Title page

Title:

Stereoselective handling of perhexiline: implications regarding accumulation within the human myocardium

Running title:

Perhexiline enantiomers in myocardium

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ABSTRACT (150-250 words)

Purpose: Perhexiline is a prophylactic anti-ischaemic agent with weak calcium antagonist effect which has been increasingly utilized in the management of refractory angina. The metabolic clearance of perhexiline is modulated by CYP2D6 metaboliser status and stereoselectivity. The current study sought to determine (1) whether the acute accumulation of perhexiline in the myocardium is stereoselective, and (2) to investigate the relationship between duration of short-term therapy and the potential stereoselective effects of perhexiline within myocardium.

Method: Patients (n=129) from the active arm of a randomised controlled trial of preoperative perhexiline in cardiac surgery, were treated with oral perhexiline for a median of 9 days. Correlates of atrial and ventricular concentrations of enantiomers were sought via univariate followed by multivariate analyses.

Results: Myocardial uptake of both (+) and (-)-perhexiline was greater in ventricles than atria, and there was more rapid clearance of (-) than (+)-perhexiline. The main determinants of atrial uptake of both (+) and (-)-perhexiline were the plasma concentrations [(+)-perhexiline: β=-0.256, p=0.015; (-)-perhexiline: β=-0.347, p=0.001] and patients' age [(+)-perhexiline: β=0.3, p=0.004; (-)-perhexiline: β=0.288, p=0.005]. Atrial uptake of (+)-enantiomer also varied directly with duration of therapy (r=0.29, p=0.004), while atrial uptake of (-)-perhexiline varied inversely with simultaneous heart rate (r=-0.22, p=0.03).

Conclusion: (1) Uptake of both perhexiline enantiomers into atrium is greater with advanced age, and displays evidence of both saturability and minor stereoselectivity. (2) Atrial uptake of (-) perhexiline may selectively modulate heart rate reduction.

(Word count: 235 words)

INTRODUCTION

Perhexiline is a metabolic modulating agent that was first introduced into clinical practice as a prophylactic anti-anginal drug in the 1970s. It exerts multiple effects which increase efficiency of myocardial metabolism: amongst these it is known to inhibit mitochondrial carnitine palmitoyl transferase-1 [1], potentially resulting in a shift from fatty acid to glucose utilisation with more adenosine triphosphate production per unit oxygen consumption.

Despite the considerable clinical efficacy of perhexiline in prophylaxis of exertional angina [2, 3], its use declined because of the substantial risk of hepato- and neuro-toxicity during chronic therapy [4-6]. However, it emerged that this toxicity reflected drug accumulation in plasma [7] which in turn resulted from inter-individual variability in CYP2D6-mediated metabolism [8, 9]. With widespread availability of therapeutic drug monitoring [10, 11] and greater understanding of its potential widespread utility for disorders of cardiac energetics [12], the clinical use of perhexiline is now increasing.

Kinetically, perhexiline is a lipophilic drug well absorbed from the gastrointestinal tract. It is highly protein bound and has a large volume of distribution. As it is subject to hepatic metabolism by CYP2D6, the plasma half-life of perhexiline ranges from several hours to several weeks [7, 13].

A number of studies have specifically evaluated short-term utility of perhexiline in the management of potential cardiac crises, such as the management of high risk

patients with unstable ischaemia and for cardioprotection during coronary revascularisation [14-16]. The recently reported CASPER trial evaluated its use as an adjunct to myocardial protection in patients undergoing coronary artery surgery but found no clear-cut beneficial effect of prophylactic perhexiline therapy [17]. During this trial, atrial and ventricular myocardial biopsies were taken at the time of surgery. We have previously reported on analyses of these samples to evaluate the relationship between plasma and myocardial drug concentrations [18]. The objective of the current analysis stems from our recent observation that the effects of racemic perhexiline may result from unequal steady-state concentrations of its two enantiomers [19].

In human liver microsomes, the intrinsic clearance of (-)-perhexiline is greater than that of the (+)-enantiomer [20], which explains the greater clearance rate of (-)-perhexiline at steady state in patients receiving racemic perhexiline [21]. Interestingly, it appears from studies in a rat model, that the safety of the two enantiomers may vary, with greater hepatotoxicity associated with the (+)-enantiomer [22].

