Institution: University of Warwick

Unit of Assessment: A1 – Clinical Medicine

Title of case study: Improving the Biocompatibility of Peritoneal Dialysis Fluids

1. Summary of the impact

Research led by Professor Paul J Thornalley since 1993, (University of Warwick, 2007-present), revealed the formation of harmful reactive dicarbonyl compounds (also known as glucose degradation products, GDPs) within the glucose osmolyte of first-generation peritoneal dialysis (PD) fluids. Clinical studies confirmed the increased damage to proteins in patients on PD therapy. In response to these findings, major manufacturers of PD fluids changed their manufacturing processes to minimise GDP content by separating glucose and buffer components within two-compartment bags for heat sterilisation, and by using osmolyte that is resistant to thermal degradation. PD fluids with low GDP content have been associated with improved clinical outcomes for patients receiving dialysis, including maintained residual renal function, decreased peritonitis, and decreased fluid infusion pain. They have been widely implemented in clinical use since 2010. Globally, approximately 240,000 patients receive PD therapy.

2. Underpinning research

Before the development of technologies to measure GDPs, it was unclear whether GDPs were formed during the thermal sterilisation of glucose-containing PD dialysis fluids. Thornalley (**Professor of Systems Biology at the University of Warwick from January 2007-present**) was the first person to develop validated analytical technology to assay levels of the GDP methylglyoxal and other GDPs and apply it to PD fluids. He demonstrated that methylglyoxal is a reactive dicarbonyl metabolite that damages proteins and DNA in physiological systems, resulting in ageing, vascular disease, glucose intolerance, and inflammation. These effects are suppressed by methylglyoxal metabolism by glyoxalase 1.[1] *In vitro* methylglyoxal was found to modify vascular type IV collagen leading to endothelial detachment and cell death, a marker of vascular inflammation and risk predictor of fatal cardiovascular disease in end stage renal disease (Dobler et al., 2006). Studies *in vitro* and in a pre-clinical model found methylglyoxal modification of low density lipoprotein (LDL) converted it to atherogenic small, dense LDL, with increased binding to arterial proteoglycan and arterial deposition *in vivo*.[2] Thornalley showed that GDPs are formed in PD fluids upon the degradation of glucose osmolyte during heat sterilization, causing protein damage (measured by levels of proteolysis products) in patients with renal failure receiving therapy with a first-generation (high-GDP) PD fluid.[3] In PD patients the flux of modification of proteins by first generation PD fluids was increased approximately 10-fold (Agalou et al., 2005). Examples of proteins modified are vascular type IV collagen and LDL (indicated above) which, through endothelial cell detachment and atherogenic transformation, increased risk of thrombosis and atherosclerosis leading to high risk (20-fold increased risk) of heart disease, a common cause of premature death in dialysis patients.

GDPs form adducts with proteins and DNA in a process that produces advanced glycation endproducts (AGEs). [1-4] MG-H1 is the most abundant AGEs linked to protein dysfunction. It is, therefore, one of the most important AGEs in human tissues and body fluids. In one study, excreted flux and plasma concentrations of MG-H1 increased 10-fold and 18-fold, respectively.[4] Reduced GDP exposure has been shown to decrease endothelial cell detachment, decrease atherogenic transformation of low-density lipoproteins and atherosclerosis, and decrease DNA damage and mutagenesis by GDPs, highlighting the clinical benefits of low-GDP PD fluids.

In 2003, with funding from the Wellcome Trust, Thornalley developed state-of-the-art analytical technology (stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry) to measure protein damage caused by GDPs[5]. Over this period, Thornalley received funding from Baxter Healthcare to research the damaging effects of GDPs and ways to avoid these effects. Thornalley has also filed a patent (WO 2005/051968 A1) for an amino acid derivative additive that

further improve the safety profile of dialysis fluids by catalyzing the degradation of GDPs. Amino-acid-containing GDP fluids where then developed by major manufacturers of PD fluid.

More recent research at the University of Warwick confirmed the profound accumulation of AGEs in experimental renal failure[6] and established analytical technologies developed by Thornalley and co-worker Dr Naila Rabbani (**Associate Professor of Experimental Systems Biology, University of Warwick, January 2007-present**) as state-of-the-art for measuring GDPs and their physiological effects.[5] Health benefits of low-GDP peritoneal dialysis fluids were supported by leading expert opinion and systematic review of randomised controlled clinical trials - see also Section 5, notes[a,b,d,e,f]. Thornalley and co-workers also showed that the GDP methylglyoxal produced peripheral neuropathy[7], which is common in renal failure patients (80% prevalence). Low GDP-fluids may also alleviate this symptom, reducing fluid infusion pain.

