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Nucleoside transporter expression profiles in human cardiac tissue show striking individual variability with overall predominance of hENT1

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ABSTRACT

Nucleoside transporters (NTs) are integral membrane transport proteins that modulate the flux of nucleosides such as adenosine across cell membranes. Two families of NTs exist, the concentrative NTs (CNTs, SLC28) and the equilibrative NTs (ENTs, SLC29). CNTs and ENTs transport anti-cancer and anti-viral nucleoside analog drugs and ENTs are also targets of drugs used to treat cardiac pathologies. Levels of some NT profiles have been shown to relate to clinical outcomes in the use of nucleoside analog drugs. However, currently, patient NT profile is not assessed prior to pharmacological administration of analog drugs. Here we describe a reliable method to determine a complete individual NT expression profile from human tissue using quantitative real-time PCR. We developed this assay on tissue (right atrial appendage, left internal mammary, aorta) from individuals undergoing cardiac surgery and compared these findings to the NT expression profiles in pooled whole heart tissue (normal and diseased). Data show that hENT1 is the most abundantly expressed NT, with highest expression levels in the aorta. However, NT expression profiles are highly variable among individuals and changes in NT expression between normal and diseased tissues were observed. These data are the first to describe the RNA expression patterns of all seven NT isoforms in the human heart. The methodology described here may be useful for quantitatively characterizing complete NT expression profiles in any human target tissue.

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1. Introduction

Nucleoside transporters (NTs), are integral membrane proteins which modulate the flux of nucleosides, nucleobases and nucleoside analog drugs across cellular membranes (Loffler et al., 2007). Two families of structurally unrelated NTs have been characterized: the concentrative NTs (CNTs), consisting of three isoforms (CNT1–3) encoded by the SLC28 gene family, and the equilibrative NTs (ENTs) consisting of four isoforms (ENT1–4) encoded by the SLC29 gene family. These proteins possess different expression profiles, substrate affinities, and transport mechanisms. CNTs are cation co-transporters and ENTs are facilitative, diffusion transporters (King et al., 2006; Loffler et al., 2007; Molina-Arcas et al., 2008; Rose and Coe, 2008). Profiles of specific NTs have been correlated with clinical outcomes in the use of nucleoside analog drugs (Santini et al., 2010) but their role in anti-cancer nucleoside analog drug resistance and cardiotoxicity is still not well understood (Damaraju et al., 2003; Floyd et al., 2005; Zhang et al., 2007). The relationship between individual NT profiles, for all seven ENTs and CNTs, and clinical treatment needs to be addressed and there is currently no standard immunohistochemical test or assay for use in screening for all NTs (or even a subset of NTs) as a diagnostic tool for developing individualized treatments on the basis of drug transporter profile.

In addition to nucleoside analog drugs, NTs transport a range of endogenous, naturally occurring nucleosides and their role in modulating the effects of the purine nucleoside, adenosine, is particularly important in the central nervous system and the cardiovasculature (Chen et al., 2007; de Jong et al., 2000; Mubagwa and Flameng, 2001; Rose et al., 2010). Adenosine is produced both intracellularly and extracellularly, particularly in response to stressors such as hypoxia, and is transported by NTs, mainly ENT1, ENT2 and CNT2. Adenosine analogs have been used clinically for over 20 years for their anti-arrhythmic effects (Mubagwa and Flameng, 2001; Pelleg et al., 2002), and for their cardioprotective effects during

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open-heart surgery (Belhomme et al., 2000). In addition, pharmacological agents, such as dipyridamole and dilazep, which inhibit ENT1 and ENT2, are also used clinically in the treatment of various cardiovascular conditions based, at least in part, on their inhibition of adenosine uptake by ENT1 and 2 which leads to increased extracellular levels of adenosine and enhanced purinergic signalling promoting cellular well-being (Kim and Liao, 2008).

Despite the significance of purinergic signalling in cardiovascular physiology, the expression patterns of the various NTs involved in regulating adenosine flux in the human heart have not been fully described, due to a lack of a simple method to determine an NT patient profile in a specific tissue. Ubiquitous distribution of some NTs in human and rodent tissues has been reported (Baldwin et al., 2004, 2005; Barnes et al., 2006; Damaraju et al., 2007; Lu et al., 2004; Pennycooke et al., 2001) and ENT1 has been shown to be highly expressed in human and mouse cardiovascular tissue (Baldwin et al., 2004; King et al., 2006; Lu et al., 2004; Pennycooke et al., 2001). While ENT2 has also been observed in cardiovascular cells and tissues, it appears to be highly expressed in skeletal muscle (Lu et al., 2004; Pennycooke et al., 2001). Both ENT4 and the intracellular isoform ENT3 have also been reported to be expressed in human and rodent hearts (Baldwin et al., 2004, 2005; Barnes et al., 2006). CNTs are not thought to be highly expressed in the cardiovasculature and were not found in the cardiomyocyte cell line, HL-1 (Chaudary et al., 2002) although CNTs do play an important role in absorptive epithelia and are abundant in cells of the kidney, intestine, and liver (Pennycooke et al., 2001). There has been no study to date which has looked at the expression profile of all seven members of the SLC28 and SLC29 families in any individual human patient tissue, including cardiovascular tissue.

To address this lack of information we have developed a panel of quantitative real-time PCR primers to investigate the mRNA expression profiles of all seven NTs in human tissue. We have used this molecular diagnostic approach to determine NT expression profiles in right atrial appendage (RAA), aorta, and left internal mammary artery (LIMA) excised from patients undergoing surgery for cardiovascular disease (CVD) and tissue obtained from healthy donors. Our data demonstrate the feasibility of using quantitative RT-PCR to determine NT expression profiles in patient tissue and demonstrate that hENT1 is the predominant isoform in cardiovascular tissue, although striking individual variability is present in terms of patient expression levels and NT profiles.