Therefore, we have re-evaluated the CASPER data in order to determine:

(1) Whether atrial and ventricular myocardial accumulation of perhexiline during short-term treatment are stereoselective, and whether this reflects similar trends in plasma enantiomer concentrations.

(2) The relationship between duration of (short-term) perhexiline therapy and the potential for stereoselective effects of perhexiline within the myocardium.

METHODS

Data were mainly derived from patients in the active treatment arm of the CASPER (Coronary Artery Surgery with PERhexiline therapy) trial (NCT00845364). In brief, this was a double-blind, randomized, placebo-controlled clinical trial evaluating whether preoperative oral perhexiline to improve myocardial protection in patients undergoing cardiac surgery. Non-diabetic patients, who were not taking CYP2D6 inhibitors, undergoing first time coronary artery bypass graft surgery were randomized to either perhexiline maleate or placebo for at least five days prior to surgery. All patients received the following medication regimen: 200mg twice daily for three days then 100mg twice daily until the morning of the surgery [17]. On-treatment heart rate was determined by preoperative resting electrocardiogram.

Following induction of anaesthesia but prior to commencing surgery, the haemodynamic status of each patient, including arterial pressures and cardiac index were determined. Plasma collected at this timepoint was centrifuged and stored at -80°C; this has previously been used to phenotype patients for CYP2D6 metaboliser status [17] according to the plasma concentration ratios of perhexiline monohydroxylated metabolite to parent drug [13]. During preparation for cardiopulmonary bypass but prior to aortic cross-clamping, right atrial and left ventricular myocardial biopsies were obtained, as previously described [17]. These biopsies were initially snap-frozen in liquid nitrogen and stored at -80°C, then later digested in 0.15 M of phosphate buffer solution (pH 6.0) using a homogenizer and tissue grinder to form a suspension (approximately 100mg tissue in 5ml buffer). Plasma and myocardial perhexiline enantiomer concentrations were determined

utilizing a modification of a previously described HPLC assay [23]. Thresholds for detection of myocardial (+) and (-) perhexiline were 0.01mg/L with sensitivity and accuracy between 0.01 and 2.00mg/L with intra-assay coefficients of variation and bias <20% at 0.01mg/L. [23]

ANALYSIS OF RESULTS

1. Determination of relative uptake of enantiomers

   Concentrations of each enantiomer in atrial and ventricular myocardium were correlated with plasma enantiomer concentrations to derive tissue to plasma concentration ratios.

   Potential stereo-selectivity of uptake was evaluated via determination of:

   I. Differential (+) to (-) enantiomer ratio in myocardium versus plasma (in order to determine whether uptake, rather than clearance, might engender stereoselective myocardial effects).

   II. Percentage of (+)-enantiomer concentrations in plasma and myocardium, in relation to time (in order to determine the net effects of stereoselective kinetics on the myocardial uptake of the drug).

2. Identification of determinants or correlates of myocardial (+) and (-)-perhexiline uptake

   Univariate (evaluated utilizing Spearman's correlation) followed by multivariate analyses were utilized for assessment of the atrial uptake of enantiomers. Parameters evaluated were plasma concentration, metaboliser status, age, weight, duration of therapy, resting heart rate, creatinine

clearance, and cardiac index. Multivariate backward stepwise analyses were performed using statistical software SPSS (version 20, Chicago).

3. Data from poor metabolisers (n=7) were excluded from analysis (unless specified otherwise) because of potential for differential clearance mechanisms and non-attainment of near-steady state kinetics.

4. Data are expressed throughout as mean ± SD for normally distributed parameters and median (interquartile range) for skewed data.

RESULTS

1. Patient demographics: metaboliser status and absence of dose titration

129 patients from the active arm of CASPER trial were included and their clinical characteristics are summarised in Table 1. While patients had well-preserved renal function, their pre-treatment cardiac function is not known, with formal estimation of cardiac indices and heart rates performed following the induction of anaesthesia for surgery; these data therefore reflect the potential interaction of perhexiline and pre-treatment status. However, the generally low cardiac indices in these patients imply some degree of systolic left ventricular dysfunction at least at the time of measurement. The median plasma concentrations of perhexiline at the time of blood sampling were 0.27mg/L (IQR: 0.13 - 0.47), with approximately one third of patients having subtherapeutic levels (i.e. <0.15mg/L).