3. References to the research

1. Rabbani, N. and Thornalley, P. J. (2011). Glyoxalase in diabetes, obesity and related disorders. Seminars in Cell & Developmental Biology. **22**, 309-317; http://dx.doi.org/10.1016/j.semcdb.2011.02.015
2. Rabbani, N., Godfrey, L., Xue, M., Shaheen, F., Geoffrion, M., Milne, R. and Thornalley, P. J. (2011). Glycation of LDL by methylglyoxal increases arterial atherogenicity a possible contributor to increased risk of cardiovascular disease in diabetes. Diabetes. **60**, 1973-1980; http://dx.doi.org/10.2337/db11-0085 **[UoA1 REF2 Submission]**.
3. Thornalley, P. J. and Rabbani, N. (2009). Highlights and Hotspots of Protein Glycation in End-Stage Renal Disease. Seminars in Dialysis. **22**, 400-404; http://dx.doi.org/10.1111/j.1525-139X.2009.00589.x
4. Thornalley, P.J. *et al*. Imidazopurinones are markers of physiological genomic damage linked to DNA instability and glyoxalase 1-associated tumour multidrug resistance. *Nucleic Acids Res.* **38**, 5432–5442 (2010); doi:10.1093/nar/gkq306
5. Rabbani, N. and Thornalley, P.J. Quantitation of markers of protein damage by glycation, oxidation and nitration in peritoneal dialysis. *Peritoneal Dialysis Internat.* **29**, Suppl. 2, S51–S56 (2009).
6. Rabbani, N. *et al*. Protein glycation, oxidation and nitration free adduct accumulation after bilateral nephrectomy and ureteral ligation. *Kidney Internat*. **72**, 1113–1121 (2007); http://dx.doi.org/10.1038/sj.ki.5002513
7. Bierhaus A, Fleming T, Stoyanov S, Leffler A, Babes A, Neacsu C*, et al.* Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nature Med* **18,** 926-933 (2012); http://dx.doi.org/10.1038/nm.2750

Related Research Grant Funding

- Awarding body: Wellcome Trust. Title of project: Structural and functional epitope mapping of proteins involved in mechanisms of disease (equipment grant with co-applicants G. Stanway and N Fernandez). Amount awarded: £220,760. Dates: 01/10/05–30/09/08.
- Awarding body: Wellcome Trust. Title of project: Enzymatic defence against dicarbonyl glycation in diabetes, obesity, renal failure and ageing. Amount awarded: award in-kind (two mutant mouse lines). Dates: 01/10/07–30/09/10.
- Awarding body: British Council [RC125]. Title of project: Functional genomic models of glyoxalase 1 in diabetic nephropathy, renal failure and ageing. Amount awarded: £34,591. Dates: 01/09/08–31/05/11.

4. Details of the impact

Changes in the industry

Thornalley presented his findings regarding protein damage with first-generation PD fluids and improved clinical outcomes with low-GDP PD fluids at several international meetings, including the 5th EuroPD conference in 2002, the American Society Nephrology Annual Renal Week meetings in 1999, 2002, and 2005, and the 12th Congress of the International Society for Peritoneal Dialysis in 2008. These meetings are attended by representatives from the major global manufacturers of dialysis fluids, including Baxter Healthcare, Gambro AB (part of Baxter Healthcare from 2013) and

Frenesius. With funding from Baxter Healthcare, Cancer Research UK, Dynamis Therapeutics Inc., Fresenius Medical Care AG, Gambro AB, GlaxoSmithKline, TransGenic Inc., Woerwag Pharma, and Chroma Therapeutics, Thornalley organized a colloquium on GDP research[a] (entitled "Enzymatic defence against glycation in health, disease and therapeutics"), which was presented at the 679th Meeting of the Biochemical Society held in Colchester in 2003. Approximately 100 clinicians, industry researchers and academics attended the colloquium. From 2002 onwards, in light of the data presented by Thornalley, major manufacturers of PD fluids, including Baxter Healthcare, Gambro AB, and Fresenius Medical Care AG, developed second-generation and third-generation PD fluids and conducted clinical trials to assess clinical outcomes such as residual renal function, risk of peritonitis, and fluid infusion pain. Second-generation PD fluids with reduced GDP content were produced using two-compartment dialysis fluid bags, which separate glucose from buffer salts during heat sterilization. Third-generation PD fluids, with even lower GDP counts, were then produced from osmolyte that is resistant to GDP formation, such as icodextrin. All of the major companies that manufacture PD fluids, including Baxter Healthcare, Gambro AB, and Fresenius Medical Care AG, have developed and marketed low-GDP solutions, and Gambro AB now produce only low-GDP PD fluids. The most recently developed PD fluids are designed to be resistant to GDP formation during transport to sites of use and storage.

