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Student Health Sciences Research Journal

The INSPIRE experience: a new student research journal, for students and by students

The issue you're about to read is the first edition of the new INSPIRE Student Health Sciences Research Journal, a publication that showcases the finest work by medical, dental and veterinary students in the South West. The journal began as a discussion between the INSPIRE leads of four medical schools, Bristol, Cardiff, Exeter and Plymouth, on how to engage students in research. The INSPIRE scheme is an initiative that provides medical schools across the UK with funding to encourage medical, dental and veterinary students to pursue research in their related fields. Most commonly, the INSPIRE funding has provided summer studentship grants for short projects and placements in research settings, and student research conferences.

In 2015, the four universities invited applications for senior editors and student peer reviewers, and since then the idea of a journal specifically for students has taken off! We greatly appreciate the input of students across the four universities in the initial brainstorming and planning of the journal. After receiving training and support from external professionals involved in scientific and medical publishing, we opened the submission process and were overwhelmed with ideas for articles. Although we would like to accept all proposed articles, space and time are limited and so we have narrowed our selection to articles that we felt would have greatest impact on a wider audience. A rigorous peer review process has enabled us to ensure the highest quality possible. Next came the graphic design and assembly of the online journal, including collaboration with the Cardiff School of Journalism. After countless hours of teleconferencing and what seemed like endless emails, we are now proudly able to present the first issue of the ISHSRJ.

We hope you enjoy reading the journal as much as we have enjoyed putting it together. And we hope to see it go from strength to strength. Please email us with new suggestions, comments and feedback at inspirestudentjournal@gmail.com.

Best wishes,

INSPIRE Student Health Sciences Research Journal Senior Editors

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Anaesthesia without needles

By Russell Hashemi

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Many patients are fearful of dental procedures such as root canals, cavity preparations, extractions and root debridements. However, the anaesthetic injections, given by the dentist to numb the immediate pain, can be equally distressing. It can lead to cancelled appointments, delayed treatment due to lack of patient consent, and in the long run, a poorer quality of life for the patient.

Given the profound (and potentially negative) effect of injections on patients' experiences, an important goal in dental research is to find an alternative means of anaesthetic drug delivery that is less invasive and painful. This would have advantages for the clinician as well if it eradicated the various hazards and undesirable factors associated with needles, such as contamination, needle stick injuries, and the cost of purchase.

Electricity instead of needles

In a recent study, researchers at the University of Sao Paulo, Brazil, investigated the efficacy of administering local anaesthetics through the use of electricity instead of the conventional use of needles.[1] They passed a low density electrical current through a gel mixture containing lidocaine and prilocaine (which are often used together as a topical anaesthetic) that had been applied to porcine mucosa as a laboratory model for the mouth. A polymer was added to the gel to help it adhere to the mucosa lining. This technique is known as iontophoresis and until now was predominantly used to treat patients suffering from hyperhidrosis, a common condition which causes excessive sweating in between one and three in every 100 people.[2]

The new research showed that the iontophoresis approach increased permeation of the locally applied anaesthetics by 12-fold compared to passive permeation following topical administration. Furthermore, the anaesthetic effect was faster-acting and longer-lasting due to the increased ion flow caused by electrical stimulation.[1]

"Needle-free administration could save costs, improve patient compliance, facilitate application and decrease the risks of intoxication and contamination," said one of the study authors, Professor Renata Fonseca Vianna Lopez.[3]

Avoiding needle stick injury

For healthcare professionals, the implementation of this technique for the administration of local anaesthetics would have a number of benefits. Perhaps the most important would be the reduced risk of needle stick injury. The major blood-borne pathogens associated with needle stick injury are the hepatitis B and C viruses and HIV. Between 2002 and 2011, there were reports of 4381 significant occupational exposures, which represents an increase from 276 in 2002 to 541 in 2011.[4] The adoption of iontophoresis for local anesthetic delivery could lead to a drastic reduction in these incidents and provide a safer working environment for healthcare staff.

And given that the NHS purchases approximately 422 million needles and syringes each year,[5] it is possible that any potential savings from the adoption of iontophoresis could be spent elsewhere. But perhaps the most important benefit of all, would be the significantly reduced discomfort for patients at all injection sites. These include the hard palate, with its tightly bound mucoperiosteum covering.[1]

The study authors also suggest that there may be other applications for this technology beyond dentistry, such as the treatment of malignant tumours. They are investigating a combination of nanotechnology, iontophoresis and sonophoresis (using sound waves to promote permeation) for drug delivery to the skin and eyes, which traditionally have posed a challenge for drug delivery.

As yet unproven

However, the study does come with caveats. The approach has yet to be tested in a human trial to assess whether it is effective and reliable in clinical practice. Furthermore, it is not certain that using iontophoresis will reduce costs, because it requires, for example, a power source, possibly from a battery pack, which itself is costly. Further analysis is therefore required to assess the overall cost effectiveness of introducing this technology.

But despite its preliminary nature, the study shows that iontophoresis could potentially have a tremendously positive impact on both patients and clinicians.

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Tooth donations – the future of dentistry?

By Christina Tran
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Using donated teeth to substitute for your own is not an entirely new concept in dentistry. In the late 18th to early 19th centuries, the wealthiest members of British society would shell out over £100 for dentures made with real human teeth. These would be savagely extracted from those financially desperate enough to sell, or harvested from the freshly deceased soldiers at Waterloo.[1]

Thankfully, dentistry has come a long way since then, with a shift in focus towards rebuilding teeth rather than replacing them. However, the most long-standing restorative material, amalgam (or 'silver fillings'), is currently being phased-out of UK dental practices, due to concerns about its contribution towards environmental mercury pollution.[2] Current alternatives available on the market are ceramics and composites, the more expensive 'white fillings'. Although they look more attractive, composites may not have the strength and therefore longevity of their amalgam counterparts.[3] On the other hand, ceramics are too strong, and wear down the opposing teeth after prolonged use.[4]

It is incredibly difficult to manufacture a synthetic material that mimics the microstructure and hence the mechanical properties of enamel and dentine. Moreover, synthetic materials do not change colour over time in a similar fashion to surrounding teeth, resulting in an artificial-looking restoration.[5] One relatively simple answer is to recycle an old question: why not reconstruct the damaged tooth using natural tooth material?

The first reported case of 'biological restoration' appeared in 1964, where a fractured incisor was restored using the patient's own tooth fragments.[6] Over the years this procedure has been refined, with introduction of the acid-etch technique (commonly-used to improve surface bonding) in 1978.[7] The term biological restoration was not coined until 1991, when decayed teeth were rebuilt using sterilised fragments of extracted teeth.[8] In 1996, the first Human Tooth Bank was established,[9] receiving donations of children's milk teeth according to the same basic rules as an organ donation centre. Such materials were used to restore both the roots and crowns of a 32-month-old boy's teeth in 2000.[10]

In 2014, Schlichting et al created the first reported biological restoration using computer-aided design (CAD) and computer-aided manufacture (CAM).[5] Their rationale was to shape the donated tooth more precisely, so that it fitted better in the recipient's mouth. Concerned about the appearance of her teeth, a 41-year-old woman opted to have a molar restored using her daughter's recently extracted wisdom tooth. Scans of the tooth that required repair were used for the restoration. The daughter's wisdom tooth was steam-autoclaved, then precisely positioned in a CAM machine using pre-prepared guides. After being soaked in espresso coffee for six hours in order to achieve the same colour as surrounding teeth, the adapted tooth was glued to the mother's damaged counterpart. It fitted well, and was not damaged during the CAM process, and remained intact after a 20-month follow up.[5]

Although further investigation is required, this report shows that a functional biological restoration can be manufactured using CAD/CAM. Being much more replicable than the freehand-shaping of tooth material, this opens the doors to a future in which biological restorations are the norm.[5] Teeth are a relatively cheap, renewable

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"As well as having similar translucency to the surrounding teeth, they also respond to staining and bleaching in a uniform manner, due to the ability of small molecules to diffuse in and out of the tooth structure"

resource, as extraction of wisdom teeth and premolars is fairly commonplace.[11] These extracted teeth can be sterilised by steam-autoclaving or gamma-irradiation, with no detectable tissue changes. As well as having similar translucency to the surrounding teeth, they also respond to staining and bleaching in a uniform manner, due to the ability of small molecules to diffuse in and out of the tooth structure. The hardness of enamel enables it to survive a lifetime of chewing with little wear, whilst the elasticity of dentine reduces the probability of fracture during function. Preserving these structures should, in theory, produce restorations with great longevity.[5]

Unfortunately, biological restorations are not without their drawbacks. By nature, natural teeth are susceptible to decay, and so giving them to patients with poor oral hygiene may be a futile operation. It may also be difficult to find donated teeth with the required dimensions,[5] and some patients may not be comfortable with the idea of receiving fragments of other people's teeth.[11] Furthermore, an increase in demand for human teeth may encourage the growth of an illegal tooth trade, prompting a return to the grave-looting ways of the 18th and 19th centuries.

In conclusion, although use of natural-looking restorations with a lifetime guarantee is an exciting concept, further studies are needed to evaluate their long-term performance. The implications for society at large should also be considered before implementing such treatments on a larger scale.

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From humans to dogs: translational research in the diagnosis of Addison's disease

By Florence Edwards
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Addison's disease, or hypoadrenocorticism, is a condition that can lead to a potentially fatal state of shock known as an Addisonian crisis, in both humans and dogs. The disease arises due to limited secretion of cortisol and aldosterone from the adrenal cortex, usually due to an autoimmune condition. Both hormones normally serve vital functions such as sodium regulation.

Hypoadrenocorticism is difficult to diagnose, owing at least partly to its relative rarity: the condition affects only around 8,400 people in the UK,[1] and only 0.36-0.5% of dogs.[2] Another is the lack of specific clinical signs. For example, chronic clinical signs in dogs include lethargy, weakness, anorexia and vomiting, while an Addisonian crisis usually involves dehydration and collapse due to hypovolaemic shock.[3] Consequently, the condition may not be diagnosed until it has become a life-threatening emergency. Therefore, a reliable test for Addison's disease is of significant research interest.