Figure 1 examines the relationship between plasma cis-OH-perhexiline/ perhexiline ratio (used to categorise metaboliser status) and plasma perhexiline concentration at the time of surgery. It is apparent that there was substantial variability in racemic perhexiline concentrations, such that therapeutic concentrations were not generally attained in rapid metabolisers. There were a total of 7 poor metabolisers, all of whom attained therapeutic or potentially toxic perhexiline concentrations.

2. Myocardial concentrations of enantiomers

The relationships between plasma and atrial or ventricular concentrations of (+) and (-)-perhexiline are depicted in Figures 2A and 2B respectively. There were strong direct correlations for both enantiomers, with slightly greater concentrations of both enantiomers in ventricle than atrium ($p<0.001$ for both, Spearman's test).

The impact of metaboliser status on these plasma: myocardial concentration relationships is summarised in Table 2. All but two plasma enantiomer concentrations were >0.02mg/L. In summary, plasma concentrations of (+)-perhexiline tended to be greater than those of (-)-perhexiline for both plasma and myocardium, irrespective of metaboliser status.

We next sought to determine whether stereoselective clearance of perhexiline and/or of its uptake into myocardium might vary with duration of therapy. The proportion of (+)-perhexiline in plasma did not vary significantly with duration of therapy (Figure 3A), while that in atria increased significantly ($p=0.004$) with time (Figure 3B). Thus the ratio of (+)-perhexiline in the atrium to that in plasma tended to increase progressively ($r=0.19$, $p=0.07$).

Concentrations of both (+)- and (-)-perhexiline into the ventricle increased significantly with time ($p=0.005$ and 0.004 respectively, Figure 4), with no significant difference between the enantiomers.

3. Multivariate correlates of uptake of enantiomer

Table 3 summarizes atrial: plasma concentration ratios, with all patients (excluding poor metabolisers) evaluated. Plasma perhexiline concentration was a strong negative correlate and age was a positive correlate of this ratio for both enantiomers. Uptake of (-)-perhexiline also varied inversely with simultaneous heart rate. Similar trends were also apparent with univariate correlations (Figure 5).

DISCUSSIONS

The current analyses complement our previously published evaluation of the uptake of racemic perhexiline into the human myocardium [18]. It is apparent from the current studies that the perhexiline dosing regimen utilized, with inadequate time available for adjustment of dosage on the basis of metaboliser status, led to wide variability in plasma and myocardial drug concentrations, but nonetheless, allowed exploration of determinants of myocardial uptake of perhexiline enantiomers.

The main findings of the current analysis are that:

1. Plasma concentrations of (+) or (-)-perhexiline and the corresponding myocardial concentrations are closely and directly correlated.
2. Just as plasma (+)-enantiomer concentration exceeds that of (-)-enantiomer, similar trends are present in myocardium, especially atrial muscle.
3. Myocardial uptake of each enantiomer also depends on plasma drug concentrations and patients' age, where a strong inverse relationship is present.
4. Atrial uptake of (+)-perhexiline also varies with on duration of therapy.
5. Atrial uptake of (-)-perhexiline varies inversely with simultaneous heart rate.

These findings carry a number of important implications regarding the myocardial handling and effects of perhexiline enantiomers.

The higher concentrations of (+) than (-)-enantiomer in plasma are consistent with more rapid clearance of the latter. These data are consistent with previous

publications regarding the stereoselective clearance of perhexiline [20, 21]. On the other hand, myocardial concentration ratios of the enantiomers generally parallel with those in plasma, suggesting absence of major stereoselectivity in myocardial uptake. The exception to this is the small but statistically significant increase in the proportion of (+)-enantiomer in the atrial myocardium with time (Figure 3B). These data suggest that myocardial uptake and efflux of (-)-perhexiline may be slightly more rapid than that of the (+)-enantiomer.

The relatively prolonged time required to reach steady-state perhexiline concentrations in the ventricular myocardium may reflect greater distribution into mitochondria [24], known to represent a site of intracellular drug accumulation for perhexiline in hepatocytes [25].

