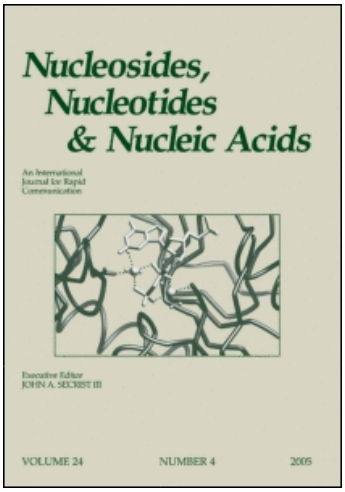

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Purine Metabolism in Pigs and Humans and Its Implications for Xenotransplantation

R. T. Smolenski[ab]; Z. Khalpey[a]; A. Yuen[a]; H. Dziewit[b]; E. M. Slominska[b]; T. Borkowski[b]; M. Zdunek[b]; Z. Kochan[b]; J. Karbowska[b]; M. Lavitrano[c]; M. H. Yacoub[a]
[a] Heart Science Centre, Imperial College at Harefield Hospital, UK [b] Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland [c] Department of Environmental and Experimental Medicine and Medical Biotechnologies, University of Milano-Bicocca, Milan, Italy

To cite this Article Smolenski, R. T. , Khalpey, Z. , Yuen, A. , Dziewit, H. , Slominska, E. M. , Borkowski, T. , Zdunek, M. , Kochan, Z. , Karbowska, J. , Lavitrano, M. and Yacoub, M. H.(2005) 'Purine Metabolism in Pigs and Humans and Its Implications for Xenotransplantation', Nucleosides, Nucleotides and Nucleic Acids, 24: 4, 263 — 266
To link to this Article: DOI: 10.1081/NCN-200059701
URL: http://dx.doi.org/10.1081/NCN-200059701

Nucleosides, Nucleotides, and Nucleic Acids, 24 (4):263–266, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059701

Taylor & Francis
Taylor & Francis Group

PURINE METABOLISM IN PIGS AND HUMANS AND ITS IMPLICATIONS FOR XENOTRANSPLANTATION

R. T. Smolenski ▫ *Heart Science Centre, Imperial College at Harefield Hospital, UK and Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland*

Z. Khalpey and A. Yuen ▫ *Heart Science Centre, Imperial College at Harefield Hospital, UK*

H. Dziewit, E. M. Slominska, T. Borkowski, M. Zdunek, Z. Kochan, and J. Karbowska ▫ *Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland*

M. Lavitrano ▫ *Department of Environmental and Experimental Medicine and Medical Biotechnologies, University of Milano-Bicocca, Milan, Italy*

M. H. Yacoub ▫ *Heart Science Centre, Imperial College at Harefield Hospital, UK*

▫ *We compared concentrations of nucleotide substrates and activities of enzymes of nucleotide metabolism in pig and human blood, heart, and kidney. The most important difference was lower ecto-5′-nucleotidase (E5′N) activity in both pig hearts and kidney. Furthermore, higher hypoxanthine, inosine, adenine, and uracil, but lower uridine and uric acid concentrations were observed in pig blood as compared to human. A twofold increase in UTP concentration has been observed in pig hearts following 4 h perfusion with human blood. Purine metabolism is an important target for genetic and pharmacological manipulation during xenotransplantations.*

Keywords Xenotransplantation, UTP, ATP, Nucleotides, Ecto-5′-Nucleotidase, Purine Metabolites

INTRODUCTION

Metabolic compatibility of animal organs with human body environment could be an important factor that may affect long-term organ survival following xenotransplantation, a procedure that may greatly improve treatment of end-stage organ failure.[1,2] Our aim was to compare concentrations of nucleotide substrates

Received 12 August 2004, accepted 10 March 2005.
Address correspondence to R. T. Smolenski, Heart Science Centre, Imperial College at Harefield Hospital, UK; E-mail: r.smolenski@Imperial.co.uk

TABLE 1 Activities of Purine Enzymes in Pig and Human Hearts and Kidney (nmol/min/mg wet wt)

	E5′N	AMPD	ADA	PNP
Human heart	1.68 ± 0.30	1.64 ± 0.18	0.73 ± 0.06	1.23 ± 0.09
Pig heart	0.46 ± 0.02	0.21 ± 0.01	0.91 ± 0.03	0.70 ± 0.19
Human kidney	0.74 ± 0.06	1.42 ± 0.11	0.43 ± 0.07	3.52 ± 0.48
Pig kidney	0.11 ± 0.56	1.11 ± 0.10	0.14 ± 0.02	12.3 ± 0.5

and activities of enzymes of nucleotide metabolism in pig and human blood, heart, and kidney. Furthermore, we studied changes in cardiac metabolite concentration following 4 h of ex vivo perfusion with human blood.