Seeking a blood test

One study has identified angiotensin II as a potential marker for Addison's. The researchers used plasma renin activity as an indirect measure of angiotensin II levels in plasma, and found that plasma renin activity was significantly higher (median 27 ng/ml/hour) in dogs with Addison's disease than in healthy dogs (median 0.8 ng/ml/hour).[4] Furthermore, the affected dogs displayed plasma renin activity which exceeded that of dogs with Addison's-like diseases. The latter animals had initially presented with symptoms similar to Addison's disease

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> "Further coordinated research between clinicians and veterinarians is required in order to advance the testing and application of these techniques to clinical practice"

such as hyponatremia, hyperkalaemia, weakness and lethargy, but their post-ACTH serum cortisol concentrations were in the normal range. Baumstark and colleagues found that these control animals had a median plasma renin activity of 1.0 ng/ml/hour. The researchers then used plasma renin activity to assess the effectiveness of various treatments for Addison's disease, thus demonstrating its potential for the diagnosis of hypoadrenocorticism in humans and dogs, and treatment evaluation.

Taking a different approach, in 2015 Boag et al have built upon clinical studies of patients with hypoadrenocorticism, showing the presence of autoantibodies against enzymes involved in the synthesis of adrenal steroid hormones.[5] The authors looked for similar autoantibodies in dogs with Addison's disease. While the majority of autoantibodies identified in humans appeared to be absent, there was detectable plasma antibody reactivity against the P450 side chain cleavage enzyme. 24% of dogs with hypoadrenocorticism were positive for the P450 autoantibody, compared to only 1.2% of healthy control animals. These results are significant for research into the potential causes of Addison's disease, but their potential as a diagnostic tool may be limited since the majority of dogs with the condition tested negative for antibodies against P450.

The search for genetic markers

There is significant evidence for genetic predisposition to Addison's disease. In fact, 50% of people diagnosed with the condition have a concurrent autoimmune condition such as autoimmune thyroid disease or Type 1 diabetes.[6] The same genetic predisposition is likely to apply to dogs, particularly in certain breeds; poodles, Portuguese water dogs and bearded collies have a higher incidence of hypoadrenocorticism than other dog breeds.[7] Research in humans has identified an association with the MHC class II gene, which is essential for immune functions such as activation of T lymphocytes.[8] Similarly, veterinary research has linked other genes involved in T lymphocyte activation (such as CTLA4 and PTPN22) to the heritability of hypoadrenocorticism in canines.[9] However, the genetic pathways associated with Addison's disease are likely to be complex, making research into new diagnostic techniques and preventative breeding programmes far from simple.

Conclusion

There has been significant progress over the past two years in the development of better diagnostic techniques for hypoadrenocorticism, in particular new blood-based tests and genetic markers. However, further coordinated research between clinicians and veterinarians is required in order to advance the testing and application of these techniques to clinical practice. Ultimately, this will give rise to more rapid and effective treatment of this potentially life-threatening condition in both humans and our canine companions.

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Remyelination therapy: a new frontier in multiple sclerosis?

By David Li, John Ng, Timothy Woo
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Multiple sclerosis (MS) afflicts millions of people globally. Its precise pathogenesis is unclear, although it involves autoimmune destruction of the myelin sheath insulating central nervous system (CNS) neurons, as well as the death of glial cells that normally support neurons.[1]

Presently, MS is treated with immune-modulatory therapies aimed at suppressing further inflammatory damage. However, these medications have limited roles in promoting the remyelination and repair of damaged myelin sheaths, a process that is highly inefficient in MS lesions.

Under normal circumstances, oligodendrocyte precursor cells (OPCs), recruited to the site of demyelination, mature to become oligodendrocytes (OLs). These extend and wrap their processes around exposed neuronal axons to form multi-layered myelin sheaths.[2] Thus, remyelination seems to allow neurons to restore full physiological function.

A new approach to treating MS is remyelination therapy, for example using Anti-LINGO 1, a monoclonal antibody developed by pharmaceutical company Biogen that targets the LINGO-1 transmembrane protein expressed on OLs.[3,4] The antibody appears to antagonise the inhibitory effect exerted by LINGO-1 on suppressing OPC maturation, which in animal studies leads to myelin repair. A phase 1 trial found that Anti-LINGO-1 was tolerated well by volunteers and patients alike.[5] In April 2015, Biogen reported results of the phase 2 RENEW trial, in which 82 patients with acute optic neuritis were treated with Anti-Lingo-1 and checked for signs of remyelination. They showed a 41% improvement in nerve signalling compared to those who received a placebo infusion. Currently, a second phase 2 trial is underway – SYNERGY – which is due for completion in 2016.[3]

Another candidate remyelination therapy is clemastine fumarate, an anti-histamine and anti-cholinergic drug that has been approved for the treatment of allergic reactions. Clemastine enhances myelin repair via an increase in mature OLs and increased expression of myelin basic protein (MBP), which is believed to promote oligodendrocyte differentiation.[6] Clemastine is currently being investigated in a phase 2 trial (ReBUILD) for its ability to promote remyelination in MS, and in the ReCOVER trial for promoting remyelination in acute optic neuritis.

At a more preliminary stage, the CNS muscarinic receptor antagonist Benztropine, which blocks the inhibition of OPC differentiation, has in vitro effects indicative of remyelination. Researchers reported a 160-fold increase in MBP expression, and a 16-fold increase in the expression of myelin oligodendroglial glycoprotein.[7]

Overall, remyelination therapy appears a very promising, albeit still unproven, possibility for the future management of MS, a highly debilitating and crippling disease. Indeed, remyelination therapy has the potential to operate in synergy with current immunomodulators that halt further damage, to restore fully the normal physiological function of myelinated neurons, and usher in a new age in the battle against MS.

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This article was written as an extension to research conducted for a poster presentation at a neurology conference, with the aim of summarising recent developments in the field of multiple sclerosis therapeutics. The project was supported by Professors Keith Hart and Dave Wilson.

Are oral contraceptives linked to congenital abnormalities?

By Zohrah Khan
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Oral contraceptives are the most common form of birth control around the world, with as many as two-thirds of young women using them in the United Kingdom.[1] With full adherence to guidelines oral contraceptives are 99% effective, but due to missed doses, drug interactions or illness, an estimated 9% of women have unplanned pregnancies whilst using oral contraceptives.[2] Other women choose to stop using contraception and become pregnant within a few menstrual cycles. In either instance, the foetus may be exposed to progestogens and oestrogens.

Early studies suggest that these exogenous sex hormones may increase plasma levels of vitamin A, and decrease plasma levels of vitamin B6, but the potential teratogenicity is not known.[3,4] Other studies had suggested an increased risk of specific congenital defects in association with oral contraceptive use, including hypoplastic left heart syndrome, gastroschisis, limb defects and urinary tract anomalies.[5,6] In 1995, Czeizel and Kodaj, for example, reported a statistically significant 1.7-fold increase in the risk of limb defects.[5] Most early studies were retrospective, involving selection of a cohort of infants with congenital defects and then questioning their mothers about contraceptive use.

Such a method may allow recall bias to affect results. Therefore, despite years of research on the safety of oral contraceptives, little is known about the true risk to the unborn infant. Could the use of oral contraceptives before, or after, the time of conception be harmful to the foetus?

To address this question in a more rigorous way, Charlton et al conducted a cohort study in Denmark to investigate whether oral contraceptives were associated with major birth defects.[7] They obtained birth-related data spanning 1 January 1997 to 31 March 2011. After accounting for birth defects with known causes or chromosomal abnormalities, the final cohort included 880 694 births. National prescription and patient registers were used to identify contraceptive use among the women who gave birth. A European classification system was used to define major birth defects.

The study primarily looked at mothers who were exposed to oral contraception less than three months before, or after, pregnancy, and found that overall, there was no significant increase in any major birth defects. Secondary analysis found no link between this subgroup and oral contraception use but small sample sizes means we should err on the side of caution when interpreting these negative findings.

The recent study does have some limitations. For example, although pharmacy registers were used to track contraceptive use, it is unknown whether the participants actually used their last prescription and therefore there is uncertainty about the period of exposure. Furthermore, the study only included singleton births and assessing the effect on twin births may have provided a more random representation of the population.

Overall, the results of the new study back the consensus of previous research and provide further reassurance on the safety of oral contraceptive use around the time of pregnancy.[8,9] Differentiating between the different types of oral contraception may warrant future research.

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Zohrah Khan is a Year 4 student in the University of Exeter Medical School BMBS programme. Her clinical interests are in paediatrics, public health, and improving quality of care. Outside of work she is interested in the history of medicine and issues of social justice.

Oral contraception is the most common method of contraception in many parts of the world, and Zohrah was interested in reviewing a new trial that looked at what happens when this method is ineffective.

Advances in the immunotherapy of cancer: the role of immune checkpoint inhibitors

By Poppy Jenkinson
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At present, more than a third of people in the UK will develop cancer at some point during their lifetime, with half of those diagnosed being expected to survive more than 10 years.[1] The three main types of treatment for cancer – surgery, chemotherapy and radiotherapy – vary hugely in their ability to eliminate malignancies and the extent of side effects or adverse reaction. This creates a need for new and novel cancer treatments.

Cancer immunotherapy is based on the concept of rallying the host immune system to destroy or prevent cancer progression. The underlying idea is to trigger and strengthen the immune response against cancer cells, whether by increasing the activity of the immune system or the immunogenicity of cancer cells, or by blocking their evasion from immune attack. The intrinsic properties of the immune system, in being able specifically to recognise, remember, and adapt, confer potential benefits of cancer immunotherapy compared to other treatments. These include minimising harm to normal cells, preventing relapse of disease and avoiding cancer cell mutations that lead to therapy resistance.

Some of these approaches have now been licensed for use, while others are showing promise in clinical trials. One very promising area is that of immune checkpoint inhibitors.

> "Other immune checkpoints are inhibitory, resulting in a dampening of the immune response"

Immune checkpoints

Immune checkpoints are ligand-receptor signalling pathways in different cells, which aim to control the degree of immune response to a specific stimulus. Some upregulate the immune response against a particular antigen, such as the CD28 molecule on T cells, which interacts with CD80 or CD86 on antigen presenting cells, stimulating T cell activation.[2] Other immune checkpoints are inhibitory, resulting in a dampening of the immune response. Cancer cells may avoid immune attack by interacting with these inhibitory receptors, blocking an immune response and initiating tumour cell tolerance.