As regards the interactions between advanced age and cardiac uptake of the enantiomers, the current data suggest that uptake of both perhexiline enantiomers is greater in older patients, perhaps related to decreased skeletal muscle mass. Indeed we have previously also shown that steady-state dosage requirements for perhexiline tend to fall with age [26], and in the current study plasma perhexiline concentrations increased with patient age ($r=0.24$, $p=0.029$). Together, these data suggest that elderly individuals may benefit from perhexiline therapy despite apparently borderline subtherapeutic plasma drug levels.

Finally, atrial uptake of (-)-perhexiline varied inversely with concurrent heart rate. This is not consistent with the usual accelerating effect of tachycardia on myocardial drug uptake [27] and suggests another mechanism for association. Perhexiline is a

weak L-type calcium antagonist [28, 29], although its effects on the myocardium have undergone only limited study. The IC_{50} for inhibition of calcium fluxes in chick embryo ventricular myocardium by racemic perhexiline was 8.3×10^{-7} M [28]. In the current study, atrial concentrations of (-)-perhexiline approximated to these values. It is therefore possible that the calcium antagonist effect of rac-perhexiline is mediated primarily by the (-)-enantiomer, despite the current limitation of the understanding of pharmacological actions of the perhexiline enantiomers.

The current study has several limitations. Most importantly, correlation with cardiac effects of perhexiline is limited by the absence of true pre-treatment data and that evaluation of the effects of the enantiomers is only extrapolated on the basis of administration of rac-perhexiline. Second, myocardial drug content after a median of 9 days of therapy reflects both uptake into and efflux from the myocardium, and there is no way to determine the precise component of each, given that steady state has not been reached. Finally, the full implications of the widely variable plasma perhexiline concentrations on variability in drug uptake cannot be fully understood without a strategy of multiple drug dosing per patient.

The current results also need to be related to the clinical context. Recently we have reported on acute loading of perhexiline in patients with severe ischaemia [14, 15]. The current data are consistent with the idea that there may be early onset of cardioprotective effects, especially in the elderly, at a time when plasma drug concentrations are notionally subtherapeutic. Furthermore, the data regarding heart rate correlations should stimulate evaluation of whether the calcium antagonist

effects of perhexiline can be dissociated from its “metabolic” cardioprotective effects [30, 31], by selective administration of the (+)-enantiomer.

TABLE AND FIGURE LEGENDS

Table 1:

Clinical characteristics of patients (n=129)

a. Expressed as median (interquartile range)

† On treatment, whilst under anaesthesia immediately prior to surgery.

Table 2:

Perhexiline enantiomer concentrations in plasma, atrial and ventricular myocardium across all metabolic phenotypes. Metabolizer status was defined by the ratio of plasma concentrations of cis-hydroxyperhexiline to perhexiline. Poor metabolizer was defined by a ratio of ≤ 0.3; intermediate metabolizer was defined by a ratio of 0.3 to 2.5; extensive metabolizer was defined by a ratio of 2.5 to 20; and ultra-rapid metabolizer was defined by a ratio of ≥ 20.

Data expressed as mean ± SD or median (interquartile range).

* $p<0.05$ vs antipode (Wilcoxon); $^{\Delta}$ $p<0.005$ vs atrial:plasma ratio

Table 3:

Correlates of the atrial: plasma ratio (net uptake) of each enantiomer on multivariate analyses (excluding poor metabolisers).

Figure 1:

The relationship between rate of perhexiline metabolism (expressed as cis-OH-perhexiline/ perhexiline ratio) and plasma perhexiline enantiomer concentrations at the time of surgery.

Data to the left of the dashed line (ratio <0.3) correspond to "poor metaboliser" status.

Figure 2:

Correlations between plasma and myocardial (atrial and ventricular) concentrations for (A) (+)-perhexiline, and (B) (-)-perhexiline. Spearman's correlations are shown.

Figure 3:

Variations in the percentage of (+)-perhexiline in (A) plasma and (B) atrial myocardium relative to duration of therapy. Spearman's correlations are shown.

Figure 4:

Impact of duration of treatment on concentrations of perhexiline enantiomers in ventricular myocardium. Spearman's correlations are shown.