2. Materials and methods

2.1. Study population and specimen collection

The study population consisted of 18 Caucasian males, who provided informed consent, between 40 and 70 years of age with a history of CVD who were undergoing coronary artery bypass or valvuloplasty surgery at Southlake Regional Health Centre (Newmarket, Ontario). Specimens were collected during surgery by cardiac surgeons and consisted of tissues which would otherwise have been discarded; RAA, aortic punches and LIMA. The tissues were immediately flash frozen in liquid nitrogen and transported to the lab where they were stored at −80 °C until further analysis.

2.2. Whole tissue RNA isolation

Total RNA was isolated from tissues weighing 25 to 100 mg, using a phenol–chloroform extraction method (Trizol, Invitrogen, Carlsbad, CA) with auto-homogenization for larger tissue samples (OMNI TH with OMNI Tip™ Clear Plastic Homogenizing Probes, 7 mm × 110 mm, OMNI, Kennesaw, GA). Once RNA purity and concentration was confirmed and quantified, cDNA synthesis was performed using Superscript reverse transcriptase (Invitrogen, Carlsbad, CA). Comparison of patient samples were made with commercial RNA samples obtained from Clontech Laboratories, Inc. (Mississauga, Canada). Normal whole heart and aortic samples consisted of pooled total RNA from 4 individuals (2 males and 2 females) between 35 and 75 years of age whose cause of death was sudden trauma. Diseased whole heart total RNA was from a single donor, 92 years of age, with severe cardiac complications resulting in death. Pooled commercial samples were used for comparison and normalization only.

2.3. Quantitative real-time PCR

Quantitative real-time PCR amplifications were conducted using Power Sybr Green PCR Master Mix (Applied Biosystems, Foster City, CA). Reaction conditions for the amplification of NTs were: 15 min at 95 °C, 40 cycles of 1 min at 94 °C, 30 s at 65 °C; dissociation step: 15 s at 95 °C, 1 min at 60 °C, 15 s at 95 °C, and 15 s at 60 °C. All samples were run in triplicate and each experiment was conducted three times. Trials included a no-template negative control and GAPDH, as an internal positive control (Applied Biosystems/Ambion, Austin, TX). Gene specific primers are shown in Table 1 and were designed using Primer Express® software (Applied Biosystems, Foster City, CA) and prepared by Sigma–Aldrich Canada (Oakville, ON). Dissociation curves revealed sharp peaks at the melting temperature of each amplicon, confirming amplification of a single gene transcript. PCR primer efficiencies for all sets of primers were between 95 and 100%. Data analysis was performed by normalizing gene expression to GAPDH and relative expression of each NT was calculated using $2^{-\Delta Ct}$ or $2^{-\Delta\Delta Ct}$ for comparative analysis between tissues and expressed as fold change

Table 1
Primers used for quantitative RT-PCR analysis of NT gene expression.

Gene	Alternative name	Primer sequences [F, forward primer; R, reverse primer]	Product size (bp)
SLC29A1	hENT1	F: 5′-TCTCCAACTCTCAGCCCACCAA R: 5′-CCTGCGATGCTGGACTTGACCT	151
SLC29A2	hENT2	F: 5′-ATGAGAACGGGATTCCCAGTAG R: 5′-GCTCTGATTCCGGCTCCTT	81
SLC29A3	hENT3	F: 5′-TCCTCAGGCCCAAGACTCAA R: 5′-GGCAGTTGTTCACCCACAGA	60
SLC29A4	hENT4	F: 5′-GGGACCTCCATCGTGTTTGA R: 5′-CTCCACCAGGACGTTGTTCA	84
SLC28A1	hCNT1	F: 5′-CATTACTGATCCGGCCCTACTT R: 5′-TGGCGTAACCTCCGGTCAT	75
SLC28A2	hCNT2	F: 5′-CTTGTGCTCTCGCCTCATCA R: 5′-TTACCCCCTCCTCACTCTTGAA	75
SLC28A3	hCNT3	F: 5′-ATTGCTGGAAGCGTGCTAGGT R: 5′-TGACGCAGGTGCTGACATAAC	90

Table 2
Demographics and CVD risk factor profiles of enrolled patients.

	Number of patients	% of patients
Patient characteristics		
Age		
40–49	3	27.3
50–59	3	27.3
60–69	5	45.4
Weight		
Underweight (BMI < 18.5)	0	0
Normal weight (BMI = 18.5–24.9)	2	18
Overweight (BMI = 25–29.9)	2	18
Obese (BMI ≥ 30)	7	64
CVD risk factor		
Hypertension (HPN)	7	63
Hyperlipidemia	7	63
Smoking history	7	63
Family history of CAD	2	18
Diabetes mellitus (DM)	4	36
DM + obesity + smoking	3	27
DM + obesity + smoking + HPN	1	9
DM + HPN	2	18
Angina	6	55
Prior ASA use	11	100
Average age (mean ± S.D), years	56.3 (±2.8)	
Weight (mean ± S.D.), kg	95.8 (±17.6)	
Body mass index (mean ± S.D.)	30.2 (±6.8)	

versus lowest expressed transcript level (one gene set to 1), shown as arbitrary units (A.U.).

2.4. Gene Expression Omnibus (GEO) database analysis

Data describing NT expression in a range of tissues from a variety of sources was obtained from the publicly accessible GEO database (Barrett et al., 2009; Edgar et al., 2002; NCBI GEO, 2009) and used to compare NT expression from independent studies against our data obtained from patient samples.

2.5. Statistical analyses

Data were compared using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. All data are expressed as mean ± S.D. with a $p < 0.05$ considered statistically significant. Statistical analyses were conducted using GraphPad Prism v4.00 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Clinical profiles

Demographics and risk factor profiles of the 11 patients contributing to the data presented here are summarized in Table 2.