One inhibitory immune checkpoint at which tumour cells may act is CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) on T cells. This receptor normally interacts with the CD80/86 ligand on antigen presenting cells and directly inhibits T cell activation. This also prevents stimulation of T cell activation by CD28 (Figure 1).

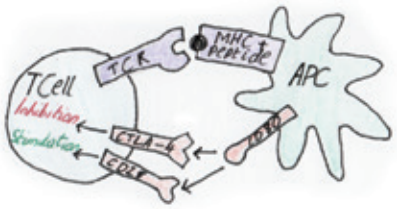

Figure 1: Opposing effects on T cell activation by two receptors for the CD80/86 ligand. A T cell expresses both receptors (CD28 and CTLA-4) for the CD80/86 ligand on the antigen presenting cell. CTLA-4 inhibits, and CD28 promotes, T cell activation following interaction between the T cell antigen receptor (TCR) and its antigen target (MHC/peptide).

Another potential target is the PD-1 receptor (programmed cell death protein 1) on T cells, which has two ligands, PD-L1 and PD-L2. Interaction between the receptor and either of its ligands is thought to reduce T effector cell proliferation and cytokine production, and cause an overall increase in T effector cell apoptosis.[3]

Immune checkpoint inhibitors

Understanding these inhibitory immune checkpoints has led to the design of therapies that block the checkpoints and antagonise the inhibitory signal, leading to an upregulation of the endogenous anti-tumour immune response. The biological targets for these therapies are the checkpoint molecules themselves: CTLA-4, PD-1 and PD-L1/2.

In 2000, clinical testing began for Ipilimumab, an anti-CTLA-4 monoclonal antibody that binds to the CTLA-4 receptor and prevents the inhibition of T cell activation and competition with CD28 for CD80/86 (Figure 2). In March 2011, it became the first immune checkpoint inhibitor to gain US Food and Drug Administration (FDA) approval and to be used worldwide for the treatment of cancer.[4]

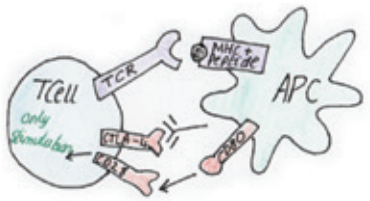

Figure 2: CTLA-4 blocked by Ipilimumab. Ipilimumab is a monoclonal antibody, shown here to block the CTLA-4 receptor and prevent its interaction with CD80/86. 'Signal one' of T cell activation (the MHC/peptide and TCR interaction) is also included for completeness.

Two anti-PD-1 monoclonal antibodies, Nivolumab and Pembrolizumab, gained FDA approval for the treatment of metastatic melanoma in 2014. During 2015, both treatments also received FDA approval for the treatment of metastatic non-small cell lung carcinoma, and

Table 1: NICE guidelines for cancer treatment using immune checkpoint inhibitors. Treatment guidelines include currently approved drugs and indications, as well as approval anticipated in the near future.[11,12]

Drug name(s)	Target molecule	Guidelines for use	Date approved
Ipilimumab	CTLA-4	Advanced melanoma after prior treatment	December 2012
		Advanced melanoma without prior treatment	July 2014
Pembrolizumab	PD-1	Advanced melanoma, after disease progression with Ipilimumab	October 2015
		Advanced melanoma, with no prior treatment with Ipilimumab	November 2015
		Non-small cell, PD-L1 positive lung cancer after chemotherapy	January 2017 (anticipated)
Nivolumab	PD-1	Advanced melanoma	February 2016
		Metastatic squamous non-small cell lung carcinoma after chemotherapy	September 2016 (anticipated)
		Advanced renal cell carcinoma, previously treated	October 2016 (anticipated)
Nivolumab + Ipilimumab	PD-1 and CTLA-4	Advanced melanoma	September 2016 (anticipated)

Nivolumab for metastatic renal cell carcinoma.[5]

Different immune checkpoints have distinct roles and mechanisms in regulating the immune response. This opens the possibility of using different inhibitors simultaneously to gain additive effects.[6] The combination of Ipilimumab and Nivolumab gives better outcomes in patients with advanced melanoma compared to either treatment alone, and gained FDA approval in September 2015.[7] It has continued to show promise, with the most recent data indicating a 26% increase in two year survival rates.[8] Many clinicians support the idea of replacing treatment based on a single immune checkpoint inhibitor with combination therapy.

A major limitation of immune checkpoint inhibitors, however, is the frequent auto-immune related side effects.[9] Releasing these normal constraints on the immune system appears to be detrimental in situations where endogenous immune inhibition is needed, as in autoimmunity. Another limitation is the need first to identify those immune checkpoints that a particular tumour is using for immune evasion, in order to define the subset of patients who will benefit from a particular therapy.

Recent findings

Clinical trials of anti-PD-1 immunotherapy are currently underway against 30 different types of cancer.[4] In April 2016, the American Association for Cancer Research annual meeting heard results of a major international trial of Nivolumab for the treatment of head and neck squamous cell carcinoma, a particularly aggressive malignancy. Nivolumab appeared to have increased patient survival rates compared to standard chemotherapy - the first result of its kind for this type of cancer.[10] This extremely encouraging finding could dramatically change the clinical management of head and neck carcinomas.

Guidelines for clinical use

The National Institute for Health and Care Excellence (NICE) guidelines reflect the current clinical benefit of immune checkpoint inhibitors as cancer treatments in the UK (Table 1).[11] Further research is needed to enhance their effectiveness, and to explore other potential immune checkpoint pathways, each of which could help to release the breaks on the immune response to cancer.

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Lessons from the French clinical trial tragedy involving BIA 10-2474

By Natalie Cheng
Year 2, Medicine, Cardiff University

How different might our lives be if the thousands of clinical trials that have taken place had never existed?[1] Thanks to clinical trials, the effectiveness and safety of medications and procedures has been verified. Patients are largely very trusting: in one survey, 72% of patient said they would like to be involved in clinical research.[2] In another study, 89% of patients perceived clinical research to be very safe or somewhat safe.[3] Earlier this year, however, the world was astounded when a drug under clinical trial in France was associated with a death and brain damage. This article explores the regulation of clinical trials and the impacts of this incident.

Background

The compound BIA 10-2474, which blocks the enzyme fatty acid amyl hydrolase, was developed by Portuguese pharmaceutical company Bial for treating neurological and psychiatric pathologies including anxiety. After testing on non-human primates and dogs, a Phase I trial began on 9 July 2015 to assess the drug's effects in humans. It involved 128 healthy volunteers aged between 18 and 55, of whom 90 received the drug without any significant problems and 38 had a placebo. Six out of the 90 began receiving multiple daily doses on 6 January 2016, with the first symptoms of headaches and blurred vision noticed in one volunteer four days later. His condition rapidly deteriorated and he was later declared brain-dead, and the trial halted. Three of the other volunteers also suffered brain damage.[4,5]

Regulation of clinical trials

Six regulatory bodies oversee clinical trials in France, including the National Security Agency for Medicines and Health Products (ANSM) and the Ethical Research Committees (CPPs).[6] In the UK, they are regulated by the UK Medicines and Healthcare products Regulatory Agency (MHRA) and the Health Research Authority (HRA). The European Medicines Agency (EMA) also regulates all clinical trials within the European Union.

The authorisation of clinical trials is subject to scientific and ethical reviews. Various aspects are evaluated, including whether a trial involves low risk, the anticipated therapeutic and public health benefits, and the risks and safety measures for participants. In the case of adverse events, the EMA specifies that reports should be submitted without delay, and compensation given for any damage suffered by trial participants. And to increase transparency, all relevant trial information has to be uploaded to a publicly accessible European database, according to the EU Clinical Trials Regulation.[1,7]

> "According to a report from the regulatory body ANSM, Biotrial and Bial were not responsible for the volunteer's death since no fault was found with the clinical trial"

Reflections on the French incident

Are the team at Biotrial (a French-based private contract research company) and Bial to blame for the French incident? According to a report from the regulatory body ANSM, Biotrial and Bial were not responsible for the volunteer's death since no fault was found with the clinical trial. However, shortcomings were identified that could have contributed to the catastrophe, including continuing to administer the experimental drug to the volunteer after the first signs of side effects had appeared.[8] In response, Biotrial declared, 'the trial was conducted in full compliance with the international regulations and Biotrial's procedures, in particular regarding the emergency procedures'.[9] In June 2016, however, the French public prosecutor's office began a manslaughter investigation.[10]

Based on the reports available, it seems unlikely likely that the tragedy was caused by violation of regulatory law. However perhaps there was a lack of well-planned emergency measures. Perhaps no one had questioned whether the headaches were anything more than a common side effect. With the benefit of hindsight, if these symptoms had been queried and handled more cautiously, the potential for injury may have been reduced. It is possible that a lack of experience or awareness of adverse events prevented staff from taking action. In addition, a drug that appears safe in animal tests isn't necessarily

"To pharmaceutical companies and clinical investigators worldwide, this incident may serve as an alarm, reminding them of the importance of adhering to official guidelines and revising safety procedures"

going to be safe in humans. Given the unpredictability of human trials, it may be that safety procedures were not fully developed for every possible outcome.

The death of the volunteer must have been devastating for his family, and for other volunteers and their families. According to an interview conducted by Channel 4 News, the dead volunteer was a 49 year old father of four children. His brother said, 'It is just a nightmare. …. My brother he was just lying in the bed. It was really awful.'[11]

To pharmaceutical companies and clinical investigators worldwide, this incident may serve as an alarm, reminding them of the importance of adhering to official guidelines and revising safety procedures. Staff training should include greater attention to minute details and understanding of their wider implications.

In order to prevent such future tragedies, tighter regulatory laws might be required. The question is, will this really help to eliminate mishaps? How many more restrictions must be imposed to guarantee the safety of trial participants? Is this even possible? It is certainly worth pondering.

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Fertility Health Summit: choice not chance

By Amy Hough
Year 4, Medicine, University of Exeter

The Fertility Health Summit* brought together a group of experts from health, education and government to discuss 'how to improve young people's knowledge of fertility and reproductive health'.