Figure 5:

Univariate comparisons (Spearman's correlation) between (A) patients' age and (B) resting heart rate and uptake ratio of each enantiomer into atrial myocardium.

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DISCLOSURE

MPF is inventor of the method of use patents for perhexiline in heart muscle diseases. GL and BCS are inventors of patent for use of enantiomers of perhexiline.

REFERENCE

[1] J. A. Kennedy, S. A. Unger, and J. D. Horowitz, "Inhibition of carnitine palmitoyltransferase-1 in rat heart and liver by perhexiline and amiodarone," *Biochem Pharmacol,* vol. 52, pp. 273-80, Jul 26 1996.

[2] L. N. Roberts and G. P. Mason, "Clinical trial of a new antianginal drug: perhexiline maleate," *J Clin Pharmacol New Drugs,* vol. 12, pp. 342-8, Aug-Sep 1972.

[3] C. J. Burns-Cox, K. P. Chandrasekhar, H. Ikram, T. H. Peirce, J. Pilcher, C. D. Quinlan, *et al.*, "Clinical evaluation of perhexiline maleate in patients with angina pectoris," *Br Med J,* vol. 4, pp. 586-8, Dec 4 1971.

[4] C. Bourrat, J. J. Viala, and J. P. Guastala, "[Letter: Peripheral neuropathy after prolonged adsorption of perhexiline maleate. 2 cases]," *Nouv Presse Med,* vol. 4, p. 2528, Oct 18 1975.

[5] D. M. Fraser, I. W. Campbell, and H. C. Miller, "Peripheral and autonomic neuropathy after treatment with perhexiline maleate," *Br Med J,* vol. 2, pp. 675-6, Sep 10 1977.

[6] G. Le Menn, D. Mabin, and P. Penther, "[Slow and incomplete regression of peripheral neuropathy due to perhexiline maleate]," *Ann Cardiol Angeiol (Paris),* vol. 26, pp. 149-50, Mar-Apr 1977.

[7] E. Singlas, M. A. Goujet, and P. Simon, "Pharmacokinetics of perhexiline maleate in anginal patients with and without peripheral neuropathy," *Eur J Clin Pharmacol,* vol. 14, pp. 195-201, Nov 27 1978.

[8] R. G. Cooper, D. A. Evans, and E. J. Whibley, "Polymorphic hydroxylation of perhexiline maleate in man," *J Med Genet,* vol. 21, pp. 27-33, Feb 1984.

[9] M. L. Barclay, S. M. Sawyers, E. J. Begg, M. Zhang, R. L. Roberts, M. A. Kennedy, *et al.*, "Correlation of CYP2D6 genotype with perhexiline phenotypic metabolizer status," *Pharmacogenetics,* vol. 13, pp. 627-32, Oct 2003.

[10] P. L. Cole, A. D. Beamer, N. McGowan, C. O. Cantillon, K. Benfell, R. A. Kelly, *et al.*, "Efficacy and safety of perhexiline maleate in refractory angina. A double-blind placebo-controlled clinical trial of a novel antianginal agent," *Circulation,* vol. 81, pp. 1260-70, Apr 1990.

[11] J. D. Horowitz, S. T. Sia, P. S. Macdonald, A. J. Goble, and W. J. Louis, "Perhexiline maleate treatment for severe angina pectoris--correlations with pharmacokinetics," *Int J Cardiol,* vol. 13, pp. 219-29, Nov 1986.

[12] R. M. Beadle and M. Frenneaux, "Modification of myocardial substrate utilisation: a new therapeutic paradigm in cardiovascular disease," *Heart,* vol. 96, pp. 824-30, Jun 2010.

[13] B. C. Sallustio, I. S. Westley, and R. G. Morris, "Pharmacokinetics of the antianginal agent perhexiline: relationship between metabolic ratio and steady-state dose," *Br J Clin Pharmacol,* vol. 54, pp. 107-14, Aug 2002.

[14] A. Philpott, S. Chandy, R. Morris, and J. D. Horowitz, "Development of a regimen for rapid initiation of perhexiline therapy in acute coronary syndromes," *Intern Med J,* vol. 34, pp. 361-3, Jun 2004.

