Institution: University of Bristol

Unit of Assessment: 5 – Biological Sciences

Title of case study: Development of treatments that protect the heart and brain from damage following heart attack and stroke

1. Summary of the impact

Research conducted at the University of Bristol has influenced the direction of research and investment of pharmaceutical companies and led to improved patient outcomes in preliminary clinical trials. Myocardial infarction (heart attack) and stroke are a major health issue for Western society and a frequent cause of premature death. Treatment of these conditions involves procedures that restore blood flow to the tissues, but there is a significant risk of further tissue damage when the blood supply returns – known as reperfusion injury – due to inflammation and oxidative stress. Since 1993, Professor Halestrap has conducted pioneering work on the role of the mitochondrial permeability transition pore (MPTP) in reperfusion injury. In 1995, he demonstrated that inhibition of the MPTP protects the rat heart from reperfusion injury and in 1998, with his collaborators he demonstrated protection in rat brains. His studies helped establish the MPTP as the most promising target for developing drugs against reperfusion injury. In 2000, pharmaceutical companies started investing in the research and development of such drugs. Subsequently, this has led to formal contracts with seven pharmaceutical companies, a patent and seven clinical trials with improved outcomes for patients in an initial Phase II trial leading to a large ongoing multi-centre Phase III trial.

2. Underpinning research

Professor Halestrap's laboratory has been at the forefront of research into the molecular mechanism of the mitochondrial permeability transition pore (MPTP) and its role in cell death for more than 20 years. Halestrap's group was the first to show that this pore opened in reperfusion injury in the heart. Since then, their research has focussed on developing a better understanding of the pore and its role in reperfusion injury. They have revealed the molecular mechanisms by which drugs can inhibit the pore, identified proteins that are good candidate targets for drug development and developed protocols that improve myocardial protection.

The key researchers in this group are Prof Andrew Halestrap, PI, mitochondrial biology (Lecturer (1975-1996), Professor of Biochemistry (1996-present)). Dr Elinor Griffiths, Co-PI, mitochondrial biology (Post-doc (1990-1993), Research Fellow (1995-1999), Lecturer/Part time lecturer (2000-2011)) and Prof Saadeh Suleiman, Co-PI, a cardiac physiologist in the School of Physiology and Pharmacology (Lecturer (1992-1994), Senior Lecturer (1994-1990), Reader (2000-2005); Professor (2005-current)).

Key findings

In 1995, Halestrap and his colleagues demonstrated that the degree of reperfusion injury correlates with the extent of MPTP opening [1]. They were the first to recognise that the drug cyclosporine A (CsA) inhibits opening of the MPTP by binding to a protein in the mitochondrial matrix known as cyclophilin or CyP-D (1993-1998). To date, this remains the only undisputed molecular component of the MPTP Halestrap was also responsible for identifying two proteins, the adenine nucleotide translocase (1994-1998) and phosphate carrier (2008), as probable pore components, which informed many subsequent studies directed towards identifying effective cardioprotective drugs [2]. In 1993, his laboratory was the first to demonstrate that CsA protects the isolated perfused rat heart from damage during reperfusion after ischaemia [3]. In the late nineties, his collaborative studies with Tadeus Wieloch (University of Lund) demonstrated CsA-mediated protection of the rat brain from damage following ischaemia/reperfusion and hypoglycaemia [4].

Halestrap and his colleagues have developed other novel cardioprotective therapies that inhibit opening of the MPTP through their extensive knowledge of the molecular mechanism of the pore and its regulation by pH, calcium and oxidative stress. These therapies include Sanglifehrin A, pyruvate and the anaesthetic propofol [5]. In 2003, Halestrap demonstrated that an extremely potent cardioprotective protocol - ischaemic preconditioning - where the blood supply to the tissue is impaired repeatedly for short periods of time prior to the prolonged ischemic period, acts by reducing the oxidative stress that induces MPTP opening [5]. More recently (2007), Halestrap and

his colleagues have developed an even more potent cardioprotective strategy, known as temperature preconditioning, which reduces oxidative stress through repeated short-term hypothermic perfusion and rewarming of the tissue [5]. By elucidating the intracellular signalling pathways involved in the preconditioning pathways the Bristol team was subsequently able to mimic this highly effective cardioprotective strategy by using a combination of two well established drugs already in clinical use, adenosine and isoproterenol. Professor Halestrap's studies were largely undertaken with isolated mitochondria and perfused rat hearts but through collaborations with academic cardiac surgeons in the Bristol Heart Institute (a university centre that integrates cardiovascular research in Bristol) the work has been validated in the open-chested pig model [6] as a prelude to clinical trials of cardioprotection in open heart surgery and balloon angioplasty.