Professor Adam Balen, chair of the British Fertility Society, presented the results of a survey of 1,000 16-24 year olds revealing large gaps in their knowledge of fertility. In 2014, for the first time, over half of women in the UK had their first child over the age of 30, and so it is important that young people are aware of fertility issues that may affect them. The key question of the day was how best to address these issues?

There were moving talks by representatives from Brook and Sexpression:UK, two charities that deliver sex and relationships education (SRE) to young people, and Lucy Emerson, coordinator of the Sex Education Forum, on the challenges of providing SRE in schools and how fertility awareness could fit into this. Professor Søren Ziebe also gave an interesting talk on what is happening in Denmark, which seems to be far ahead of the UK in terms of SRE and fertility awareness. He runs a clinic where patients can self-refer for free fertility assessment and counselling. They also have easy access to IVF (9% of all babies are born by IVF), famous fertility ambassadors, and large public health campaigns surrounding fertility awareness.

Alex Jones, co-host of The One Show, gave the keynote speech on her own experiences of fertility issues, and what she discovered while making the television documentary entitled '*The Truth About Fertility*'. After many more inspiring speakers, the day concluded with a lively Q&A and plans for the future direction of the Fertility Health Task Force, who hosted the event.

Read more about the conference here: https://britishfertilitysociety.org.uk/downloads/ms_20955.pdf

* Fertility Health Summit, hosted on 16th April 2016 by the Fertility Health Task Force, a special interest group of the British Fertility Society, Royal College of Obstetricians and Gynaecologists.

"In 2014, for the first time, over half of women in the UK had their first child over the age of 30, and so it is important that young people are aware of fertility issues that may affect them"

Bristol One Health Conference

By Holly Ravenhall
Year 4, School of Veterinary Sciences, University of Bristol

Back Row - Isobel Hutchinson, Andy Grist, Jamison Suter, Professor Abigail Wood, Dr Martin Hetzel, Dr Sandra Corr, Nigel Gibbens (CVO), Robin Hargreaves

Front Row - Cherry Phypers, Collette Taylor, Emily Logie, Holly Ravenhall, Poppy Simonson

The Bristol One Health Conference, held at Langford Veterinary Services on 5 March 2016, saw 98 delegates come for a full day of lectures and debates.

The day started with Professor Abigail Wood taking us through the history of One Health. The concept has been around for a surprisingly long time: Aristotle practised One Health as early as the third century! But the term One Health was not coined until much later.

The second speaker, Martin Hetzel, discussed current methods in treating tuberculosis (TB), and surprised everyone with the estimate that one-third of the world population is harbouring latent TB. It means that we could face a resurgence in TB over the coming years as the world population ages and life expectancy increases.

Equally fascinating was Sandra Corr's presentation about the link that is emerging between pet- and owner obesity, before Andy Grist (University of Bristol) and Imogen Hutchinson (Animal Aid) battled it out in a vegan debate, centred around human health, the environment, food sustainability and slaughter. The debate highlighted many pro-vegan points, especially on animal welfare, but also the questions of whether veganism is sustainable for large populations, and where exactly do we draw the line? Are MMR vaccines grown in animal-derived cell culture acceptable for vegans?

After lunch Chief Veterinary Officer Nigel Gibbens discussed the issue of food safety, and Jamison Suter of the Environmental Foundation for Africa emphasised that the environmental aspect of One Health needs greater awareness. Understanding ecology, he said, especially in light of disease spread, may help us deal not only with outbreaks such as Ebola, but will also help prevent diseases from emerging and causing wide-scale crisis.

Robin Hargreaves, former British Veterinary Association President, gave a touching insight into approaching the difficult topic of pet euthanasia with clients. He was followed by the final speaker of the day, Heather Thompson from Medical Detection Dogs, who described the incredible work of dogs involved in assistance and disease scent research.

All in all, it was a very informative, thought provoking and interesting day. The committee would like to thank all the speakers and delegates for coming and hope to see them all again in 2017.

The author would like to acknowledge the University of Bristol Veterinary Sciences Staff for their on-going support.

Thanks also to the Bristol One Health Committee: Holly Ravenhall, Cherry Phypers, Poppy Simonson, Emily Logie and Collette Taylor.

INSPIRE Intercalators' Research Conference 2015

By Andy Jones and Anna Taylor
Year 4, Medicine, University of Bristol

The first National INSPIRE Intercalators' Research Conference took place in Bristol in October 2015. Attendees came from across the UK, with 150 students coming from Cardiff, Exeter, Plymouth, Sheffield, Birmingham, Oxford, Cambridge and Bristol - mostly medical students, as well as dental and veterinary students. Prof George Banting, Dean of the Faculty of Biomedical Science, opened the conference, followed by a keynote speech from Dr Tony Pickering, academic, anaesthetist and joint INSPIRE lead. He described how his intercalated degree became a springboard to further research as a student, and for helping to obtain grants. It was a long-term perspective on how intercalation can kick-start an academic career.

Previous intercalators spoke in a series of themed programme talks, covering cancer, immunology and infection; cardiorespiratory, endocrine and neuroscience; and epidemiology, general practice and global Health. Delegates chose which stream to attend and voted on which presentations were of sufficient quality to win prizes.

Lunchtime included themed poster sessions, in which presenters had two minutes to summarise the main points of their project before being questioned by a facilitator and student delegates. Prospective intercalators had the opportunity to talk with intercalation programme leads from across the South West.

The final part of the day involved young clinical academics talking about their careers beyond intercalation. Specialties included veterinary practice, core surgical training, GP training and medical leadership. They talked about opportunities, challenges faced when getting clinical and academic jobs, decisions about changing career paths, and future plans. Several students commented that it was reassuring to hear that a strict career plan was not needed in order to succeed in academia!

Professor Sandy closed the conference, awarding prizes to students from across the country. A wine reception followed hosted by Bristol Medics'Wine-Tasting, with the opportunity to network with senior academics.

The variety of projects presented formed an impressive array of student research. The event served as an excellent introduction to the world of conference presentation and was very informative about what to expect post-intercalation. The opportunity to meet intercalators from other universities revealed how student research is conducted nationally and the story of serendipity in academic careers was a relief to many delegates.

The conference opened our minds to the opportunities of intercalating, including at another medical school, or intercalating between third and fourth years in order to acquire the option of working towards a masters degree. Intercalated degrees included courses in biochemistry, bioethics, global health, medical humanities and even childhood studies, providing a refreshing change from medical studies. Many students reported that intercalating gave them skills in research, data acquisition and presentation. Being able to talk personally to the course leaders really makes a difference when deciding whether or not to intercalate.

Thank you to Dr Tony Pickering, Dr Liz Coulthard, Elle Chilton-Knight and Kay Russell for organising the conference!

The next INSPIRE Intercalators' Research Conference takes place on Saturday 15 October 2016 in Exeter. To register see http://medicine.exeter.ac.uk/bmbs/inspire

Intercalators' Conference 2016

INSPIRE National Intercalators' Research Conference

09.30-17.15 Saturday 15th October 2016

Peter Chalk Conference Centre, University of Exeter, Stocker Road, Exeter, EX4 4QD

Open to **Medical, Veterinary** and **Dental** students who have already intercalated and to those considering intercalation. The conference will feature:

- Student oral presentations.
- Student poster discussion session.
- Information fair for intercalation programmes.
- A keynote address.
- Talks from more junior professionals on how intercalation has influenced their subsequent career tracks.
- Networking over lunch

Prizes for the best poster and oral presentations in each category (£25-50). Free coaches from Truro, Plymouth, Bristol and Cardiff to Exeter. Competitive travel bursaries (£75) available for presenters travelling further afield. Registration £10. To register and submit an abstract- http://medicine.exeter.ac.uk/bmbs/inspire/. For further details contact: j.tarr@exeter.ac.uk . Deadline for registration **10th Oct.**

ISCAID 2016

October 2016 will see Bristol host the 4th International Society for Companion Animal Infectious Diseases (ISCAID) Forum, in conjunction with the International Feline Retrovirus Research Society. This is the first time this biennial conference will be held in the UK following successful meetings in the USA, Canada and France. Registration is now open at iscaid2016.eventbrite.com

The conference will feature keynote speakers Professor Adrian Hill of The Jenner Institute ('Novel strategies for vaccine development'), Professor David Price of Cardiff University School of Medicine (Deciphering the T-cell immune response to HIV-1), Professor Jonathan Fogle of North Carolina State University (CD8+ T cell dysfunction in FIV, CD8+ T cell epigenetics and micro-RNAs), and Professor Cynda Crawford of University of Florida (Canine influenza virus – old and new). The symposium has a further broad array of topical lectures delivered by influential and respected authorities in the field of companion animal infectious diseases.

The symposium is generously sponsored allowing this symposium to be excellent value for money.
Those not local to Bristol will get time to explore the beautiful city and all its landmarks, and the symposium dinner on the famous SS Great Britain promises to be a fantastic and rare treat that few people are ever lucky enough to experience.

Please visit the website for further information on the programme and symposium www.iscaid.org/meeting-info. Early registration is encouraged.

Frances Keane

GU and HIV consultant, Royal Cornwall Hospitals Trust

By Amy Hough
Year 4, Medicine, University of Exeter

How did you get into research?

I was always quite interested in medical microbiology, so after my third year of medical school I took a year out and did an intercalated degree in medical microbiology. After a couple of years as a registrar I decided I would try and get into a bit of sexually transmitted infections (STI) research and worked as an MRC research fellow for David Taylor Robinson at St Mary's Hospital in London. He was the person who discovered *Mycoplasma genitalium*. I did a project on the association of bacterial vaginosis and non-gonnococcal urethritis, including *mycoplasma genitalium*.

What is your current research about?

Most of the research I have done since I moved to Cornwall has been service-based, looking at how we can improve services for patients. What we are looking at the moment is how patients would feel about using an online booking system and having asymptomatic screening.

You mentioned your supervisor earlier, was he the person that inspired you, or has there been a particular mentor that inspired you to get into research?

He was very inspirational actually. He'd discovered so much and was very unassuming, but had a very good scientific mind, I learnt a lot from him.

What is the most valuable piece of advice you've received?

To trust your gut instinct, and always do what you think is best for the patient. Keep the patient at the centre of everything.

Within GU medicine what would you say are the most exciting current advances?

