Targeted disruption of the heat shock protein 20–phosphodiesterase 4D (PDE4D) interaction protects against pathological cardiac remodelling in a mouse model of hypertrophy

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1. Introduction

The small heat shock protein, HSP20 is transiently up-regulated during stress/injury to the heart and underpins a plethora of cardio-protective properties including protection against apoptosis, inflammatory signalling, β-agonist-induced remodelling, and regulation of Ca²⁺ cycling [2]. HSP20 confers its protective properties via a number of diverse signalling systems including, prevention of caspase activity, enhancement of Akt/PKB signalling, inhibition of NF-κB signalling, reduction in cardiac myocyte apoptosis and necrosis, stabilisation of the cytoskeleton and increased cardiac myocyte shortening rates due to faster calcium transients [3]. Crucially, phosphorylation of HSP20 at Ser16 by PKA is critical for its protective actions [4], as illustrated by a variety of overexpression studies either using genetic engineering [5] or phospho-HSP20 analogues [6]. Cellular PKA activity is tightly regulated both spatially and temporally by the compartmentalisation of cAMP [7,8]. In cardiac myocytes, cAMP is a pivotal second messenger influencing inotropic and chronotropic activities, as well as hypertrophy and apoptosis [9]. Targeted intracellular hydrolysis by cAMP-specific phosphodiesterases (PDEs) enables the creation of subcellular compartments with high levels of cAMP relative to the surrounding environment [10]. Recent research has shown that HSP20 sequesters the PDE4D sub-family, with targeted disruption of this complex resulting in hyper-phosphorylation of HSP20 and protection of neonatal cardiac myocytes from β-adrenergic-induced hypertrophy [11]. Disruption of the HSP20–PDE4 complex was achieved following mapping of the protein–protein interface by peptide array, which allowed selection of a peptide (called peptide 906) of PDE4 sequence that was capable of interfering with HSP20 binding [11]. Phosphorylation of HSP20 by PKA is also facilitated by virtue of association to the PKA anchor protein, AKAP Lbc [12]. Here, using in vitro and in vivo techniques, we show that peptide 906-mediated disruption of the HSP20–PDE4D complex normalises ISO-induced elongation of action potential duration and reduces the development of the hypertrophic response in aortic-banded mice by attenuating cardiac remodelling, improving left ventricular function and decreasing cardiac fibrosis.
2. Methods

2.1. Action potential measurements in human induced pluripotent stem cell-derived cardiomyocytes

Human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs; obtained from Cellular Dynamics Inc.) were maintained in culture for 10 days. Cells were incubated for 2 h in the presence of vehicle (PBS), bs906 (10 μM) or scrambled control peptide (10 μM) or scrambled control peptide (10 μM) followed by 24 h co-incubation with ISO (10 μM). Cells were loaded with 3 μM di-4-ANEPPS and electrical activity of spontaneously beating cardiomyocytes was registered using CellOPTIQ platform (Clyde Biosciences Ltd.). (A) Example AP traces (grey trace baseline, black trace 24 h 10 μM ISO/Vehicle). (B) (i) Average values of APD at 50% (APD50) and (ii) 90% of repolarisation (APD90) from baseline (white columns) or after treatment (black columns). Data represents mean ± S.E.M, measured from 15 areas per treatment from n = 3 separate cultures, *p < 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA post hoc Tukey’s test.

Fig. 1. HSP20-PDE4D peptide disruptor modulates APD in hiPSC-CMs. hiPSC-CMs (Cellular Dynamics International, Madison, Wisconsin) were pre-treated for 2 h with vehicle (PBS), bs906 (10 μM) or control peptide (10 μM), followed by 24 h co-incubation with ISO (10 μM). Cells were loaded with 3 μM di-4-ANEPPS and electrical activity of spontaneously beating cardiomyocytes was registered using CellOPTIQ platform (Clyde Biosciences Ltd.). (A) Example AP traces (grey trace baseline, black trace 24 h 10 μM ISO/Vehicle). (B) (i) Average values of APD at 50% (APD50) and (ii) 90% of repolarisation (APD90) from baseline (white columns) or after treatment (black columns). Data represents mean ± S.E.M, measured from 15 areas per treatment from n = 3 separate cultures, *p < 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA post hoc Tukey’s test.
2.2. MTAB surgery and transthoracic echocardiography