3. References to the research

Outputs:

[1] Griffiths, E.J. & Halestrap, A.P. (1995) 'Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion', *Biochemical Journal,* 307: 93-98. URL: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1136749/pdf/biochemj00066-0097.pdf> *(595 citations*)*

[2] Halestrap, A.P. (2009) 'What is the mitochondrial permeability transition pore?', *Journal of Molecular and Cellular Cardiology* 46: 821-31. DOI: 10.1016/j.yjmcc.2009.02.021. *(296 citations)*

[3] Griffiths, E.J & Halestrap, A.P. (1993) 'Protection by cyclosporin A of ischemia reperfusion-induced damage in isolated rat hearts', *Journal of Molecular and Cellular Cardiology,* 25: 1461-1469. DOI: 10.1006/jmcc.1993.1162. *(403 citations)*

[4] Friberg, H., Ferrand-Drake, M., Bengtsson, F., Halestrap, A.P. & Wieloch, T. (1998) 'Cyclosporin A, but not FK 506, protects mitochondria and neurons against hypoglycemic damage and implicates the mitochondrial permeability transition in cell death', *Journal of Neuroscience,* 18: 5151-5159. URL: <http://www.jneurosci.org/content/18/14/5151.full> *(318 citations)*

[5] Halestrap, A.P. (2010) 'A pore way to die: the role of mitochondria in reperfusion injury and cardioprotection', *Biochemical Society Transactions,* 38: 841-860. DOI:10.1042/BST0380841 *(89 citations)*

[6] Lim, K.H., Halestrap, A.P., Angelini, G.D. & Suleiman, M-S. (2005) 'Propofol is cardioprotective in a clinically relevant model of normothermic blood cardioplegic arrest and cardiopulmonary bypass', *Experimental Biology and Medicine,* 230: 413-420. URL: <http://ebm.sagepub.com/content/230/6/413.full> *(41 citations)*

**All citations are as of September 5th, 2013 on Google Scholar*

Funding:

This work has been funded by 16 peer-reviewed grants and 2 industrial contracts totalling £3.38M. Illustrative grants are listed below.

[7] Halestrap (1995-1998) Inhibitors of mitochondrial pore opening as protective agents against reperfusion injury in different animal models" BHF Project, £114,177.

[8] Halestrap (1997-2000) The molecular mechanism and role of the mitochondrial non-specific pore, a critical factor in cell death. MRC Project, £160,661.

[9] Halestrap (2001-2003) The molecular mechanism of the mitochondrial permeability transition and its role in reperfusion injury in the heart. BHF Project, £140,780.

[10] Halestrap (2001-2004) The role of mitochondria in ischaemic preconditioning. BHF Project, £168,871.

[11] Halestrap (2004-2008) Mitochondria in the life and death of the heart – from molecule to man. BHF Programme, £670,000.

[12] Halestrap (2009-2013) The role of mitochondria in the life and death of the heart BHF Programme, £834,000.

4. Details of the impact

Despite initial scepticism, the importance of the MPTP in a range of pathologies has now been confirmed in many laboratories. This has resulted in a paradigm shift in cardioprotection research, where drug development and clinical trials are now focused on the MPTP as a key therapeutic

target [a] – a shift in which Halestrap and Griffiths are widely credited as being pioneers [a, pg 160].

Industry invests in research and development:
The pharmaceutical industry has been consulting Halestrap since 2000 regarding the exploitation of the MPTP as a therapeutic target. Bristol has entered into formal contracts (Material Transfer Agreements (MTAs) and Confidentiality agreements) with several pharmaceutical companies including MitoKor, Novartis, DebioPharm, Hoffman-Laroche and most recently with Trophos (2010) and Congenia (2011). The French clinical stage pharmaceutical company, Trophos, views the collaboration with Bristol as "*mutually beneficial*" and credits it for enabling them to "*bring the concept of MPTP inhibition and cardioprotection from bench to bedside*" [b]. Halestrap's research was a "*major influence in [their] decision to focus on inhibition of MPTP opening as the most promising target for protecting the heart from reperfusion injury*" and it is currently the principle target of all compounds being developed by Trophos, with 21 R&D employees and 27 employees in total dedicated to this area of research [b]. In 2011, Trophos was awarded a US Patent for their novel cardioprotective compound TRO40303 [c].

In March 2013, the Swedish-based company NeuroVive Pharmaceutical AB, which specialises in the commercialisation of cyclosporine-based drugs, acquired a portfolio of novel cyclophilin inhibitors from the UK biotech company Biotica Ltd. The financial figures for this investment are confidential, but the strategic acquisition will "*allow NeuroVive to broaden and deepen its pipeline of novel mitochondrial medicines*", including those that reduce reperfusion injury [d].

Government and industry invest in clinical trials:
Since 2008, seven clinical trials involving over 2,000 patients in nine European countries, have been initiated to investigate drugs that target the MPTP either directly or indirectly [e-h,j]. In January 2011, the European Commission awarded a grant of €6 million for a 2.5 year international, translational medicine project led by Trophos. Professor Halestrap acts as a work package leader on this project to investigate the efficacy and safety of TRO40303, which was shown to be cardioprotective in a rat model of ischemia/reperfusion injury (IRI) and has successfully completed Phase I clinical trials and is now in Phase II with 180 patients [e].

Trophos is also testing their neuronal active drug (Olesoxime), which is another MPTP inhibitor. This drug is in Phase II/III, multicenter, randomized clinical trials to assess its safety and efficacy in treatment of Amyotrophic Lateral Sclerosis (ALS) patients [f] and in Phase II trials for Spinal muscular atrophy [g].