Well, I think new drug advances in HIV therapy are always very interesting and very welcome! The other thing that has made a real difference to patients is the development of very good nucleic acid amplification tests for organisms like gonorrhea and chlamydia, which means that we can offer non invasive testing to people.

What should students know about pursuing a career in research?

The whole attitude to research is changing. It's becoming much more mainstream in medicine, so rather than people specifically branching off into research, they're much keener to get everybody involved and actively thinking about what they can do with the patient sitting in front of them that might make a difference to their care. You've got to have quite an analytical mind and a real interest if you want to pursue research as your main career.

Are there any opportunities for students to get involved with GU research?

We normally run a research SSU (student selected unit) every year so that a student very much gets involved in a project. I like the student to do a lot of the project themselves, so that rather than just slotting in, they tend to do the first piece of work, the literature search, and they often help us to analyse the data and produce a poster. We usually then carry on and collect more data, and prepare for our spring meeting. So they usually get quite a lot out of it.

Do you have any advice for students interested in research?

Go ahead and have a go, and see if you enjoy it!

Rebecca Sims

Research Associate, Institute of Psychological Medicine and Clinical Neurosciences, and the MRC Centre for Neuropsychiatric Genetics & Genomics, Cardiff University

By Helena Jones
Year 4, Medicine, Cardiff University

What brought you to your current research on Alzheimer's Disease?

Since school I have had an interest in biology, particularly the human body. I undertook a biology degree and quickly realised that the key to many of my questions lay in genetics. After gaining a BSc in genetics I was fortunate enough to be offered a PhD studying the interplay of genetics and the behavioural aspects of Alzheimer's disease (AD) – a disease which has had a significant impact on my own family. I was a research assistant and then a research associate in Julie Williams' group at the MRC Centre, Cardiff University, where we identified two novel AD risk genes, the first ones for nearly 20 years! In 2012, I secured an Alzheimer's Society fellowship to investigate the effect of rare variation in AD. I am nearing completion of this fellowship and have identified two novel risk variants for disease (manuscript in preparation). I have recently been awarded two European research grants, with which to study a cohort of young onset dementia sufferers, using next generation sequencing technology to further elucidate the genetics of AD.

Why did you decide to pursue a career in research?

During my undergraduate degree I was lucky enough to be offered a professional training year (PTY) in schizophrenia research. I learnt a lot during this time and got the research bug – so I chose to pursue a Ph.D. in genetics after graduating (I obtained a B.Sc. class 2:1).

What was your first research project?

My first project was during my PTY, investigating genetic variation within the DISC1 gene as potential schizophrenia risk variants.

What are your most valuable discoveries in Alzheimer's disease research?

I have been part of a world-leading group who through collaboration identified over 20 risk genes for late-onset AD. These highlight novel biological pathways that contribute to the pathophysiology of the disease.

How do you hope to contribute to the future of AD?

My current and future research aims to identify rare genetic variation that significantly affects disease risk, through a combination of genotyping and sequencing technology. In addition, I am looking to collaborate with scientists from various disciplines to translate our genetic findings into disease-relevant knowledge.

What are the three most valuable skills you have learnt in research?

Communication, problem solving, and initiative.

What advice would you give to a student about a research career?

Get as much experience as you can - do work experience placements, etc. And get your face and name known to people who may be able to help progress your career.

What would make a student stand out amongst others?

Experience on their curriculum vitae, academic achievement, and a well-constructed and clear application that shows motivation and enthusiasm.

Helena Jones took the opportunity to intercalate between her third and fourth year of study, to do a BSc. in Psychology and Medicine, including a research project titled 'Genome-wide association study of Alzheimer's disease with symptoms of agitation'. With the burden of Alzheimer's disease (AD) increasing in our ageing population,[1] understanding its genetic basis could provide the key to advances in diagnosis and treatment. Although more than 25 novel risk loci for AD have been identified since 2006 using genome-wide association studies (GWAS), these account for only 30% of genetic variation in late-onset AD.[2] Helena's work involved investigating heritability linked to the sub-phenotype of AD: Alzheimer's disease with agitation.[3]

"Rebecca Sims was my supervisor, and her dedication to her fascinating research on the genetic and behavioural aspects of AD was truly inspiring," says Helena.

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How to make your academic CV flourish

By Sophie Herbert
Year 4, Medicine, University of Bristol

Creating and updating your curriculum vitae (CV) as a medical student will help you to stand out amongst your peers. With the increasingly competitive Academic Foundation Programme and greater demands at medical school, it is important to work on your CV little and often.

This article discusses important topics such as intercalation, presentations and publication but also discusses other ways to boost your CV including vacation studentships, paid work, audits, teaching, committee experience, and prizes. All can be used in the white space areas on an AFP application form.

The earlier you start work on your CV the better.[1] Keeping it updated will save time in future and you won't forget your achievements. But don't get stressed over CV-building; you will be surprised how much you have accomplished already. Here are areas to consider:

Intercalation

Approximately one third of medics will intercalate during their studies,[2] but if you haven't yet intercalated, why not consider applying to a different University? Or complete a Masters degree instead of a BSc?

There are many scholarships available to help with the extra tuition fees and other costs. Organisations such as Kidney Research UK and The Wolfson Foundation provide scholarships on a range of topics including neurology, dermatology, and medical ethics. 'Money 4 med students' has lists of intercalation scholarships available.[3]

Publications

It is important to weigh up the amount of time you would spend trying to get a publication against the points awarded. Remember, the Academic Foundation Programme states that you do not need to be first author to be awarded points, provided that your work has a PubMed Identifier number.[4]

Have you come across an interesting case on the wards? Writing case reports can show your interest in a particular speciality, and the experience will help you to improve your writing skills and shows initiative on your CV.

Vacation studentships

Vacation studentships offer the opportunity to complete research whilst earning money throughout the holidays. They also provide insight into intercalation. However, for many, the summer holidays are sacred, and it can be difficult to decide whether to work or relax.

"The INSPIRE vacation studentship opens a door into the world of research. Many labs I applied to said, "you need prior research experience." Well this scheme allows you to gain that first experience! It is great for your CV, but more importantly, it shapes your understanding of research."

Andrea Cordaro, Year 5 Medicine, University of Bristol

Presentations

Poster and oral presentations can be daunting, but they look great on a CV. They don't have to just be on a scientific topic - medical education and Quality Improvement Projects count too. Presentations can include data from a Student Selected Component, audit, vacation studentship or intercalation, and offers a great opportunity to present work in your desired field. Completing the poster is the hard work, so why not present it again?

Audits

Audits can be completed at the bedside, whether you are setting up and running your own audit or helping others to collect data. Organisations such as Starsurg and Globalsurg invite students to take part in international audits, without heavy time commitments. Don't forget, you may then have the opportunity to present your work or even to get a publication.

Committee experience

Team work, leadership and organisational skills are important for practicing doctors. Being part of a committee allows students to demonstrate these skills, whether it is the netball committee or research society. Running for national committees gives your CV the extra push.

Prizes

The 'Prizes' section of a CV is difficult to fill. While it is hard to get a University prize, there are other ways to gain awards, such as essay competitions, enabling students both to develop writing skills and win money. Many students have extra-curricular studies or an interesting patient that could provide a starting point.

"Presentations can include data from a Student Selected Component, audit, vacation studentship or intercalation, and offers a great opportunity to present work in your desired field"

Teaching

Teaching ability is an important skill for doctors. You can attend courses on teaching, and practice by tutoring younger students. Alternatively, you could get involved in medical school interviews and open days, or visit your old school to talk about the application process for medicine and life as a medical student.

Paid work

Try combining paid work with medical studies? Working as a health care assistant, a phlebotomist, or summarising doctors notes may help you to develop and enhance skills.

The personal touch

Remember to display personal interests such as sport, music, and art. General advice from clinicians is to work on your CV little and often. The hardest part is the first step. Ask lecturers and clinicians if they have any projects you can get involved with, and remember to use work you have completed throughout your degree to enhance your CV.

Remember too that you can say 'no' to projects that do not appear to be of future benefit. CV-boosting is an extra-curricular activity, and is not a substitution for poor grades. Therefore remember to focus on what is important at medical school.

If there is a particular specialty you are interested in, be proactive. Why not this summer complete an essay competition in it, and do a SSC on it next year. Join a society committee in that area of specialty, and email clinicians to see if they have any audits or projects that you can get involved in. These steps will help you along the way for a presentation and a publication. Applying for jobs with a competitive CV demonstrating commitment to a specialty from an early age will make the process easier. If you aren't sure which specialty is for you, try to keep your CV as broad as possible. Getting involved in different specialities may shed light on the best career for you.

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Obstetric care in rural India

Student Selected Component

Shashank Chaganti
Year 2, Medicine, Plymouth University Peninsula Schools of Medicine and Dentistry

"During a gap year in October 2014, I developed an interest in obstetrics and visited Christian Hospital Mungeli, in Mungeli, India, as part of a sponsored observation programme. This report explores the high incidence of maternal mortality affecting the region. It also provides us with an opportunity to appreciate the UK's National Health Service for its value-driven service, which is much needed at a time of national and political uncertainty."

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Abstract

Christian Hospital Mungeli (CHM) sees a high number of women with complications during delivery. Contributing factors include a lack of a sustained programme of vital pre-natal care for pregnant women as well as mothers who present in a distressed state after having been in labour for hours at home. This, coupled with other issues such as malnutrition and anaemia, has led to a large number of emergency caesarean sections (C-section) being performed.

Current efforts to tackle maternal mortality have focused on developing a new incentive-based programme that aims to increase the number of mothers seeking prenatal visits. The initiative provides women with a 'baby box' containing essential baby care items for free when they attend four prenatal visits, and represents an initial step in tackling maternal mortality in the region.

Introduction

CHM is a 120-bed secondary care hospital that serves the diverse rural community of Mungeli and the surrounding districts of the Chhattisgarh state in India, which is home to a poor, marginalized and largely illiterate population. Established in 1896, the hospital provides 11 different medical services from general surgery and dentistry to intensive care.[2]

During my time in the Obstetrics Unit, I was struck by the high number of maternal complications during childbirth, particularly as this is an area with one of the highest maternal mortality rates in India.[4] Furthermore, in 2015, CHM recorded 45 intra-uterine deaths out of 799 deliveries.[5] In comparison, the stillbirth rate in the UK for 2015 was 3.8 deaths per 1,000. And giving birth in the UK remains safer than ever for the mother too, with a maternal mortality rate of nine out of 100,000 live births in 2015, compared to 174 deaths per 100,000

in India.[6] In light of such disparity, it is important to understand better the factors contributing to maternal mortality in India and to seek sustainable public health measures to combat these.