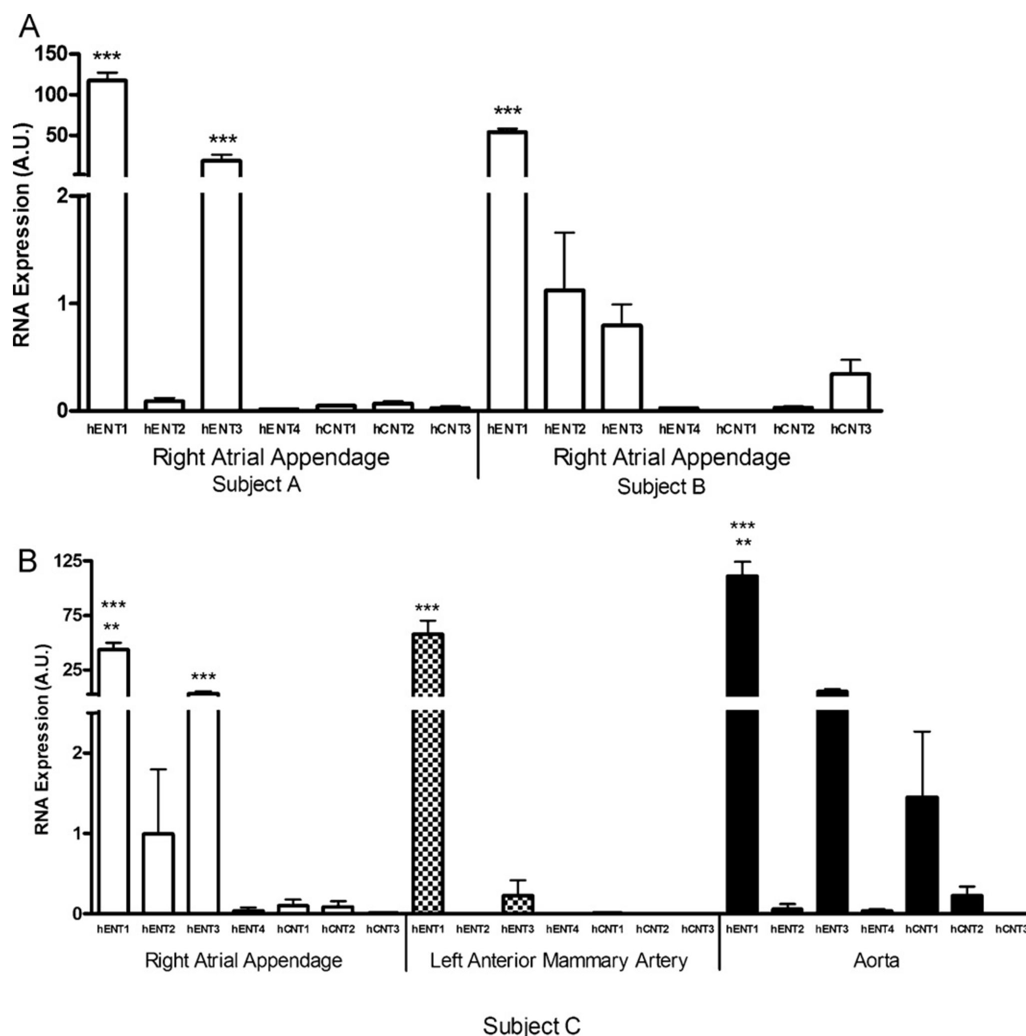

Fig. 1. Variable NT expression among individuals and in cardiovascular tissue. NT expression is highly variable among patients (panel A) and within a patient's cardiovascular tissue, however hENT1 was overall predominant in a patient sample (**$p < 0.01$ hENT1 vs. hENT3, ***$p < 0.001$, hENT1 or hENT3 vs. all other NTs).