Healthy adult male C57BL/6J mice (Harlan, UK) weighing between 25 and 30 g were used for these experiments. Procedures conformed to the UK Animals (Scientific Procedures) Act 1986 and were approved by institutional ethical review committees. MTAB and sham surgical protocols were performed as previously described [13]. Immediately following surgery, animals were injected subcutaneously with either HSP20–PDE4 disruptor peptide [11] (bs906) or a scrambled control peptide (10 mg/kg) twice weekly and were left for 4 weeks to allow cardiac remodeling to occur. Echocardiographic assessment of left ventricular function was performed 4 weeks after MTAB or sham surgery, as described previously [13]. LV end systolic dimension (LVESD) and LV end diastolic dimension (LVEDD) were assessed from M-mode traces and % fractional shortening calculated. Fractional shortening (FS) is expressed as \[
\frac{(LVEDD - LVESD) \times 100}{LVEDD}
\]
An average of three measurements of each variable was used.

2.3. Post-mortem measurements

After 4 weeks, animals were euthanised with an intravenous injection of pentobarbital sodium (Euthatal, 200 mg/kg). Terminal anaesthesia was confirmed by testing loss of the pedal reflex. The heart, liver and lungs were rapidly excised and washed thoroughly in ice-cold Ca\(^{2+}\)-free Krebs solution (120 mM NaCl, 5.4 mM KCl, 0.52 mM NaH\(_2\)PO\(_4\), 20 mM HEPES, 11.1 mM glucose, 3.5 mM MgCl\(_2\), 20 mM Taurine, 10 mM Creatine; pH 7.4). Tissue was either homogenised or processed as described in the following sections.

2.4. Picro-sirius red staining

LV tissue was processed, embedded in paraffin and sectioned. Slides were stained for 1 h in picrosirius red solution containing 0.1% (w/v) direct red 80 dye in a saturated aqueous solution of 1.3% picric acid as previously described [14]. Five random sections per heart (5 µm sections with a distance of 200 µm between sections), and 5 areas of interest per section were photographed at 10× magnification using non-polarised light with a Leica DM LB2 microscope and a Leica DFC 320 camera (Leica Microsystems, Germany). Quantification of picrosirius red staining used Image-ProPlus software (version 5.0; MediaCybernetics), with stained area expressed as a percentage of the total area of interest. Values were averaged to give one representative value per heart.

2.5. Statistics

Results were expressed as the mean ± S.E.M. of \(n\) observations, where \(n\) refers to the number of animals or samples. Statistical comparisons were performed using the student’s unpaired \(t\) test and one-way ANOVA, unless otherwise stated. \(p < 0.05\) was considered statistically significant.

3. Results

3.1. Attenuation of isoprenaline-induced action potential elongation by bs906

The hypertrophied heart displays remodelling of its metabolic, biological, and electrophysiological properties, all of which are risk factors for ventricular arrhythmias and cardiac failure [15]. Recently, HSP20 has been shown to regulate Ca\(^{2+}\) cycling and SR Ca\(^{2+}\) load [16], with transgenic mice overexpressing HSP20 displaying enhanced basal cardiac function and protection against \(\beta\)-adrenergic-induced LV dysfunction [2,17]. Tight regulation of SR Ca\(^{2+}\) transients is critical for normal/synchronised cardiac cycles and action potential duration (APDs). While rodent neonatal and adult cardiomyocytes are appropriate for mechanistic studies of HSP20–PDE4 disruption, the use of a human-derived cell line may be more appropriate to evaluate the clinical relevance of drug effects [18]. To this end, we pre-treated human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs) in a similar manner to
that used previously for mouse neonatal cardiac myocytes [11], that is for 2 h with either vehicle (PBS), bs906 (10 μM) or scrambled control peptide (10 μM), followed by 24 h in the presence or absence of ISO (10 μM) to determine if disruption of the HSP20–PDE4D interaction could reverse hypertrophy and associated electrical remodelling (Fig. 1). ISO-induced increases in APD at 50% and 90% repolarisation (i.e. a hypertrophic phenotype) were reversed when cells were pre-treated with bs906, but not with scrambled peptide (50%: 463.9 ± 22.4 ms cf. 542.8 ± 27.0 ms, 90%: 581.3 ± 20.9 ms cf. 665.2 ± 19.6 ms, ISO + bs906 and ISO + scrambled peptide, respectively, \( p < 0.05 \); Fig. 1A and B respectively,). Importantly, there was no effect on APD 50 or 90 when cells were pre-treated with bs906 or scrambled peptide in the absence of ISO (50%: 446.1 ± 23.5 ms cf. 441.2 ± 59.5 ms, 90%: 531.8 ± 22.2 ms cf. 543.5 ± 62.9 ms, bs906 and scrambled peptide, respectively, \( p > 0.05 \); Fig. 1A and B respectively.). Importantly, there was no effect on APD 50 or 90 when cells were pre-treated with bs906 or scrambled peptide in the absence of ISO (50%: 446.1 ± 23.5 ms cf. 441.2 ± 59.5 ms, 90%: 531.8 ± 22.2 ms cf. 543.5 ± 62.9 ms, bs906 and scrambled peptide, respectively, \( p > 0.05 \)), suggesting that bs906 acts only to normalise hypertrophic induced changes in electrical signalling in hiPSC-CMs, possibly via prolonged inhibition of protein phosphatase 1 (PP1) by HSP20[16].