In this report, I present my observations and lessons learned from the Obstetrics Unit at CHM, as well as a summary of efforts being made to tackle this major global health issue.

The clinical picture of maternal mortality

As part of my clinical observation, I saw a large number of women presenting with severe eclamptic seizures who were often unconsciousness, needing immediate medical intervention in order to deliver the baby. A high prevalence of unsuccessful attempts to give birth at home meant that 78% of these cases were not booked for a hospital birth.[5] Therefore, due to the general urgency of the situation, the medical team would often deliver by emergency C-sections.

Emergency C-sections make up the majority of the deliveries in the Obstetrics Unit. In 2015, 78% of deliveries at CHM were C-sections.[5] The clinical decision-making behind this procedure is complex, as the medical team has to balance the associated risks and benefits.

A combination of malnourishment in women and a high prevalence of adolescent pregnancy were also major determinants for emergency C-sections. In these cases, young women would present with under-developed hips, leading to an increased prevalence of cephalopelvic disproportion - a risk factor for maternal mortality in the form of obstructed labour.[8]

Malnutrition means that 43% of pregnant women are anaemic,[9] most likely due to a diet lacking in folate. For these women, a C-section would not be recommended due to the increased risk of mortality,[8] and yet, the medical team would have to take this risk in order to deliver the baby.

Plymouth University: 'baby box' initiative

In order to reduce maternal mortality, a possible solution might be to try to reduce the number of emergency presentations.

Through my clinical observation, communication is not possible because women often present in labour. Establishing an efficient interactive model that enables close monitoring of a woman during pregnancy could play a large role in reducing the risk of maternal mortality.

So how do we achieve this? In many developed nations, prenatal care plays a key role in monitoring pregnancy and identifying any potential issues, and reduces the risk of maternal mortality.[10] Given that 90% of deliveries at CHM are unplanned, there are many missed opportunities for these pregnant women to gain vital advice and screening through pre-natal care.[5]

By building on a welfare programme from Finland begun in 1938,[11] the Enactus team at Plymouth University, Edify, is working with CHM on a 'Baby Box' initiative. Through this incentive-based programme, the hospital hopes to increase the number of women seeking pre-natal care. A woman will receive a 'baby box' when she completes four prenatal visits, with at least one before the fourth month of pregnancy and delivers in the hospital. This baby box will contain essential items such as cloth diapers, blankets, mattress, baby clothes, baby soap and visual educational materials advising families on contraception, family planning and nutrition.

These items are unaffordable for the average Mungeli family, and so by providing them for free, the aim is to see an increase in pre-natal care. This outcome is similar to the one observed in the original Finish programme which contributed to a dramatic reduction in infant mortality, from 65 out of 100,000 in the 1930s to the current 3.4 out of 100,000.[11,12] CHM is therefore confident that providing prenatal care will result in better management of pregnancy and prevention of complications such as eclampsia, ultimately reducing the risk of maternal mortality.[13]

Conclusion

Through my clinical observation, I have attempted to identify the underlying factors contributing to the high risk of maternal mortality at CHM. A combination of late presentation, malnutrition amongst adolescent females, lack of prenatal care and a high rate of unplanned C-sections, appears to be responsible.

One possible solution is culturally adapting the highly successful 1938 Finnish baby box programme to rural Chhattisgarh, in order to increase the number of women gaining prenatal care.

Finally, maternal mortality is a global health issue that in this case may have a local solution. However, although such local solutions show promise, there needs to be an increased concerted effort by non-government organisations, governments, corporate and philanthropic organisations, community hospitals such as CHM and other bodies, in order to tackle what may also be interpreted as a sociocultural problem. In Mungeli, with four out of five women stating that they do not feel that they have control over their health,[5] this may also be viewed as an issue around the empowerment of women.

For the time being, the prospects for women giving birth at CHM look positive. The implementation of the new baby box scheme in May 2016 is a progressive starting point for reforming obstetric care in rural India.

> "CHM is therefore confident that providing prenatal care will result in better management of pregnancy and prevention of complications such as eclampsia, ultimately reducing the risk of maternal mortality"

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Management of pregnant women with overt hypothyroidism

Case Report

By Nimarta Idnani
Year 5, Medicine, Plymouth University Peninsula Schools of Medicine and Dentistry

"With a keen interest in obstetrics and gynaecology, I undertook a placement in the Early Pregnancy Unit where I met this patient. Being of a similar age to her, I was able to relate to her but unable to imagine the psychological stress she endured after two miscarriages. Intrigued by her case, I started to research the link between hypothyroidism and miscarriage and found discrepancies in the guidelines. This article highlights and summarises the controversies to enable the development of a single care pathway for similar women in the future."

Abstract

Hypothyroidism is present in one in every 200 pregnancies. Most cases are diagnosed before pregnancy and treated with replacement thyroxine therapy. If the condition is mismanaged during pregnancy, consequences can include spontaneous abortion, pre-eclampsia, gestational diabetes, gestational hypertension, and decreased IQ in the child. All of these outcomes can be psychologically stressful for a mother. The patient described in this case report suffered two miscarriages; thyroid function tests (TFTs) were not conducted in either of her pregnancies. There are no data to indicate whether her levothyroxine treatment was of a sufficient dose to maintain thyrotropin (TSH) and free thyroxine (T4) within the appropriate range during pregnancy, and any link to management of the patient's condition remains uncertain. Controversy exists over the upper threshold for TSH levels during treatment of hypothyroidism, and whether the levothyroxine dose should be increased empirically by 25-50 µg in pregnancy. There remains a lack of clear guidelines from the Royal College of Obstetricians and Gynaecologists regarding treatment of overt hypothyroidism in pregnancy.

Introduction

Dysfunction in the thyroid gland significantly reduces a woman's ability to conceive, and can also adversely affect the outcome of pregnancy for both mother and foetus.[1] This article focuses on the consequences of an under-active thyroid gland (hypothyroidism). Overt hypothyroidism during pregnancy is defined as a low TSH level and high T4 level, according to trimester-specific reference ranges.[2] The condition is present in 0.5% of all pregnancies[3] and has been associated with miscarriage, because until ten weeks of gestation, the foetus is dependent on placental transfer of T4 from the mother.[4] A case report illustrating this association described a hypothyroid patient who had two missed miscarriages, defined as the loss or failed development of the foetus before 24 weeks gestation.[5] Pre-pregnancy counselling and regular monitoring of the thyroid function tests (TFTs) may have prevented her second miscarriage.

Methodology

Search engines such as Google Scholar, and the PubMed and TRIP databases were used to obtain papers. Guidelines were consulted from the National Institute for Health and Care Excellence and Care (NICE), British Thyroid Association (BTA) and British Thyroid Foundation. The search terms "overt hypothyroidism", "missed miscarriage", "levothyroxine" and "pregnancy" were used and the search was limited to papers written in the last 20 years, with priority given to randomised controlled trials and meta-analyses.

Case Presentation

The patient was a 21-year-old trainee dispenser, with a BMI of 28 kg/m^2, who presented at the early pregnancy unit with a three-day history of small, painless fresh vaginal bleeding and brown discharge. Her last menstrual period was twelve weeks previously and she had had a positive urinary pregnancy test four weeks later. Prior to this pregnancy, she was on the oral contraceptive pill, Cerazette. Her obstetric history was three pregnancies in total, two of which ended in miscarriage, and one abortion (gravida, 3, para 0, abortus 1). The outcomes of her last two pregnancies include a missed miscarriage one year ago at seven weeks of gestation, which resolved with expectant management, and a termination of pregnancy two years ago, which was surgically managed. Her medical history included hypothyroidism for which she was on 100µg thyroxine for the past two years. She had also taken folic acid for this pregnancy and was on no other medications. She did not drink alcohol and was a non-smoker.

Management and Outcome

A trans-vaginal ultrasound scan performed on her retroverted uterus showed a seven weeks gestational sac, but there was no yolk sac, amniotic sac or foetal heart activity present (Figure 1). A diagnosis of missed miscarriage was made and she opted for surgical management. Histology confirmed normal products of conception.

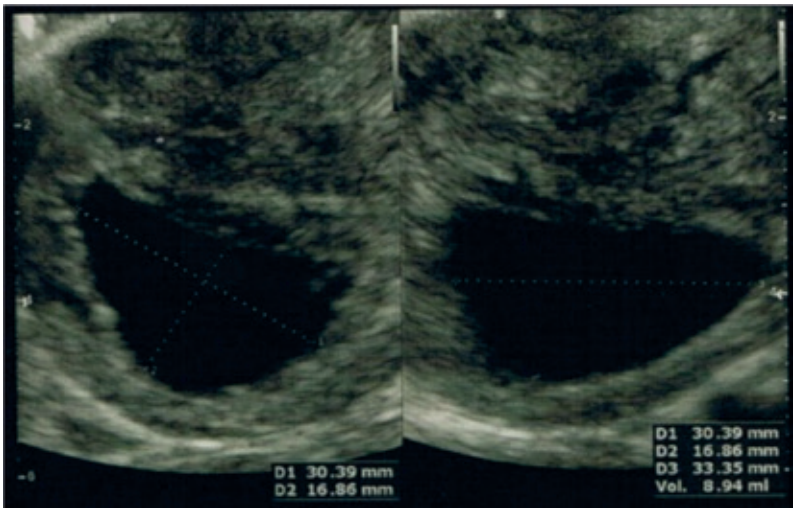

Figure 1: Image captured during trans-vaginal ultrasound scanning

Discussion

Guidelines on management of pregnant women with overt hypothyroidism

According to the NICE guidelines,[6] which are based on BTA guidelines,[7] pregnant women with pre-existing hypothyroidism should firstly have an immediate increase in their levothyroxine dose of at least 25-50 µg. Secondly, treatment should aim to maintain TSH in the low-normal range (0.4 mU/L to 2.0 mU/L) and a free T4 in the upper reference range. TFTs, monitoring TSH and free T4, should be performed every four weeks during the first trimester and subsequently at 16 and 28 weeks gestation. However, questions over the strength of evidence behind these two recommendations have led to significant debate among obstetricians and endocrinologists.[8]

Recommendation 1: TSH cut-off value in pregnancy

In interpreting TFTs in pregnancy, trimester-specific reference ranges should be used (Table 1).