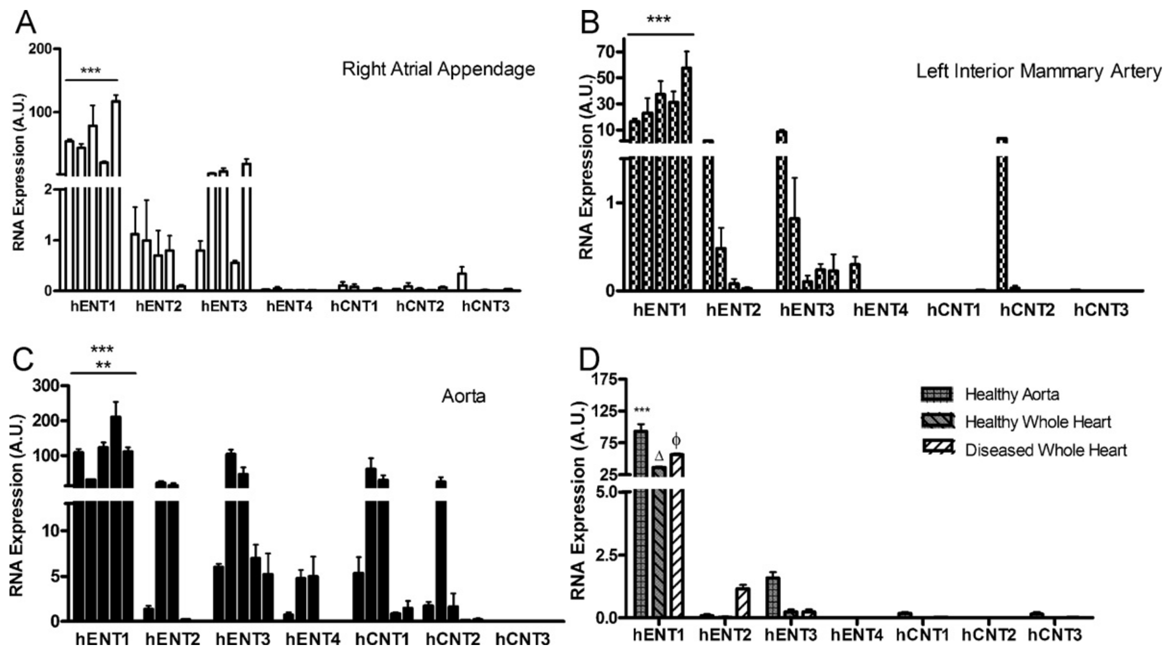

Fig. 2. hENT1 is the predominant NT expressed in all patients. NT expression profiles vary between patients but hENT1 is the predominant NT expressed in RAA (panel A), LIMA (panel B) and aorta (panel C) (**$p < 0.01$ hENT1 vs. hENT3, ***$p < 0.001$, hENT1 vs. all other NTs, five patients per tissue, data pooled from 3 experiments performed, each in triplicate). Panel D: NT expression profiles in pooled healthy aorta, pooled healthy whole heart and diseased whole heart show significantly higher hENT1 expression compared to all hENT and hCNT isoforms (***$p < 0.001$, Δ $p < 0.001$, ϕ $p < 0.001$). Healthy aortic and whole heart RNA were pooled from four donors, diseased whole heart RNA was from one donor. Data are pooled from three experiments, each performed in triplicate, normalized to GAPDH and expressed as mean ± S.D.

The mean age of patients was 56.3 ± 2.8 years. Average body mass index (BMI) was 30.2 ± 6.8, including 2 overweight and 7 obese patients (based on BMI values). Hypertension was defined as a diastolic blood pressure ≥90 mm Hg, systolic blood pressure ≥140 mm Hg, or reported use of antihypertensive drugs. Cardiovascular disease was diagnosed by angiography, ECG, and effort test. The risk factors for CVD, apart from age, were present in all patients: 7 were smokers, 7 had hyperlipidemia, 7 had hypertension, 4 had diabetes, 2 had a family history of CVD and 6 had a history of chest pain consistent with angina. Nearly one quarter of patients (27%) exhibited diabetes, a history of smoking, and obesity combined. The majority of patients enrolled in the present study had ≥50% stenosis in at least one of their coronary arteries and were undergoing coronary artery bypass graft surgery.

3.2. Variable NT expression profiles among individual patients

Initial analysis of NT expression in individuals suggested that hENT1 is the predominant NT expressed in human heart tissue; however complete NT expression profiles were strikingly variable across patients (Fig. 1A). Analysis of NT profiles of one individual in different heart tissues revealed variable expression even within an individual patient (Fig. 1B) and prompted a complete analysis of a NT expression across CVD patients and across cardiovascular tissue.

3.3. Relative expression of NTs in cardiovascular samples

Of the 18 enrolled patients, 11 surgeries yielded the minimum tissue sample size required for sufficient repeated analyses (25 mg) and we used tissue from a number of different subsets of five patients for each analysis. Analysis of NT expression patterns across patients ($n = 5$ patients for each tissue type, each experiment performed in triplicate) of RAA (Fig. 2A), LIMA (Fig. 2B) and aorta (Fig. 2C) showed consistently higher hENT1 expression than all other NTs in each individual. However, individual variation in NT expression profiles among patients was clearly present. There was no obvious correlation between NT profile and clinical profile for this relatively small group of patients. Other NTs were present at lower levels compared to hENT1 in all patients and tissues. A general trend of hENT1 > hENT2 ≈ hENT3 > hCNT3 > hCNT1 ≈ hCNT2 > hENT4 was observed across all tissue samples from all patients (Fig. 2A–C). To determine if this expression profile was consistent in other tissue samples obtained from other sources under different conditions, we analyzed the NT expression in diseased whole heart, healthy whole heart and healthy aorta. We noted the same NT expression profile in all samples (Fig. 2D). We then determined the fold change in NT expression levels between our reference healthy aortic tissue and the patient samples. With the exception of hENT1 and hCNT3, all other NTs showed significantly greater expression of NT mRNA relative to healthy aorta (Fig. 3A). A similar pattern was noted for RAA (data not shown). We also noted that hENT2 expression was significantly greater in diseased whole heart relative to healthy whole heart (Fig. 3B) although we cannot confirm that the nature of the disease in this individual is similar to that of our patient population and therefore cannot, at this time, draw any conclusions about changes in NT expression profiles in relation to disease state or progression.

3.4. Differential expression of NTs in human heart tissue

To compare the relative levels of NTs expressed in RAA, aorta and LIMA tissues, we determined the differential expression of each individual NT, and expressed these data as fold change normalized to NT expression in commercial healthy whole heart tissue RNA (Fig. 3C). Our findings show that hENT1–4 and hCNT1–2 expression was significantly greater in the aorta than in RAA or LIMA and the general trend aorta > RAA > LIMA was observed. In contrast, the trend for hCNT3 expression was observed to be RAA > aorta > LIMA.