3.2. Preserved cardiac function in bs906-treated hypertrophic mice

Another key feature of the hypertrophied and remodelling heart is impaired LV function. To investigate whether HSP20–PDE4D complex disruption could protect against LV dysfunction and remodelling, we used a minimally invasive transverse aortic banding (MTAB) model of pressure overload hypertrophy. Following MTAB, mice were injected with either 10 mg/kg bs906 or scrambled peptide over a 4-week period, followed by echocardiography to determine the extent of LV remodelling. As shown in Fig. 2A(i)), twice weekly injections of bs906, but not control, prevented MTAB-induced decreases in left ventricular (LV) contractility (fractional shortening: 47.1 ± 1.7% cf. 29.6 ± 2.0%, \( p < 0.0001 \), MTAB + bs906 and MTAB + control, respectively, and 47.1 ± 1.7% cf. 46.1 ± 1.1%, \( p > 0.05 \), MTAB + bs906 and sham + bs906, respectively). Interestingly, a one-weekly dose of bs906 proved sufficient in preventing LV dysfunction in MTAB mice (Fig. 2A(ii)), 38.5 ± 2.5% cf. 29.2 ± 2.2%, \( p < 0.5 \), MTAB + bs906 and MTAB + control, respectively, and 38.5 ± 2.5% cf. 40.4 ± 1.3%, \( p > 0.05 \) MTAB + bs906 and sham + bs906, respectively).

3.3. Attenuation of MTAB-induced cardiac remodelling

Aberrant chronic and prolonged β-adrenergic stimulation is a key feature of the diseased and remodelling heart. Cardiac hypertrophy is a physiological response to pressure/volume overload that results in an increase in cardiac myocyte size. We have previously shown that bs906 (but not scrambled control) could attenuate cardiac myocyte hypertrophy in culture [11]; however,
our current work has now validated this approach in an animal model (Fig. 2B). Pressure-overload-mediated cardiac remodelling was attenuated with twice-weekly doses of bs906 (Fig 2B(i)); 5.2 ± 0.3 cf. 4.6 ± 0.07, n ≥ 6, p < 0.001, MTAB + scrambled and sham + scrambled, respectively and 4.8 ± 0.1 cf. 4.7 ± 0.09, n = 8, p > 0.05, MTAB + bs906 and sham + bs906, respectively). In fact, one weekly dose of bs906 was adequate in reducing MTAB induced remodelling (Fig. 2B(ii); 4.9 ± 0.2 cf. 6.0 ± 0.6, n ≥ 3, p < 0.05, MTAB + bs906 and MTAB + scrambled, respectively, and 4.9 ± 0.2 cf. 4.8 ± 0.1, n ≥ 3, p > 0.05, MTAB + bs906 and sham + bs906, respectively).