	TSH (mU/L)	Free T4 (pmol/L)
Non-pregnant	0.27-4.20	12.0-22.0
First Trimester	0.00-5.50	10.0-16.0
Second Trimester	0.50-3.50	9.0-15.5
Third Trimester	0.50-4.00	8.0-14.5

Table 1 – Pregnancy, trimester-specific reference ranges for TFTs.[9]

International guidelines recommend using a lower TSH cut-off value of 2.5 mU/L for treating overt hypothyroidism in pregnancy. However, where ethnicity has been considered, studies have shown a wider normative range for TSH. In the UK, cohort studies of pregnant women without pre-existing thyroid disease demonstrated first trimester TSH concentrations as high as 5.5 mU/L.[9] Thus, there is a move towards interpreting TSH results based on local trimester specific reference ranges rather than a universal threshold of 2.5 mU/L.

Recommendation 2: Empirical increase in levothyroxine dose

The evidence for an empirical increase in levothyroxine dose is based primarily on a small cohort study of 19 patients, of whom six had thyroid cancer. For these patients, an increase in levothyroxine dose would be justified in order to suppress TSH, rather than maintain it in the reference range.[10] The evidence is therefore insufficient for using TFTs to justify increasing the levothyroxine dose in pregnancy. There is a theoretical risk in increasing the levothyroxine dose and insufficient data on the benefits of doing so.[11]

A routine increase in the levothyroxine dose is not normally required, and it is only necessary for 25% of women in response to abnormal TFT results.[12]

Further research

Large cohort studies are needed in order to resolve these controversies. Firstly, population-based studies that take ethnicity into account are required to determine trimester-specific reference ranges for TSH and T4 concentrations in pregnancy. Secondly, studies are required which examine the effect of an empirical increase in levothyroxine dose on pregnancy outcomes including miscarriage, compared with dose adjustment (based on TFT results). Lastly, more investigation is needed into the potential long-term side effects of an empirical increase of levothyroxine dose.

Conclusion

The treatment of overt hypothyroidism in pregnancy is essential for both maternal and foetal health. Regular monitoring of thyroid function is warranted. Guidelines from NICE, BTA and Thyroid UK recommend aiming for a TSH of <2.5 mU/L and empirically increasing the levothyroxine dose by 25-50 µg. Debate exists on whether the cut-off value of 2.5 mU/L leads to better pregnancy outcomes in comparison to the trimester-specific reference ranges. Furthermore, there is controversy over whether to increase empirically the levothyroxine dose and make adjustments based on thyroid function tests. Large cohort studies are required to validate the international guidelines. An important take-home message includes advising women with hypothyroidism to have pre-conception counselling, to enable appropriate dose adjustments to be made.

"International guidelines recommend using a lower TSH cut-off value of 2.5 mU/L for treating overt hypothyroidism in pregnancy"

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Genome-wide association study of Alzheimer's disease with symptoms of agitation

Intercalation Project Report

Helena Jones
Year 5, Medicine, and Rebecca Sims, supervisor, Cardiff University

"I undertook an Intercalated BSc last year as part of my medical degree. I thoroughly enjoyed my research project, and am keen to show other students, with this article, the kind of inspiring research they can become involved in throughout medical school."

Abstract

Genome-wide association studies (GWAS) have identified more than 25 novel risk loci for late-onset Alzheimer's disease (LOAD). However, a proportion of the genetic heritability of Alzheimer's disease is not yet fully understood. This study explores the behavioural sub-type known as LOAD with agitation, to aid detection of novel risk genes.

The analysis aimed to identify loci that a) predisposed to the development of the Alzheimer's disease sub-type of LOAD with agitation (heterogeneity model); or b) modified the risk of subsequent agitation in patients with LOAD (disease modifier model). Single nucleotide polymorphisms (SNPs) were tested for association with LOAD using logistic regression.

The most significant evidence for association was identified at the well-known apolipoprotein E (APOE) region on chromosome 19. Four SNPs in close proximity to the APOE region showed genome-wide significant associations ($p \leq 5\times10^{-8}$). Additionally, 13 SNPs outside of the APOE region showed suggestive significant evidence ($P < 1\times10^{-5}$) for association.

This study does not identify any novel SNPs that meet strict criteria for genome-wide association. However, a number of associations were identified at a suggestive significance level, which show stronger patterns of effect than those typically observed in LOAD GWAS.

Introduction

In 2015 there were 44.3 million cases of dementia globally,[1] the most common cause being Alzheimer's disease (AD).[2] With an ageing population, prevalence is forecast to triple worldwide by 2050.[1] It is therefore imperative that we advance our methods of diagnosis and improve the efficacy of treatment. Understanding the genetic basis of this complex disease is likely to play an important role in this.[3]

Genome-wide association studies (GWAS) are a tool used to identify the genetic basis of susceptibility to common complex diseases such as AD.[4] Whole genomes from a large sample of people with a particular disease are scanned in order to identify associated genetic variations. This information is used to enhance methods of detection, treatment, and prevention of disease.[5]

Although GWAS have identified more than 25 novel risk loci for LOAD since 2006, the SNPs identified in AD GWAS account for only 30% of genetic variation in LOAD.[4] GWAS are likely to continue to be an efficient way of investigating the missing heritability, particularly if based on more precise phenotypes.[6] This study explored the sub-phenotype of LOAD with agitation, one of the behavioural and psychological symptoms of dementia (BPSD), and was a largely hypothesis-generating analysis.

Materials and methods

We used a sample set previously collected as part of the Medical Research Council (MRC) genetic resource for LOAD, from the GERAD 1 GWAS dataset. The sample set comprised 481 AD cases with agitation (AD+A), 200 cases without agitation (AD-A), and 968 healthy control individuals.

BPSD were assessed in all cases using the Neuropsychiatric Inventory (NPI). The NPI is the most commonly used scale for the detection of BPSD.[7] AD+A was defined as either the presence of agitation *and* irritability on the NPI, or using criteria from previous clinical trials,[8] where if only one symptom was present, a frequency rating of ≥ 2 ('often') and a severity rating of ≥ 2 ('moderate') was required. AD cases with agitation and irritability scores of 0 were coded as AD-A. Individuals with intermediate scores were excluded from the analysis. AD was ruled out in elderly controls either at neuropathological examination, or using Mini Mental State Examination (MMSE) or The Alzheimer's Disease Assessment Scale – Cognition (ADAS-cog) as screening tools.

The study undertook separate analyses to compare:

a) AD+A to AD-A cases, (disease heterogeneity model) and

b) AD+A cases with unaffected controls (disease modifier model).

The study tested SNPs for association with AD using a logistic regression model in PLINK v1.50: a tool set for whole-genome association and population-based linkage analyses.[9]

Results

The final analyses of the 'AD+A versus AD-A' and 'AD+A Versus Control' datasets included 528,279 and 528,635 SNPs respectively. The most significant evidence for association was identified at the APOE region on chromosome 19. The most relevant known risk factor is the APOE ε4 allele.[10]

Results are plotted on Manhattan plots. Each SNP is plotted on the x-axis across the human chromosomes. The y-axis corresponds to the strength of association (p-value).

Analysis of AD+A Versus AD-A

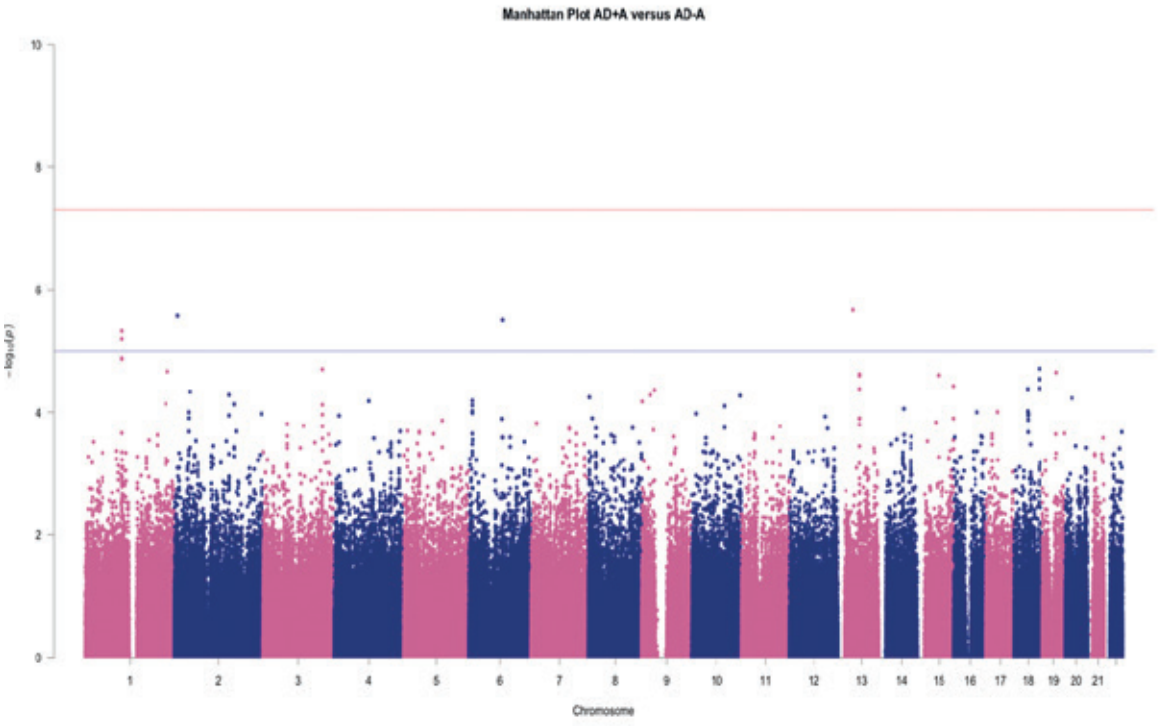

Figure 1: Manhattan plot of chromosomal position (x axis) against $-\log_{10}$ GWAS P value (y axis) for 'AD+AvAD-A'. The red horizontal line indicates the threshold for genome-wide significance ($p \leq 5\times10^{-8}$). The blue horizontal line indicates suggestive significance ($P \leq 1\times10^{-5}$). Plots were produced using R console: http://www.r-project.org. Five SNPs lie above the blue horizontal line.