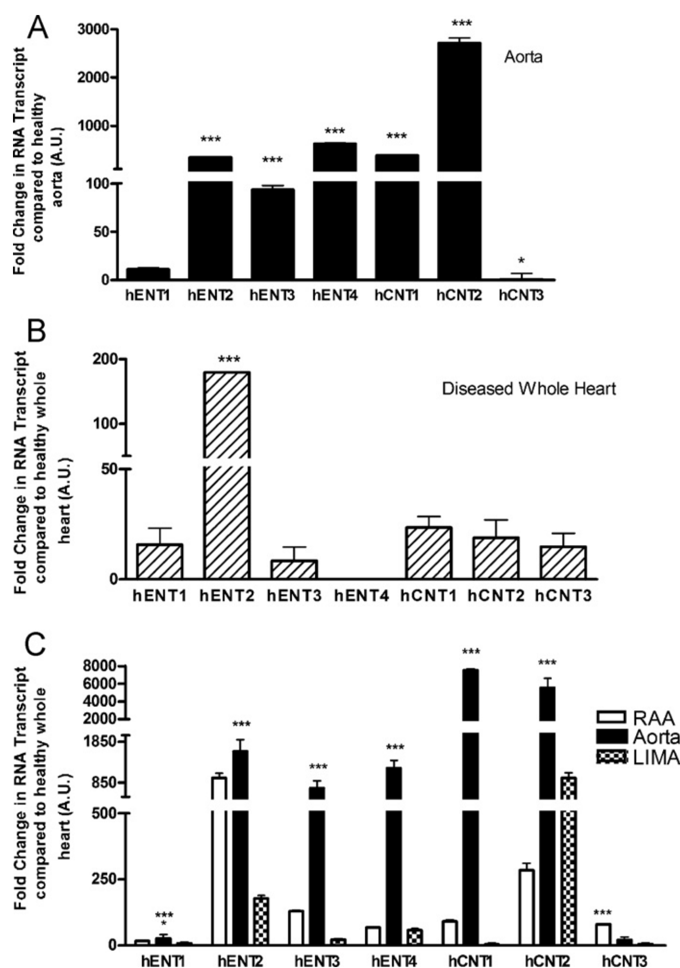

Fig. 3. Fold change in NT expression profiles between patient and healthy tissues. hENT1 expression is effectively unchanged when patient tissues are compared to the healthy pooled tissue while changes in expression levels of other NTs are noted. Panel A: Patient aorta tissue demonstrated significantly higher hENT2–4 and hCNT1–2 ($^{***}p < 0.001$) expression relative to healthy aortic NT expression. hCNT3 expression decreased in patient tissues compared to healthy aorta ($^{*}p < 0.05$). Pooled data from five patient aorta samples and four healthy aorta donors. Data are pooled from three experiments, each performed in triplicate and expressed as mean ± S.D. Panel B: NT expression profiles in diseased whole heart relative to healthy whole heart show significantly increased hENT2 expression compared to all other NT isoforms ($^{***}p < 0.001$). Pooled data from three experiments (one patient for diseased whole heart, four pooled patients for healthy whole heart), each experiment performed in triplicate expressed as mean ± S.D. Panel C: NT expression profiles in RAA, aorta, and LIMA of patient relative to healthy whole heart show significantly greater levels of all NTs in the aorta compared to RAA and LIMA. Aortic hENT1 expression was significantly greater than RAA ($^{*}p < 0.05$) and LIMA ($^{***}p < 0.001$) as were hENT2–4 and hCNT1–2 expression ($^{***}p < 0.001$). hCNT3 expression was greater in RAA compared to aorta and LIMA ($^{***}p < 0.001$). Data were pooled from five patients, normalized to GAPDH and show the mean ± S.D. expressed as fold change versus the transcript expressed at the lowest level in healthy whole heart. Data represent three experiments each performed in triplicate.

3.5. Gene Expression Omnibus database

To determine if these results were representative of the broader human population, we examined NT expression profiles deposited into the GEO database. This database provides information on patterns of gene expression in different human tissues from DNA microarray experiments conducted by independent researchers (Barrett et al., 2009; Edgar et al., 2002; NCBI GEO, 2009). To determine the abundance of each NT in the heart compared to other tissues, we compared the normalized average expression levels in heart tissues to the average expression in all tissues combined from four single-channel DNA microarray datasets (GDS422, 596, 1096, 181) (Barrett et al., 2009; Edgar et al., 2002; NCBI GEO, 2009). The average expression of hENT1 in the heart was consistently higher compared to all other tissues (Fig. 4). All other NTs exhibited variability in their abundance and did not exhibit consistently higher than average expression. In addition, we analyzed datasets from dual-channel microarrays for NT expression in normal heart tissues. These arrays demonstrate the difference in NT expression between normal heart tissue and human universal total RNA (pooled from numerous cell lines). As with our previous findings, hENT1 was identified as the predominant NT in the heart (Fig. 4). Interestingly, hCNT1, which was not detected in any of the single-channel experiments, was shown to be elevated over universal levels in all heart arrays from one dataset (GDS2206) and in 2 of 4 arrays in another dataset (GDS 1088) (Barrett et al., 2009; Edgar et al., 2002; NCBI GEO, 2009). An elevated level of hENT4 mRNA was observed in only one heart array (GDS2206, GSM 82403).

4. Discussion

NTs are important in cellular homeostasis and contribute to the regulation of cellular concentrations of endogenous nucleosides as well the cellular uptake of nucleoside analog drugs (Beck et al., 1993; King et al., 2006; Kong et al., 2004; Griffith and Jarvis, 1996; Naydenova et al., 2008; Pastor-Anglada et al., 2005). Their essential, but varying, roles in the translocation of nucleoside analog drugs used in antiviral and anticancer therapy means that presence and profile of NTs in human tissue can significantly influence the efficacy and toxicity of these chemotherapeutic agents (Damaraju et al., 2003; Floyd et al., 2005; Pastor-Anglada et al., 2005; Santini et al., 2010; Zhang et al., 2007). While some NT profiles of a variety of human tissues and cell lines have been described (Archer et al., 2004; Baldwin et al., 2004, 2005; Barnes et al., 2006; Chaudary et al., 2002; Damaraju et al., 2007; Griffith and Jarvis, 1996; Govindarajan et al., 2007; Kong et al., 2004; Lu et al., 2004; Naydenova et al., 2008; Pennycooke et al., 2001) this study is the first to report (to our knowledge) the development of a reliable diagnostic tool to determine NT patient profiles directly from small surgical tissue samples using quantitative real-time PCR. We describe the mRNA expression profiles and individual variability patterns of all seven human NTs across multiple cardiovascular tissues derived from patients, including diseased and healthy tissue. Since antibodies against all seven NTs do not exist, analysis of expression profiles is currently the only way to analyze the presence and level of all potential contributors to nucleoside drug uptake in parallel from very small amounts of tissue.