Neurovive Pharmaceutical AB began clinical trials of their novel formulation of cyclosporine, CicloMulsion®, in 2008. They began a Phase III clinical trial with 1,000 patients in 2011 [h]. NeuroVive entered a collaboration with Sihuan Pharmaceutical Holdings Group Ltd in 2012 to develop and commercialise CicloMulsion® and another CsA formulation, NeuroSTAT® [EudraCT Number: 2012-000756-34], in China. The launch of these products could generate more than 2 billion Chinese Yuan annually [i].

In 2009, a clinical trial involving 96 patients began in Bristol to investigate the cardioprotective effect of the anaesthetic propofol when added to the solution used to induce cardiac arrest during heart surgery [j].

Halestrap's research was a "*major stimulus*" for Antipodean Pharmaceutical's decision to develop the mitochondria-targeted antioxidant drug MitoQ [k], which is a good candidate for protecting the heart and other tissues from reperfusion injury through reducing mitochondrial oxidative damage.

A clinical trial in Bristol has been agreed with Antipodean Pharmaceuticals to test the efficacy of MitoQ in protecting the heart from reperfusion injury during complex cardiac surgery that involves stopping the heart and using cardiopulmonary bypass (a heart-lung machine) to maintain blood flow [k].

Though the details of investment costs for each drug and each company are commercially sensitive, recent estimates have suggested that the out-of-pocket costs for industry to take a single drug from Phase I to Phase III clinical trials is around US$215-220 million (in 2011 USD) [l]. This suggests a significant investment since 2008 by the pharmaceutical industry in the development of

drugs that target the MPTP.

5. Sources to corroborate the impact

[a] Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM (2012) 'Paradigm shifts in cardioprotection research: the importance of the MPTP as a therapeutic target', *Cardiovascular Research,* 96: 160-164. DOI:10.1093/cvr/cvs174. Independent review that credits Halestrap and Griffiths as helping to lead a paradigm shift in cardioprotection research.

[b] Chief Scientific Officer, Trophos**.**

[c] Pruss, R., Buisson, B. and Bordet, T. (2011) *Use of 3,5-seco-4-nor-cholestane derivatives for obtaining a cytoprotective drug*, US Patent Number 7,985,774 <http://www.archpatent.com/patents/7985774>

[d] NeuroVive (March 11, 2013) *NeuroVive: NeuroVive acquires highly potent, novel cyclophilin inhibitors from Biotica,* Press release, <http://publish.ne.cision.com/Release/ViewReleaseHtml/F691632B8093D938> Illustrates commercial investment in the research area.

[e] Trophos (2011) *Phase II, multicenter, randomized, double-blind, placebo controlled study to assess safety and efficacy of TRO40303 for reduction of reperfusion injury...,* EudraCT Number: 2010-024616-33 <https://www.clinicaltrialsregister.eu/ctr-search/search?query=2010-024616-33>.

[f] Trophos (2009) *Phase II/III, multicenter, randomized, parallel group, double-blind, placebo controlled study to assess safety and efficacy of TRO19622 in ALS patients...,* EudraCT Number: 2008-007320-25 < https://www.clinicaltrialsregister.eu/ctr-search/search?query=2008-007320-25>.

[g] Trophos (2010) *Phase II, multicenter, randomized, adaptive, double-blind, placebo controlled study to assess safety and efficacy of olesoxime (TRO19622) in 3-25 year old Spinal Muscular Atrophy patients,* EudraCT Number: 2010-020386-24 <https://www.clinicaltrialsregister.eu/ctr-search/search?query=2010-020386-24>

[h] Hospices Civils de Lyon (2010) *Does Cyclosporine improve clinical outcome in ST elevation mycordial infarction patients (CIRCUS study),* EudraCT Number: 2009-013713-99 https://www.clinicaltrialsregister.eu/ctr-search/search?query=2009-013713-99.

[i] PR Newswire (November 20, 2012) *NeuroVive and Sihuan Pharmaceutical to develop and commercialise CicloMulsion® and NeuroSTAT®, for Cardio- and Neuroprotection in China.* Press release, <http://www.prnewswire.com/news-releases/neurovive-and-sihuan-pharmaceutical-to-develop-and-commercialise-ciclomulsion-and-neurostat-for-cardio--and-neuroprotection-in-china-180110111.html> Evidence of financial gains from commercialisation of two CsA formulations.

[j] University Hospitals Bristol NHS Foundation Trust (2009) *A single-centre randomised controlled trial of propofol cardioplegia on blood and myocardial biomarkers of stress and injury...,* EudraCT Number: 2009-015779-28. <https://www.clinicaltrialsregister.eu/ctr-search/search?query=2009-015779-28>

[k] CEO, Antipodean Pharmaceuticals Inc.

[l] Mestre-Ferrandiz, J., Sussex, J. and Towse, A. (2012) *The R&D Cost of a New Medicine,* Office of Health Economics, London. <http://ohematerials.org/NMECost/index.html#/0> Supports financial figures estimating industry investment in this research.