[15] S. Stewart, D. W. Voss, D. L. Northey, and J. D. Horowitz, "Relationship between plasma perhexiline concentration and symptomatic status during short-term perhexiline therapy," *Ther Drug Monit,* vol. 18, pp. 635-9, Dec 1996.

[16] E. A. Liberts, S. R. Willoughby, J. A. Kennedy, and J. D. Horowitz, "Effects of perhexiline and nitroglycerin on vascular, neutrophil and platelet function in patients with stable angina pectoris," *Eur J Pharmacol,* vol. 560, pp. 49-55, Mar 29 2007.

[17] N. E. Drury, N. J. Howell, M. J. Calvert, R. J. Weber, E. L. Senanayake, M. E. Lewis, *et al.*, "The effect of perhexiline on myocardial protection during coronary artery surgery: a two-centre, randomized, double-blind, placebo-controlled trial," *Eur J Cardiothorac Surg,* Jun 19 2014.

[18] N. E. Drury, G. Licari, C. R. Chong, N. J. Howell, M. P. Frenneaux, J. D. Horowitz, *et al.*, "Relationship between plasma, atrial and ventricular perhexiline concentrations in humans: insights into factors affecting myocardial uptake," *Br J Clin Pharmacol,* vol. 77, pp. 789-95, May 2014.

[19] B. J. Davies, J. K. Coller, H. M. James, A. A. Somogyi, J. D. Horowitz, and B. C. Sallustio, "The influence of CYP2D6 genotype on trough plasma perhexiline and cis-OH-perhexiline concentrations following a standard loading regimen in patients with myocardial ischaemia," *Br J Clin Pharmacol,* vol. 61, pp. 321-5, Mar 2006.

[20] B. J. Davies, J. K. Coller, A. A. Somogyi, R. W. Milne, and B. C. Sallustio, "CYP2B6, CYP2D6, and CYP3A4 catalyze the primary oxidative metabolism of perhexiline enantiomers by human liver microsomes," *Drug Metab Dispos,* vol. 35, pp. 128-38, Jan 2007.

[21] B. J. Gould, A. G. Amoah, and D. V. Parke, "Stereoselective pharmacokinetics of perhexiline," *Xenobiotica,* vol. 16, pp. 491-502, May 1986.

[22] G. Licari, Sallustio B.C., Somogyi A.A., Milne R.W., "The enantiomers of the myocardial metabolic agent perhexiline display divergent effets on hepatic energy metabolism and peripheral neural function in rats," *Global Heart,* vol. 9, p. e272, 2014 2014.

[23] B. J. Davies, M. K. Herbert, J. A. Culbert, S. M. Pyke, J. K. Coller, A. A. Somogyi, *et al.*, "Enantioselective assay for the determination of perhexiline enantiomers in human plasma by liquid chromatography," *J Chromatogr B Analyt Technol Biomed Life Sci,* vol. 832, pp. 114-20, Feb 17 2006.

[24] M. J. Legato, "Ultrastructure of the atrial, ventricular, and Purkinje cell, with special reference to the genesis of arrhythmias," *Circulation,* vol. 47, pp. 178-89, Jan 1973.

[25] D. Deschamps, V. DeBeco, C. Fisch, B. Fromenty, A. Guillouzo, and D. Pessayre, "Inhibition by perhexiline of oxidative phosphorylation and the beta-oxidation of fatty acids: possible role in pseudoalcoholic liver lesions," *Hepatology,* vol. 19, pp. 948-61, Apr 1994.

[26] L. H. Ling, W. Chik, P. Averbuj, P. K. Pati, A. L. Sverdlov, D. T. Ngo, *et al.*, "Effects of aging, renal dysfunction, left ventricular systolic impairment, and weight on steady state pharmacokinetics of perhexiline," *Ther Drug Monit,* vol. 33, pp. 251-6, Apr 2011.

[27] J. D. Horowitz and A. C. Powell, "Myocardial uptake of drugs and clinical effects," *Clin Pharmacokinet,* vol. 11, pp. 354-71, Sep-Oct 1986.