Levels of hENT1 (either mRNA or protein) have been correlated with clinical outcomes (Damaraju et al., 2009; Santini et al., 2010) suggesting that hENT1 may be a useful predictive biomarker for nucleoside therapy. However, since the presence or absence of other NTs can contribute to drug uptake (Damaraju et al., 2003, 2009) the overall NT profile within any human tissue may be an important factor in evaluating potential response to a drug regimen. Therefore the presence and relative levels of all NTs within a single tissue need to be evaluated in parallel for a complete profile and this is particularly important given the striking variability of NT profiles we observed in this study, which is consistent with previous findings in other tissues (Pennycooke et al., 2001).

Despite the established importance of purinergic signalling in the cardiovasculature, very little is known about NTs in the human heart. Our data are consistent with previous reports of abundant ENT1 expression in human and mouse cardiovascular tissues (Archer et al., 2004; Baldwin et al., 2004; Chaudary et

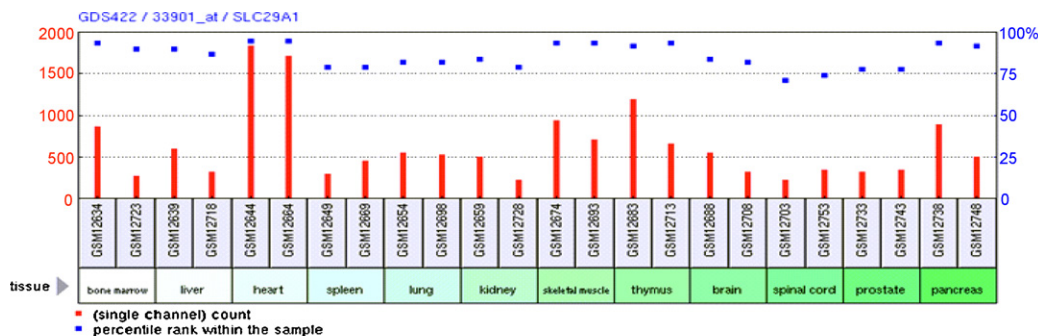

Fig. 4. NT expression profiles in the Gene Expression Omnibus (GEO) database show elevated hENT1 expression in the human heart. Representative GEO gene profile chart of hENT1 in different human tissues. Red bars indicate normalized mRNA expression values. Blue boxes show percentile rank and indicate the expression level of that gene with respect to all other genes on each array. Note elevated hENT1 expression in human heart. (GEO Dataset: GDS422). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

al., 2002; Handa et al., 2001; Lu et al., 2004; Pennycooke et al., 2001; Rose et al., 2010) and also with data extracted from the GEO database, which represents work by numerous independent investigators (Barrett et al., 2009; Edgar et al., 2002). The predominant expression of hENT1 in cardiovascular tissues is indicative of the important role of this transporter in cardiovascular physiology (Loffler et al., 2007; Rose et al., 2010; Young et al., 2008). We have recently shown that ENT1 is an essential component of purinergic cardioprotection in response to coronary occlusion, and propose this is due to its central role in regulating adenosine flux and signalling (Rose et al., 2010). However, since hENT2 and hENT3 are both clearly expressed at measurable levels in human cardiovascular tissue, it is possible that other NTs may contribute to modulation of purinergic-signalling pathways and overall cardiovascular physiology. This would be consistent with observations that an ENT1 knock-out is not lethal (Choi et al., 2004) and that ENT2 and inosine have been shown to contribute to cardioprotection in cultured myocytes (Naydenova et al., 2008). The presence of hENT4 confirms previous findings but was present at much lower levels (Barnes et al., 2006). The precise role of hENT4 in any cell type remains unclear and variable expression levels of NTs in different tissues clearly demonstrates that more work needs to be done on their relative contributions to cardiovascular physiology and particularly the relationship between NT expression levels, NT protein levels and NT functional activity. In addition, while the samples of tissue used in this study were removed with surgical precision, a mixture of cell types (endothelial, epithelial, etc.) is almost certainly present in each tissue sample and since expression levels can vary between cell types, we cannot rule out the cellular compositions of samples as being a source of variability between individuals.

The physiological significance of higher levels of NTs (excluding hCNT3) in the aorta remains to be determined but highlight the importance of this tissue as a target for drugs that interact with NTs. There was a striking increase in the relative levels of NTs (other than hENT1 and hCNT3) in the aorta, suggesting a dynamic transcriptional regulation of NTs, possibly correlating with disease progression or state. Increased levels of ENT1, ENT2 and CNT2 have been reported in the ileum and colon of patients suffering from inflammatory bowel disease (Wojtal et al., 2009) suggesting transcriptional regulation of NT expression occurs in response to disease and possibly inflammation. While there is some information on the promoter structure and transcriptional regulation of ENT1 (Abdulla and Coe, 2007; Eltzschig et al., 2005) and ENT2 (Morote-Garcia et al., 2009), virtually nothing is known about the transcriptional regulation of any other NTs and clearly this is an area for more research given the importance of NTs as both drug targets and transporters.

Increased presence of hENT1 is associated with increased cellular uptake of nucleoside analog drugs used in the treatment of various cancers (Damaraju et al., 2003; Santini et al., 2010; Zhang et al., 2007), and abundance of hENT1 in plasma membranes has been correlated with increased sensitivity to various nucleoside anticancer drugs (Zhang et al., 2007). Moreover, a deficiency of these transporters contributes to resistance to cytotoxicity (Damaraju et al., 2003; Floyd et al., 2005; Zhang et al., 2007). Chemotherapeutic agents such as Fludarabine, Clofarabine, Capecitabine, Cladribine, Cytarabine and Gemcitabine, all pose the rare but serious threat of cardiotoxicity. Among the complications that have been reported are arrhythmias, thrombosis, myocardial infarction, and congestive heart failure (Floyd et al., 2005; Peres et al., 2010). Therefore, the predominance of hENT1 in the cardiovasculature may contribute to the uptake of these agents in the heart and could provide a possible explanation for their adverse cardiovascular effects. The individuality of NT expression may account for the variability of response to nucleoside analog drugs and could be a future predictive indicator of treatment and surgical outcomes. The assay described here, using a complete primer set for all NTs, will aid in developing a clearer picture of individual variability in NT expression, which might account for differences in drug response and serve to predict possible toxic effects. Moreover, such individual profiling will provide a basis for the development of future patient-centered therapies, which will optimize therapeutic impact and minimize toxic side effects.