Clinical benefits

Clinical evidence of the benefits of low-GDP PD fluids has emerged since 2008[b,c], including data to show improved preservation of residual renal function and decreased peritonitis, fluid infusion pain, and vascular inflammation. The British Renal Society now recommends that all patients experiencing infusion pain, and preferably all other patients (provided there is no cost penalty), should be given low-GDP PD solutions. The European Pediatric Dialysis Working Group[d] recommends low-GDP fluid use in all paediatric patients receiving PD. Current usage of low-PD fluids in Europe is 60% of patients receiving PD dialysis, although all patients receiving dialysis would probably benefit from the use of low-GDP PD fluids. The use of PD therapy for patients with renal failure is likely to increase at the rate of 5–6% per annum as diseases linked to renal failure become more prevalent and access to dialysis improves, highlighting the continued importance of PD fluid development. An additional benefit to patients is that PD treatment with low-GDP PD fluids allows PD to continue for longer, reducing the need for haemodialysis.

Economic benefits

The total global market for PD therapy is around US$2.7 billion per year, for the dialysis of approximately 240,000 patients. The use of low-GDP PD fluids has been shown to reduce treatment costs and increase efficacy.

Other benefits

In addition to their impact on PD fluid composition, Thornalley's findings led the food industry to develop glyoxalase-1-inducer-based foods for healthy ageing (Technology Strategy Board project with Unilever; funding £1.1 million). Selected dietary bioactive compounds at dietary exposure levels induce glyoxalase 1 through Nrf2 transcriptional signalling (Xue, M., Rabbani, N., Momiji, H., Imbasi, P., Anwar, M. M., Kitteringham, N. R., Park, B. K., Souma, T., Moriguchi, T., Yamamoto, M. and Thornalley, P. J. 2012 Transcriptional control of glyoxalase 1 by Nrf2 provides a stress responsive defence against dicarbonyl glycation. Biochem. J. **443**, 213-222). [This is not to be confused with certain other dietary bioactive compounds at markedly higher exposures/doses than dietary, which have been reported to inhibit glyoxalase 1 in some cell model systems *in vitro*]. Other research from Thornalley identified glyoxalase amplification in multidrug resistant (MDR) tumours and sensitivity of such tumours to glyoxalase 1 inhibitors. This led pharmaceutical companies such as AstraZeneca to develop glyoxalase 1 inhibitors for the treatment of multidrug-resistant tumours.[e]

5. Sources to corroborate the impact

- **Supporting Statement**: from Chair of the European Uremic Toxin Network (EUTox) - a workgroup of the European Society of Artificial Organs (ESAO), the European Renal Association (ERA), and the Renal Dialysis and Transplantation Association (EDTA).

(Identifier 1). Specifically acknowledges Thornalley's research contribution in this area, and high level of achievement and impact in terms of advances in provision of improved care for patients with renal failure.

- **Supporting Statement:** from the Medical Director of Baxter Healthcare, Deerfield, USA. (Identifier 2). Corroborates the clinical relevance of Thornalley's research, the formation of which has been subsequently widely explored and clinical practice has changed as a result. Also specifically confirms that Thornalley's research helped lead to the development, registration and clinical use of the low-GDP fluid, Physioneal™, across Europe, the Middle East, Russia, Canada, Australia, New Zealand and Korea, and that global use of this fluid continues to increase.

Corroborating Sources

a) Enzymatic defence against glycation in health, disease and therapeutics (http://212.250.180.38/bst/031/1341/0311341.pdf)

b) Jorres A. Novel Peritoneal Dialysis Solutions - What Are the Clinical Implications? Blood Purif 33, 153-159 (2012). DOI: 10.1159/000342712

c) Cho, Y., Johnson, D. W., Badve, S., Craig, J. C., Strippoli, G. F. K. and Wiggins, K. J. (2013) Impact of icodextrin on clinical outcomes in peritoneal dialysis: a systematic review of randomized controlled trials. Nephrology Dialysis Transplantation. **28**, 1899-1907; 10.1093/ndt/gft050

d) Schmitt, C. P. *et al*. Solutions for peritoneal dialysis in children: recommendations by the European Pediatric Dialysis Working Group. *Pediatr. Nephrol.* 26, 1137–1147 (2011). DOI: 10.1007/s00467-011-1863-4

e) **Person who can be contacted**: (Acting) Director of Structure & Biophysics, AstraZeneca, Alderley Park, Cheshire, UK. (Identifier 3).