3.4. Reduced MTAB-induced interstitial fibrosis by bs906

Cardiac fibrosis is another important feature of the remodelling heart that contributes to the pathogenesis of diastolic dysfunction [13]. Cardiac fibrosis is the result of aberrant fibroblast proliferation, remodelling of the extracellular matrix and increased collagen synthesis and deposition [19]. Analysis of picrosirius red stained LV sections showed increased collagen deposition in MTAB hearts treated with scrambled peptide twice weekly (Fig. 3A and B; % stained area, 5.5 ± 0.9% cf. 1.7 ± 0.4%, n ≥ 6, p < 0.001, MTAB + scrambled and sham + scrambled, respectively), in correspondence with previous published data [13]. By contrast, MTAB mice treated twice weekly with bs906 showed a significant reduction in fibrosis (Fig. 3A and B; 2.4 ± 0.2% cf. 5.5 ± 0.9%, n ≥ 6, p < 0.001, MTAB + bs906 and MTAB + scrambled, respectively, and 2.4 ± 0.2% cf. 1.5 ± 0.2%, n ≥ 7, p > 0.5, MTAB + bs906 and sham + bs906, respectively). This observation is gratifyingly similar to studies by other labs that have shown that excessive collagen deposition can be reversed following treatment with a phosphopeptide-analogue of HSP20 [20] or cardiac overexpression of HSP20 [17].

4. Discussion

In summary, we have shown that targeted disruption of the HSP20–PDE4D interaction attenuates myocardial remodelling following cardiac insult in a minimally invasive transverse aortic banding model of pressure overload hypertrophy. This strategy [10], previously validated in cellular systems [11], has advantages over simple cell permeable phosphate-peptide mimics of HSP20 [6,21] as these contain a labile phosphate group which can easily be targeted by phosphatases and are difficult to mass produce. High-throughput screening of small molecule libraries could also be applied to unlock the cardio-protective potential of HSP20. This approach has been used to discover a small molecule modulator of HSP20 that promotes relaxation of human airway smooth muscle cells and intact tissue ex vivo [1]. Our novel approach in targeting the interaction of HSP20 with an inhibitory protein (PDE4D) may represent a new therapeutic strategy for the treatment of a myriad of cardiovascular disorders, including cardiac hypertrophy.

Acknowledgments

This work was supported by Heart Research UK (Grant No: RG2610). G.S.B. is supported by MRC grant (MR/J007412/1). We are extremely grateful to Mr Graeme MacKenzie for technical assistance. MPH-V is recipient of a postdoctoral fellowship from Fundacion Alfonso Martin Escudero, Spain. GSB and SC conceived and designed the project, TPM, MPV-H, JEF and CE acquired and analyzed the data, TPM, GSB and SC wrote the paper.

