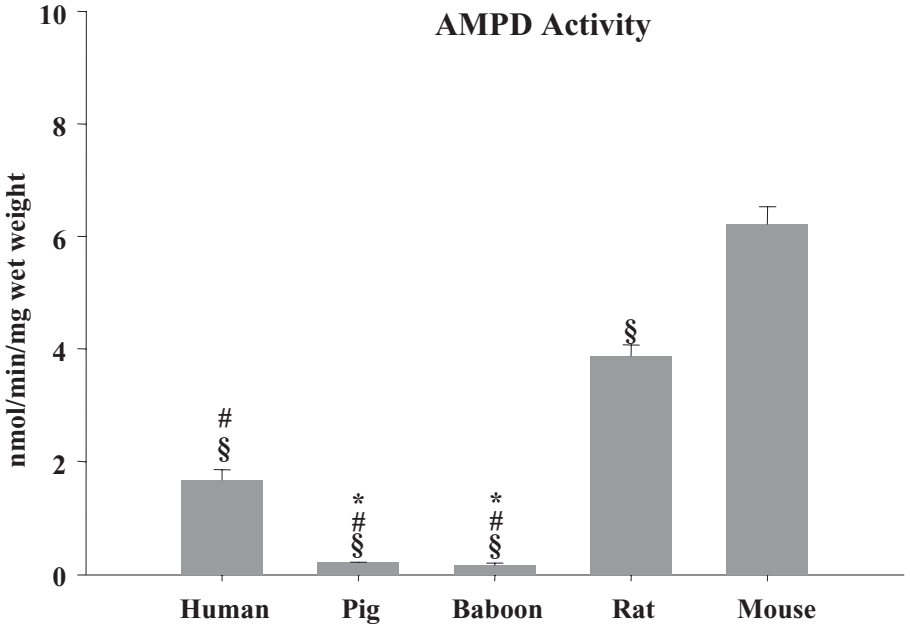

FIGURE 2 AMP-deaminase activity in different species ($^{*}p < 0.05$ in comparison with human; $^{\#}p < 0.05$ in comparison with rat; $^{\S}p < 0.05$ in comparison with mouse).

and 0.46 ± 0.03 nmol/min/mg wet weight respectively). Mouse PNP activity (13.54 ± 1.19 nmol/min/mg wet weight) was significantly higher than human, baboon, rat and pig (1.23 ± 0.09, 2.99 ± 0.71, 1.45 ± 0.25, and 0.70 ± 0.19 nmol/min/mg wet weight, respectively ($p < 0.05$)).

DISCUSSION

We have shown here that there are significant differences in activities of enzymes of adenosine metabolism in the heart of different species. These differences may not only play a role in the rejection of pig organs in humans but also may affect experimental models of xenotransplantation. Widely accepted preclinical pig-to-baboon experiments may not fully characterize pig-to-human xenotransplantation due to the differences in enzyme activities. Similar low activity of E5′N in pig and baboon hearts may result in lack of the responses that will occur after pig organ transplantation into humans. On the other hand mouse to rat model may be appropriate to study the role of E5′N in xenotransplantation as this will reproduce transplantation of the low E5′N cardiac activity organ into high cardiac E5′N activity species. The activity of E5'N is especially interesting in context of xenotransplantation since lower activity of E5′N in pig hearts may attenuate production of adenosine and its cytoprotective, immunosuppressive and thrombo-inhibitory properties leading to augmented xenograft acute vascular rejection.

We also have shown other important differences in activities of enzymes of nucleotide metabolism such as AMPDA, ADA, and PNP. These differences may affect intracellular nucleotide metabolism and in particular ability of the cells in the transplanted organ to maintain adequate nucleotide pool. Nucleotide metabolism requires therefore particular attention in context of xenotransplantation.

REFERENCES

1. Ogata, K.; Platt, J.L. Potential applications and prospects for cardiac xenotransplantation. *J. Heart Lung Transplant.* **2004**, 23, 515–526.
2. Cooper, D.K.; Gollackner, B.; Sachs, D.H. Will the pig solve the transplantation backlog?. *Annu. Rev. Med.* **2002**, 53, 133–147.
3. Appel, J.Z. III.; Buhler, L.; Cooper, D.K. The pig as a source of cardiac xenografts. *J. Card Surg.* **2001**, 16, 345–356.
4. Bach, F.H.; Soares, M.; Lin, Y.; Ferran, C. Barriers to xenotransplantation. *Transplant. Proc.* **1999**, 31, 1819–1820.
5. Kochan, Z.; Smolenski, R.T.; Yacoub, M.H.; Seymour, A.-M.L. Nucleotide and adenosine metabolism in different cell types in human and rat heart. *J. Mol. Cell. Cardiol.* **1994**, 26, 1497–1503.
6. Smolenski, R.T.; Lachno, D.R.; Ledingham, S.J.M.; Yacoub, M.H. Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in hearts for transplantation. *J. Chromatogr.* **1990**, 527, 414–420.