Investigating pharmacological modification of cardiac pyruvate metabolism using hyperpolarized magnetic resonance

Lydia M Le Page[1], Michael S Dodd[1], Daniel R Ball[1], Vicky Ball[1], Huw B Jones[2], Edvin Johansson[3], and Damian J Tyler[1]
[1]*University of Oxford, Oxford, United Kingdom,* [2]*AstraZeneca, Alderley Park, Macclesfield, United Kingdom,* [3]*AstraZeneca, Mölndal, Sweden*

Introduction: The pyruvate dehydrogenase (PDH) enzyme is a major control point for metabolism, especially within the heart. This is of particular relevance in type 2 diabetes, where one proposed method of treatment is the reduction of blood sugar levels via increased flux through PDH. This can be achieved by inhibiting the action of the associated enzyme pyruvate dehydrogenase kinase (PDK). Hyperpolarized ^{13}C-labelled pyruvate enables visualisation of real-time flux through PDH both *ex vivo*, in the isolated perfused heart, and *in vivo*. In this study we have demonstrated an increase in PDH flux in both of these situations due to the administration of dichloroacetate (DCA), a non-specific PDK inhibitor, a result which was not mirrored when a PDK2 specific inhibitor (AZD7545[1]) was applied.

Materials and Methods: The acute and chronic effects of both DCA and AZD7545 on cardiac function and metabolism were assessed as follows.

Ex vivo Approximately 10 mg of [1-^{13}C]pyruvic acid doped with 15 mM trityl radical (OX063, GE Healthcare) and a trace amount of Dotarem (Guerbet, France) was hyperpolarized in a Hypersense polarizer (Oxford Instruments, Abingdon), with 40 min of microwave irradiation. The sample was subsequently dissolved in a pressurised and heated alkaline solution, containing 100 mg/L EDTA. Once dissolved in 175 ml Krebs-Henseleit (KH) buffer, this mixture yielded a solution of 0.625 mM hyperpolarized sodium [1-^{13}C]pyruvate with a polarization of ~30% at physiological temperature and pH. Hearts from male Wistar rats (n = 6 DCA, n = 5 AZD7545) were perfused in the Langendorff mode as previously described[2] but with KH buffer containing 0.625 mM pyruvate, and placed in an 11.7 T vertical bore magnet system (Bruker, Germany) to achieve a perfusion pressure of 85 mmHg.

Perfusion protocol: Following a period of stabilization, where the hearts were perfused with the standard KH buffer, a hyperpolarized ^{13}C pyruvate dissolution was carried out. Acquisition of ^{13}C spectra began immediately after infusion of the hyperpolarized solution, which continued throughout a two minute acquisition with a spectrum acquired every second. This was followed by a fully relaxed ^{31}P MRS acquisition and a series of 35 one minute partially saturated ^{31}P scans. The control KH buffer was switched 10 minutes into the series of partially saturated ^{31}P scans for KH buffer containing either 1 mM DCA or 0.1 µM AZD7545. At the end of the 35 minutes of ^{31}P acquisitions, a second hyperpolarized ^{13}C dissolution and acquisition was performed. This process was then repeated and the concentration of compound increased: DCA to 10 mM or AZD7545 to 30 µM. Heart function data was continuously measured via a polyethylene balloon that had been inserted into the left ventricle. ^{13}C spectra were referenced to the [1-^{13}C]pyruvate resonance and fitted using the AMARES algorithm in the jMRUI software package[3]. Spectra were corrected for DC offset using the last half of acquired points. The metabolite maximum peak areas were then expressed relative to the maximum pyruvate peak area. ^{31}P spectra were analysed as previously described[4].

In vivo AZD7545 was administered by gavage to male Wistar rats (n = 10) once daily with a suspension of AZD7545 (300 mg/kg/day) in 0.5% w/v hydroxymethylcellulose (HPMC) in 0.1% w/v aqueous polysorbate 80. Control animals (n = 10) were dosed with HPMC in polysorbate 80. DCA (n = 8) was administered via the animals' water supply (0.75 g/L, neutralised to pH 7.2 with NaOH). Following 28 days of dosing, *in vivo* PDH flux was assessed using hyperpolarized [1-^{13}C]pyruvate as previously described[5]. On the following day *in vivo* cardiac structure and function were assessed using cine-MRI as previously described[6].