References

[1] An, S.S., Askovich, P.S., Zarembinski, T.I., Ahn, K., Peltier, J.M., von Rechenberg, M., Sahasrabudhe, S. and Fredberg, J.J. (2011) A novel small molecule target in human airway smooth muscle for potential treatment of obstructive lung diseases: a staged high-throughput biophysical screening. Respir. Res. 12, 8.
[2] Fan, G.C., Chu, G. and Kranaes, E.G. (2005) Hsp20 and its cardioprotection. Trends Cardiovasc. Med. 15, 138–141.
[3] Edwards, H.V., Cameron, K.T. and Baillie, G.S. (2011) The emerging role of HSP20 as a multifunctional protective agent. Cell Signal. 23, 1447–1454.
[4] Edwards, H.V., Scott, J.D. and Baillie, G.S. (2012) Phosphorylation of the small heat-shock protein Hsp20 enhances its cardioprotective effects. Biochem. Soc. Trans. 40, 210–214.
[5] Wang, X., Zingarelli, B., O’Connor, M., Zhang, P., Adeyemo, A., Kranaes, E.G., Wang, Y. and Fan, G.C. (2009) Overexpression of Hsp20 prevents endotoxin-induced myocardial dysfunction and apoptosis via inhibition of NF-kappaB activation. J. Mol. Cell. Cardiol. 47, 382–390.
[6] McMenemy, E.C., Tessori, D.J., Flynn, C.R., Furnish, E.J., Komalavilas, P., Thresher, J.S., Joshi, L., Stone, W.M., Fowle, R.J. and Brophy, C.M. (2004) Transducible recombiant small heat-shock related protein, HSP20, inhibits vasospass and platelet aggregation. Surgery 136, 573–578.
[7] Baillie, G.S. (2009) Compartmentalized signalling: spatial regulation of cAMP by the action of compartmentalized phosphodiesterases. FEBS J. 276, 1790–1799.
[8] McCormick, K. and Baillie, G.S. (2014) Compartmentalisation of second messenger signalling pathways. Curr. Opin. Genet. Dev. 27, 20–25.
[9] Tomita, H., Nazmy, M., Kajimoto, K., Yehia, G., Molina, C.A. and Sadoshima, J. (2003) Inducible cAMP early repressor (ICER) is a negative-feedback regulator of cardiy hypertrophy and an important mediator of cardiac myocyte apoptosis in response to beta-adrenergic receptor stimulation. Circ. Res. 93, 12–22.
[10] Lee, L.C., Maurice, D.H. and Baillie, G.S. (2013) Targeting protein–protein interactions within the cyclic AMP signalling system as a therapeutic strategy for cardiovascular disease. Future Med. Chem. 5, 451–464.
[11] Sin, Y.Y., Edwards, H.V., Li, X., Day, J.P., Christian, F., Dunlop, A.J., Adams, D.R., Zaccolo, M., Houslay, M.D. and Baillie, G.S. (2011) Disruption of the cyclic AMP phosphodiesterase-4 (PDE4)-HSP20 complex attenuates the beta-agonist induced hypertrophic response in cardiac myocytes. J. Mol. Cell. Cardiol. 50, 872–883.
[12] Edwards, H.V., Scott, J.D. and Baillie, G.S. (2012) The A-kinase-anchor protein AKAP-Lbc facilitates cardioprotective PKA phosphorylation of Hsp20 on Ser(16). Biochem. J. 446, 437–443.
[13] Martin, T.P., Robinson, E., Harvey, A.P., MacDonald, M., Grieve, D.J., Paul, A. and Currie, S. (2012) Surgical optimization and characterization of a minimally invasive aortic banding procedure to induce cardiac hypertrophy in mice. Exp. Physiol. 97, 822–832.
[14] Junqueira, L.C., Bignolas, G. and Brentani, R.R. (1979) Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem. J. 11, 447–455.
[15] Szentandrassy, N., Farkas, V., Barandi, L., Hegyi, B., Ruzsnavszky, F., Horvath, B., Banyasz, T., Magyar, J., Marton, I. and Nanas, P.P. (2012) Role of action potential configuration and the contribution of C(2)(+)a and K(+) currents to isoprenaline-induced changes in canine ventricular cells. Br. J. Pharmacol. 167, 599–611.
[16] Qian, J., Yafaiadaki, E., Florea, S.M., Singh, V.P., Song, W., Lam, C.K., Wang, Y., Yuan, Q., Pritchard, T.J., Cai, W., Haghighi, K., Rodriguez, P., Wang, H.S., Sanoudou, D., Fan, G.C. and Kranaes, E.G. (2011) Small heat shock protein 20 interacts with protein-phosphatase-1 and enhances sarcoplasmic reticulum calcium cycling. Circ. Res. 108, 1429–1438.
[17] Fan, G.C., Yuan, Q., Song, G., Wang, Y., Chen, G., Qian, J., Zhou, X., Lee, Y.J., Ashraf, M. and Kranaes, E.G. (2006) Small heat shock protein 20 attenuates beta-agonist-mediated cardiac remodeling through apoptosis signal-regulating kinase 1. Circ. Res. 99, 1233–1242.
[18] Schweikart, K., Guo, L., Shuler, Z., Abrams, R., Chiao, E.T., Kolaja, K.L. and Davis, M. (2013) The effects of jaspamide on human cardiomyocyte function and cardiac ion channel activity. Toxicol. In Vitro 27, 745–751.
[19] Moreo, A., Ambrosio, G., De Chiara, B., Pu, M., Tran, T., Mauri, F. and Ramani, S.V. (2009) Influence of myocardial fibrosis on left ventricular diastolic function: noninvasive assessment by cardiac magnetic resonance and echo. Circ. Cardiovasc. Imaging 2, 437–443.
[20] Lopes, L.B., Furnish, E.J., Komalavilas, P., Flynn, C.R., Ashby, H., Hansen, A., Ly, D.P., Yang, G.P., Longaker, M.T., Panitch, A. and Brophy, C.M. (2009) Cell permeant peptide analogues of the small heat shock protein, HSP20, reduce TGF-beta-induced CTGF expression in keloid fibroblasts. J. Invest. Dermatol. 129, 590–598.
[21] Dreiza, C.M., Brophy, C.M., Komalavilas, P., Furnish, E.J., Joshi, L., Pallero, M.A., Murphy-Ullrich, J.E., von Rechenberg, M., Ho, Y.S., Richardson, B., Xu, N., Zhen, Y., Peltier, J.M. and Panitch, A. (2005) Transducible heat shock protein 20 (HSP20) phosphopeptide alters cytoskeletal dynamics. FASEB J. 19, 261–263.