MATERIALS AND METHODS

Enzyme activities and metabolite concentrations were measured using HPLC according to our procedures described in detail previously.[3,4] Specimens collected from unused donor human heart or kidneys and blood of healthy subjects were used for enzyme activities and metabolic determinations. Pig tissue specimens and blood samples were collected from adult (70–120 kg) animals used for cardiac surgery experiments. Pig samples were treated in the same way as specimens collected from human hearts or kidneys. Analysis of the effect of perfusion of pig hearts with human blood was performed during perfusion of transgenic pig hearts expressing human decay accelerating factor (hDAF)[5] with human blood. The system used for perfusion was retrograde and constant flow and the hearts were perfused under physiological work load at 37°C for 4 h. Cardiac biopsies were collected using Tru-Cut Travenol biopsy needles and were immediately frozen in liquid nitrogen.

RESULTS AND DISCUSSION

Results of purine enzymes activity determination are presented in Table 1. The most prominent and important difference in cardiac enzymes was in ecto-5′-nucleotidase (E5′N) activity. This activity was much lower in pig hearts compared tohuman. A significant difference was also observed between the activities of E5′N in pig and human kidneys. This difference may have important implications because of the regulatory role that extracellular nucleotides and adenosine play in inflammation, immune response, and platelet aggregation. Nucleotides such as

TABLE 2 Concentrations of ATP, GTP, UTP, and CTP in Pig Hearts at the Beginning and After 4 h of Perfusion of the hDAF Transgenic Pig Hearts with Human Blood

	ATP	GTP	CTP	UTP
Start of perfusion	21.53 ± 2.45	0.64 ± 0.03	0.16 ± 0.02	0.48 ± 0.04
4 h of perfusion	21.09 ± 1.10	0.60 ± 0.02	0.19 ± 0.01	0.87 ± 0.05

Values (μmol/g dry wt) are means ± SEM, $n = 5–6$.

TABLE 3 Concentrations of Purine Metabolites in Pig and Human Whole Blood

	Hypoxanth	Inosine	Adenine	Uracil	Uridine	Uric acid
Human blood	0.95 ± 0.08	0.10 ± 0.10	0.07 ± 0.02	0.35 ± 0.09	2.80 ± 0.30	290 ± 16
Pig blood	17.7 ± 1.5	6.45 ± 1.42	3.95 ± 0.14	17.6 ± 0.9	1.41 ± 0.13	15.0 ± 1.9

Values (μM) are means ± SEM, $n = 5–7$.

ATP or, in particular, ADP trigger immune and inflammatory responses and platelet aggregation, whereas adenosine opposes these effects. Therefore, a lower activity of E5′N may trigger unfavorable responses in the organs following xenotransplantation. Furthermore, the activity of AMP deaminase (AMPD) was markedly lower in the pig hearts. However, this difference could be beneficial as low activity of this enzyme was associated with improved cardiac function (Kalsi et al. and Yuen et al. this issue).

Concentrations of ATP, GTP, UTP, and CTP in transgenic pig hearts at the beginning and at the end of 4 h perfusion with human blood are presented in Table 2. A twofold increase in UTP concentration has been observed in pig hearts following 4 h perfusion with human blood, whereas GTP, CTP, and ATP concentrations remained stable.

Table 3 presents a comparison of concentrations of purine and pyrimidine metabolites in pig and human blood. There were profound differences in blood purine and pyrimidine concentrations such as manifold higher hypoxanthine hypoxanthine, inosine, adenine, and uracil concentrations in pig blood compared to human, but higher uridine and uric acid concentration in human blood compared to pig.

CONCLUSION

We conclude that there are fundamental differences in purine metabolism between pigs and humans that may have implications for xenotransplantation. Low activity of E5′N activity in pigs may lead to retention of pro-inflammatory and pro-aggregatory nucleotides and attenuation of the formation of anti-inflammatory adenosine that exert opposite effects. The higher level of uridine in human blood was the most likely factor that induced a supraphysiological increase in UTP concentration in the pig heart after 4 h of perfusion with human blood. The implications of significant differences in concentration of nucleotide precursors have to be considered during xenotransplantation and treatment, e.g., by allopurinol, in order to adjust the purine profile to be closer to that of pigs.

REFERENCES

1. Bach, F.H. Xenotransplantation: problems and prospects. Annu. Rev. Med. **1998**, 49, 301–310.
2. Cooper, D.K.; Gollackner, B.; Sachs, D.H. Will the pig solve the transplantation backlog? Annu. Rev. Med. **2002**, 53, 133–147.
3. 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.

4. 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.
5. Lavitrano, M.; Bacci, M.L.; Forni, M.; Lazzereschi, D.; Di Stefano, C.; Fioretti, D.; Giancotti, P.; Marfe, G.; Pucci, L.; Renzi, L.; Wang, H.; Stoppacciaro, A.; Stassi, G.; Sargiacomo, M.; Sinibaldi, P.; Turchi, V.; Giovannoni, R.; Della Casa, G.; Seren, E.; Rossi, G. Efficient production by sperm-mediated gene transfer of human decay accelerating factor (hDAF) transgenic pigs for xenotransplantation. Proc. Natl. Acad. Sci. U. S. A. **2002**, 99, 14230–14235.