Results and Discussion: A significant increase in PDH flux was seen on application of DCA to the isolated perfused heart, both at 1 mM and 10 mM concentrations (Fig. 1A, *p<0.05). However, this result was not repeated in the AZD7545 perfusions (Fig. 1B). Whilst the ^{31}P MRS data demonstrated no energetic alteration between control and experimental conditions for either compound at either concentration, a decrease in heart function was seen on delivery of 30 µM AZD7545 to the isolated perfused heart. In agreement with the *ex vivo* data, *in vivo* hyperpolarized ^{13}C data showed an increase in PDH flux in DCA treated animals (Fig. 1C, *p<0.05), accompanied by a decrease in lactate and alanine. Again, this was not replicated in AZD7545 treated animals (Fig. 1D). Cine-MRI data taken at the end of the dosing period showed no changes in cardiac structure or function for either compound when compared with control animals (Fig. 2).

These data show that application of DCA successfully increased pyruvate metabolism both in the isolated heart and *in vivo*, but specific inhibition of PDK2 by AZD7545 was not able to mimic this effect in either situation. This may be due to the other isoforms of PDK present in the heart counteracting the isoform specific effects of AZD7545.

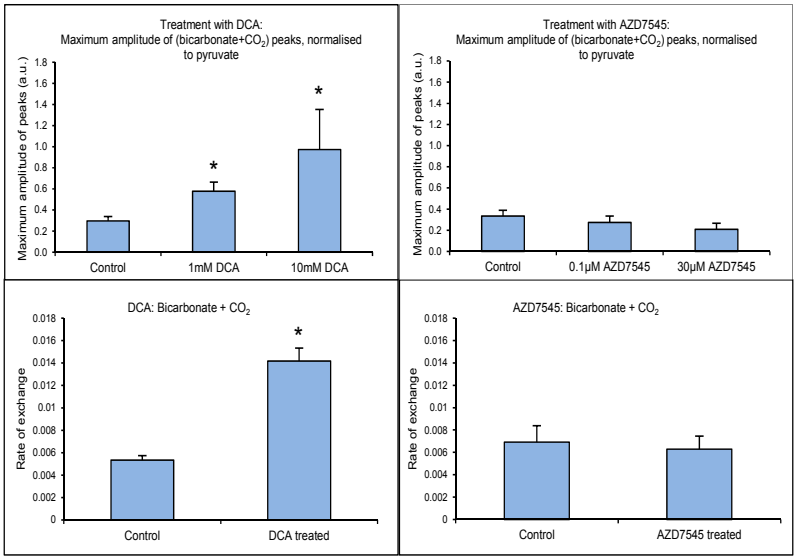

Figure 1 *PDH flux changes in the isolated perfused heart, with A) DCA and B) AZD7545, and in vivo with C) DCA and D) AZD7545*

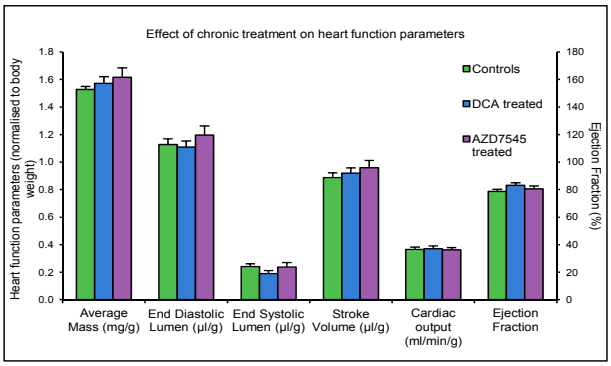

Figure 2 *Heart function measurements following 28 days administration of DCA or AZD7545*

Conclusions: In this study, we have demonstrated the ability to investigate the acute and chronic effects of PDK inhibition on cardiac function and metabolism using a perfused heart model and *in vivo* techniques. PDK2 specific inhibition with AZD7545 is not sufficient to increase PDH flux either *ex vivo* or *in vivo*, but increased PDH flux can be demonstrated using dichloroacetate in either situation.

References: [1]Morrell JA, *et al.* (2003) *Biochem. Soc. Trans.* 31(6):1168-1170. [2]Heather L, *et al.* (2006) *Cardiovascular Research* 72(3):430-437. [3]Naressi A, *et al.* (2001) *Computers in Biology and Medicine* 31(4):269-286. [4]Schroeder MA, *et al*. (2009) *The FASEB Journal* 23(8):2529-2538. [5]Atherton H, *et al.* (2011) *NMR in Biomedicine*. [6]Tyler D, *et al*. (2006) *J Cardiovasc Magn Reson.* 8(2):327-333.

Acknowledgements: This study was supported by the EPSRC, the British Heart Foundation, AstraZeneca and GE Healthcare.