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References

Abdulla, P., Coe, I.R., 2007. Characterization and functional analysis of the promoter for the human equilibrative nucleoside transporter gene, hENT1. Nucleosides Nucleotides Nucleic Acids 26 (1), 99–110.

Archer, R.G., Pitelka, V., Hammond, J.R., 2004. Nucleoside transporter subtype expression and function in rat skeletal muscle microvascular endothelial cells. Br. J. Pharmacol. 143, 202–214.

Baldwin, S.A., Beal, P.R., Yao, S.Y.M., King, A.E., Cass, C.E., Yong, J.D., 2004. The equilibrative nucleoside transporter family, SLC29. Pflugers Arch. 447 (5), 735–743.

Baldwin, S.A., Yao, S.Y.M., Hyde, R.J., Ng, A.M.L., Foppolo, S., Barnes, K., Ritzel, M.W., Cass, C.E., Young, J.D., 2005. Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. J. Biol. Chem. 280 (16), 15880–15887.

Barnes, K., Dobrzynkski, H., Foppolo, S., Beal, P.R., Ismat, F., Scullion, E.R., Sun, L., Tellez, J., Ritzel, M.W., Claycomb, W.C., Cass, C.E., Young, J.D., Billeter-Clark, R., Boyett, M.R., Baldwin, S.A., 2006. Distribution and functional characterization of equilibrative nucleoside transporter-4, a novel cardiac adenosine transporter activated at acidic pH. Circ. Res. 99 (5), 510–519.

Barrett, T., Troup, D.B., Wilhite, S.E., Ledoux, P., Rudnev, D., Evangelista, C., Kim, I.F., Soboleva, A., Tomashevsky, M., Marshall, K.A., Phillippy, K.H., Sherman, P.M., Muertter, R.N., Edgar, R., 2009. NCBI GEO: archive for high-throughput functional genomic data. Nucleic Acids Res. 37, D5–1518.

Beck, D.W., Vinters, H.V., Moore, S.A., Hart, M.N., Cancilla, P.A., 1993. Uptake of adenosine by cultured cerebral vascular smooth muscle cells. J. Neurochem. 41, 939–941.

Belhomme, D., Peynet, J., Florens, E., Tibourtine, O., Kitakaze, M., Menasché, P., 2000. Is adenosine preconditioning truly cardioprotective in coronary artery bypass surgery. Ann. Thorac. Surg. 70, 590–594.

Chaudary, N., Shuralyova, I., Liron, T., Sweeney, G., Coe, I.R., 2002. Transport characteristics of HL-1 cells: a new model for the study of adenosine physiology in cardiomyocytes. Biochem. Cell Biol. 80, 655–665.

Chen, J., Rinaldo, L., Lim, S.J., Young, H., Messing, R.O., Choi, D.S., 2007. The type 1 equilibrative nucleoside transporter regulates anxiety-like behavior in mice. Genes Brain Behav. 6 (8), 776–783.

Choi, D.S., Cascini, M.G., Mailliard, W., Young, H., Paredes, P., McMahon, T., Diamond, I., Bonci, I., Messing, R.O., 2004. The type 1 equilibrative nucleoside transporter regulates ethanol intoxication and preference. Nat. Neurosci. 7 (8), 855–861.

Damaraju, V.L., Damaraju, S., Young, J.D., Baldwin, S.A., Mackey, J., Sawyer, M.B., Cass, C.E., 2003. Nucleoside anticancer drugs: the role of nucleoside transporter in resistance to cancer chemotherapy. Oncogene 22, 7524–7536.

Damaraju, V.L., Elwi, A.N., Hunter, C., Carpenter, P., Santos, C., Barron, G.M., Sun, X., Baldwin, S.A., Young, J.D., Mackey, J.R., Sawyer, M.B., Cass, C.E., 2007. Localization of broadly selective equilibrative and concentrative nucleoside transporters, hENT1 and hCNT3, in human kidney. Am. J. Physiol. Renal Physiol. 293, F200–F211.

Damaraju, V.L., Sawyer, M.B., Mackey, J.R., Young, J.D., Cass, C.E., 2009. Human nucleoside transporters: biomarkers for response to nucleoside drugs. Nucleosides Nucleotides Nucleic Acids 28, 450–463.

de Jong, J.W., de Jonge, R., Keijzer, E., Bradamante, S., 2000. The role of adenosine in preconditioning. Pharmacol. Ther. 9, 141–147.

Edgar, R., Domrachev, M., Lash, A.E., 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30 (1), 207–210.

Eltzschig, H.K., Abdulla, P., Hoffman, E., Hamilton, K.E., Daniels, D., Schonfeld, C., Löffler, M., Reyes, G., Duszenko, M., Karhausen, J., Robinson, A., Westerman, K.A., Coe, I.R., Colgan, S.P., 2005. HIF-1-dependent repression of endothelial equilibrative nucleoside transporter (ENT) in hypoxia. J. Exp. Med. 202 (11), 1493–1505.

Floyd, J.D., Nguyen, D.T., Lobins, R.L., Bashir, Q., Doll, D.C., Perry, M.C., 2005. Cardiotoxicity of cancer therapy. J. Clin. Oncol. 23, 7685–7696.