[28] W. H. Barry, J. D. Horowitz, and T. W. Smith, "Comparison of negative inotropic potency, reversibility, and effects on calcium influx of six calcium channel antagonists in cultured myocardial cells," *Br J Pharmacol,* vol. 85, pp. 51-9, May 1985.

[29] H. Ono and M. Kimura, "Effect of Ca2+-antagonistic vasodilators, diltiazem, nifedipine, perhexiline and verapamil, on platelet aggregation in vitro," *Arzneimittelforschung,* vol. 31, pp. 1131-4, 1981.

[30] K. Abozguia, P. Elliott, W. McKenna, T. T. Phan, G. Nallur-Shivu, I. Ahmed, *et al.*, "Metabolic modulator perhexiline corrects energy deficiency and improves exercise

capacity in symptomatic hypertrophic cardiomyopathy," *Circulation*, vol. 122, pp. 1562-9, Oct 19 2010.

[31] L. Lee, R. Campbell, M. Scheuermann-Freestone, R. Taylor, P. Gunaruwan, L. Williams, *et al.*, "Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment," *Circulation*, vol. 112, pp. 3280-8, Nov 22 2005.

Table 1:

Clinical characteristics of patients (n=129)

a. Expressed as median (interquartile range)

† On treatment, whilst under anaesthesia immediately prior to surgery.

Parameters	
Age (years) [a]	66 (58-73) [a]
Weight (kg)	83 ± 14
Creatinine clearance (ml/min) [a]	70 (60 - 85) [a]
Baseline cardiac index	2.04 (1.78 - 2.34) [a]
Resting heart rate (beats per minute) †	59 ± 10
Duration of therapy (days)[a]	9 (6 - 12) [a]

Table 2:

Perhexiline enantiomer concentrations in plasma, atrial and ventricular myocardium across all metabolic phenotypes. Metabolizer status was defined by the ratio of plasma concentrations of cis-hydroxyperhexiline to perhexiline. Poor metabolizer was defined by a ratio of ≤ 0.3; intermediate metabolizer was defined by a ratio of 0.3 to 2.5; extensive metabolizer was defined by a ratio of 2.5 to 20; and ultra-rapid metabolizer was defined by a ratio of ≥ 20.

Data expressed as mean ± SD or median (interquartile range).

* p<0.05 vs antipode (Wilcoxon); $^{\Delta}$ p<0.005 vs atrial:plasma ratio

Metaboliser status	**Plasma concentrations (mg/L)**		**Atrial concentrations (mg/kg)**		**Ventricular concentrations (mg/kg)**	
	(+)	**(-)**	**(+)**	**(-)**	**(+)**	**(-)**
Poor metaboliser	n=7		n=3		n=1	
	0.67 ± 0.32 *	0.48 ± 0.21	16.57 ± 6.57	13.22 ± 4.89	17.5	12.5
Intermediate metaboliser	n =28		n =19		n =6	
	0.28 (0.24-0.37) *	0.23 (0.18-0.29)	6.17 (5.45-8.16) *	5.11 (4.28-6.58)	11.07 ± 5.41$^{\Delta}$	9.08 ± 4.54 $^{\Delta}$
Extensive metabolisers	n =85		n =64		n =21	
	0.11 (0.07-	0.08 (0.05-	2.77 (1.50-	2.17 (1.26-	5.16 ± 2.48 $^{\Delta}$	3.55 ± 2.17 $^{\Delta}$

	0.17) *	0.13)	3.99) *	3.24)		
Ultrarapid	n =9		n =8		n =2	
metabolisers	0.03 (0.026-0.037) *	0.023 (0.017-0.027)	0.84 ± 0.31	0.623 ± 0.314	3.41 ± 0.41	1.56 ± 2.21

Table 3:

Correlates of the atrial: plasma ratio (net uptake) of each enantiomer on multivariate analyses (excluding poor metabolisers).

Determinant	Correlates	β coefficient	p-value
Atrial:plasma ratio of (+)-perhexiline	Plasma (+)-perhexiline concentration	-0.256	0.015
	Duration of therapy	0.228	0.025
	Age	0.300	0.004
Atrial: plasma ratio of (-)-perhexiline	Plasma (-)-perhexiline concentration	-0.347	0.001
	Age	0.288	0.005
	On-treatment resting heart rate	-0.240	0.015