Govindarajan, R., Bakken, A.H., Hudkins, K.L., Lai, Y., Casado, F.J., Pastor-Anglada, M., Tse, C.M., Hayashi, J., Unadkat, J.D., 2007. In situ hybridization and immunolocalization of concentrative and equilibrative nucleoside transporters in the human intestine, liver, kidneys, and the placenta. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, R1809–R1822.

Griffith, D.A., Jarvis, S.M., 1996. Nucleoside and nucleobase transport systems in mammalian cells. Biochem. Biophys. Acta. 1286, 153–218.

Handa, M., Choi, D.S., Caldeiro, R.M., Messing, R.O., Gordon, A.S., Diamond, I., 2001. Cloning of a novel isoform of the mouse NBMPR-sensitive equilibrative nucleoside transporter (ENT1) lacking a putative phosphorylation site. Gene 262, 301–307.

Kim, H.H., Liao, J.K., 2008. Translational therapeutics of dipyridamole. Arterioscler. Thromb. Vasc. Biol. 28, s39–s42.

King, A.E., Ackley, M.A., Cass, C.E., Young, J.D., Baldwin, S.A., 2006. Nucleoside transporters: from scavengers to novel therapeutic targets. Trends Pharmacol. Sci. 27, 416–425.

Kong, W., Engel, K., Wang, J., 2004. Mammalian nucleoside transporters. Curr. Drug Metab. 5, 63–84.

Loffler, M., Morote-Garcia, J.C., Eltzschig, S.A., Coe, I.R., Eltzschig, H.K., 2007. Physiological roles of vascular nucleoside transporters. Arterioscler. Thromb. Vasc. Biol. 27, 1004–1013.

Lu, H., Chen, C., Klaassen, C., 2004. Tissue distribution of concentrative and equilibrative nucleoside transporters in male and female rats and mice. Drug Metab. Dispos. 32, 1455–1461.

Molina-Arcas, M., Trigueros-Motos, L., Casado, J.F., Pastor-Anglada, M., 2008. Physiological and pharmacological roles of nucleoside transporter proteins. Nucleosides Nucleotides Nucleic Acids 27, 769–778.

Morote-Garcia, J.C., Rosenberger, P., Nivillac, N.I., Coe, I.R., Eltzschig, H.K., 2009. HIF-dependent repression of nucleoside transporter ENT2 attenuates mucosal inflammation during intestinal hypoxia. Gastroenterology 136 (2), 607–618.

Mubagwa, K., Flameng, W., 2001. Adenosine, adenosine receptors and myocardial protection: an updated overview. Cardiovasc. Res. 52, 25–39.

Naydenova, Z., Rose, J., Coe, I.R., 2008. Inosine and equilibrative nucleoside transporter 2 (ENT2) contribute to hypoxic preconditioning in the murine cariomyocyte cell line, HL-1. Am. J. Physiol. Heart 294 (6), H2687–H2692.

NCBI, 2009. Gene Expression omnibus database. GEO, GSM or GDS; 422, 596, 1096, 181, 2206 and 82403 (accessed August 2009) http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE.

Pastor-Anglada, M., Cano-Soldado, P., Molina-Arcas, Lostao, M.P., Larrayoz, I., Martinez-Picado, J., Casado, F.J., 2005. Cell entry and export of nucleoside analogues. Virus Res. 107, 151–164.

Pelleg, A., Pennock, R.S., Kutalek, S., 2002. Proarrhythmic effects of adenosine: one decade of clinical data. Am. J. Ther. 9, 141–147.

Pennycooke, M., Chaudary, N., Shuralyova, I., Zhang, Y., Coe, I.R., 2001. Differential expression of human nucleoside transporters in normal and tumor tissue. Biochem. Biophys. Res. Commun. 280 (3), 951–959.

Peres, E., Levine, J.E., Khaled, Y.A., Ibrahim, R.B., Braun, T.M., Krijanovski, O.I., Mineishi, S., Abidi, M.H., 2010. Cardiac complications in patients undergoing a reduced-intensity conditioning hematopoietic stem cell transplantation. Bone Marrow Transplant. 45 (1), 149–152.

Rose, J.B., Coe, I.R., 2008. Physiology of nucleoside transporters: back to the future.. Physiology 23, 41–48.

Rose, J.B., Naydenova, Z., Bang, A., Eguchi, M., Sweeney, G., Choi, D., Hammond, J.R., Coe, I.R., 2010. Equilibrative nucleoside transporter 1 (ENT1) plays an essential role in cardioprotection. Am. J. Physiol. Heart Circ. Physiol. 298 (3), H771–H777.

Santini, D., Vincenzi, B., Fratto, M.E., Perrone, G., Lai, R., Catalano, V., Cass, C., Ruffini, P.A., Spoto, C., Muretto, P., Rizzo, S., Muda, A.O., Mackey, J.R., Russo, A., Tonini, G., Graziano, F., 2010. Prognostic role of human equilibrative transporter 1 (hENT1) in patients with resected gastric cancer. J. Cell Physiol. 223 (2), 384–388.

Wojtal, K.A., Eloranta, J.J., Hruz, P., Gutman, H., Drewe, J., Staumann, A., Beglinger, C., Fried, M., Kullak-Ublick, G.A., Vavricka, S.R., 2009. Changes in mRNA expression levels of solute carrier transporters in inflammatory bowel disease patients. Drug Metab. Dispos. 37 (9), 1871–1877.

Young, J.D., Yao, S.Y.M., Sun, L., Cass, C.E., Baldwin, S.A., 2008. Human equilibrative nucleoside transporter (ENT) family of nucleoside and nucleobase transporter proteins. Xenobiotica 38 (7–8), 995–1021.

Zhang, J., Visser, F., King, K.M., Baldwin, S.A., Young, J.D., Cass, C.E., 2007. The role of nucleoside transporters in cancer chemotherapy with nucleoside drugs. Cancer Metastasis Rev. 26, 85–110.