In the Manhattan plot of the 'AD+A versus AD-A' analysis five SNPs lie above the blue horizontal line. This illustrates that five SNPs, located on chromosomes 1, 2, 6, and 13 show "suggestive significance" (P<1x10-5) of association with AD+A when compared with AD-A. Only two of these are intragenic, located within genes ID2-AS1 and BC037927. Neither of these genes have a biologically plausible link to agitation in AD.

Analysis of AD+A Versus Controls

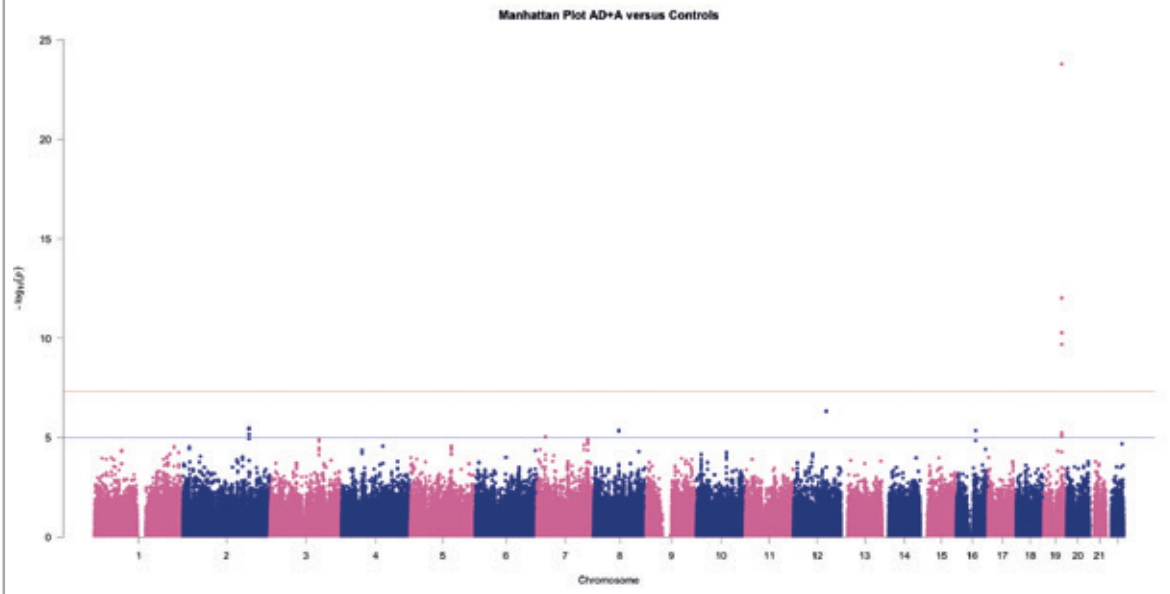

Figure 2: Manhattan plot of chromosomal position (x axis) against $-\log_{10}$ GWAS P value (y axis) for 'AD+AvControl' The threshold for genome-wide significance (p ≤ 5×10−8) is indicated by the red horizontal line. Plots produced using R console http://www.r-project.org . 4 SNP's lie above the red horizontal line, and 10 SNP's lie above the horizontal blue line ($P \leq 1x10^{-5}$).

On the Manhattan plot of the 'AD+A versus Controls' analysis, four SNPs lie above the red horizontal line, and 10 SNPs lie above the blue horizontal line. This illustrates that four SNPs, all located at the APOE locus on chromosome 19, show genome-wide significance of association with AD+A when compared with controls. These have all been previously identified in AD GWAS. An additional 10 SNPs show suggestive significance ($P<1x10^{-5}$) when compared with healthy controls, with six inside the APOE region. Of the remaining four, three are intragenic and do not have a biologically plausible link to agitation in AD.

Variation at DRD1, DRD4, DAT, TPH1, TPH2, and 5HT-2A receptor genes may be involved in the development of agitation in AD. We did not find any associations with these genes that met the stringent levels of significance used in this study. However, four SNPs in the Dopamine Transporter (DAT) gene were identified with p-values <0.05. Given limited study power, SNPs in the current study failing to reach the stringent levels of statistical significance could reflect true disease loci. If these SNPs on the DAT gene were to show a true association with AD+A, this could imply a role in the development of AD+A.

Discussion and conclusion

This study was largely a hypothesis-generating analysis. It did not identify any novel SNPs with genome-wide significant evidence for association; however, a number of associations were observed at a level of suggestive significance. These SNPs showed patterns of effect that are stronger than those generally observed in AD GWAS.

In addition, a number of associations were observed at lower levels of significance (p<0.05) in a gene previously proposed to be involved in behavioural and emotional control processes in non-demented individuals, and in the development of AD+A. Dopamine is implicated in emotional and behavioral control processes, and agitation in AD may be related to deficits in dopaminergic neurotransmission.[11] This is therefore a biologically plausible candidate gene for the development of agitation in AD, showing effects in both the disease modifier- and disease-heterogeneity models. SNPs in the current study failing to reach the stringent levels of statistical significance may reflect true disease loci.

Further investigation is needed into larger, more appropriately powered sample sets, in which the presence of agitation symptoms in AD is well characterised.

"Dopamine is implicated in emotional and behavioral control processes, and agitation in AD may be related to deficits in dopaminergic neurotransmission"

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Neurosurgery and stress: how much stress are surgeons under?

Rory Dyke
Year 5, Medicine, and Mr Paul Leach, supervisor, Cardiff University

"I am interested in a career in surgery, in particular neurosurgery. When the time came to do a Student Selected Component project I looked at the role of surgeons and began to wonder about their levels of stress, and whether this was an under-researched area. To my surprise the literature on stress in surgeons was very sparse, so I thought it would be a great opportunity to look into this myself. I chose Biodots technology as a viable method for measuring stress, mainly because any neurosurgeon would be mad to stop mid-operation for serum or urine cortisol measurements. Consequently I came up with this study on neurosurgery and stress."

Abstract

This pilot study investigated levels of stress experienced by neurosurgeons both pre- and post-surgery. Other studies have failed so far to establish whether or not surgeons are physiologically stressed during operations. Biodots technology, which measures skin temperature and can thus be an indicator of physiological stress, was used to assess the levels of stress experienced by neurosurgeons and registrars before and after performing operations. Our data demonstrate that the surgeons are significantly more relaxed after operations than before ($P<0.0001$), whilst during surgery registrars are significantly more relaxed than consultants ($P<0.05$).

Introduction

Safe surgical practice is dependent upon a combination of technical and non-technical skills, both of which may be influenced by the stress experienced by physicians during an operation. This is of particular relevance to non-technical skills such as communication, teamwork and decision-making, which are particularly prone to influence by stress.[1] Stress refers to the processes experienced by the body when demands exceed the perceived ability to cope, and there is a well-established negative correlation between stress among hospital staff and the occurrence of adverse effects in patients such as wound infections or unexpected bleeds.[2]

Common causes of stress, or 'stressors', include time pressure, interruptions to work, increased workload or technical problems, all of which may be experienced by an operating surgeon. Several studies have attempted to assess stress as experienced by surgeons, but these have thus far failed to demonstrate satisfactorily any extrapolation to surgery in practice. The largest study of stress among surgeons concluded that administrative factors, interference of work with personal life, and outpatient clinics, were the largest stressors for surgeons.[3] The study was questionnaire-based, however, and focused on general job stressors as opposed to intra-operative stressors. To be more directly relevant to adversities during surgery, other studies have looked at the impact of intra-operative stressors, using interviews to analyse intra-operative stress. One study conducted interviews and concluded that surgeons should learn coping strategies during surgical training due to the stress of on-the-job performance.[4] Another examined the heart rates and urine adrenaline levels of operating surgeons and their assistants and found that stress levels were highest among those with greatest experience.[5] Both studies, however, were limited by their small sample size. A further similar study measured pulse rate and blood pressure among neurosurgeons and demonstrated that their haemodynamic stress changes were greater during surgery than following vigorous exercise.[6] Klimo et al used surveys to identify several

significant stressors stemming from the impact of lifestyle on a career in neurosurgery, and found a "burnout rate" of 27% due to stress.

In our study, we aimed to avoid the use of invasive methods, which could be cumbersome and involve interruptions to surgery. We therefore adopted the non-invasive method of stress measurement using Biodots technology (Stresswise UK, Holyhead, Gwynedd).

In a normal physiological stress response, blood is directed away from the peripheral circulation and skin in order to increase blood flow to essential organs, accompanied by a drop in surface temperature of the arms and hands. This biofeedback mechanism follows sympathetic nervous inhibition of the periphery in response to stressful stimuli.[8] Biodots technology exploits this phenomenon and quantifies the stress response by recording colour changes in the Biodots that correspond to changes in skin temperature.[9] Skin temperature and conductivity changes appear to be the best physiological signs of stress including among nurses.[10,11,12] Biodots have been used in hospitals in Gaza to assess stress in children affected by conflict.[13] Our study is the first to use this method for assessing peri-operative stress in surgeons.

Aims

The primary aim of this pilot study was to compare pre- and post-operative stress in neurosurgeons, at both registrar and consultant grade. The secondary aim was to determine whether the nature of the operation affects stress levels, particularly if it is a craniotomy or other neurosurgical operation.

Methods

Neurosurgeons of registrar or consultant grade volunteered as study participants. We obtained ethical approval from the School of Medicine Research Committee of Cardiff University. We monitored the stress levels of the lead operating surgeon by placing Biodots in each antecubital fossa and allowing 20 minutes for the temperature and hence colour of the Biodots to reach equilibrium. Immediately before pre-surgical scrubbing the temperature of the Biodots was read according to a reference colour chart and temperature scale. The Biodots were then removed and a new pair applied after scrubbing but before donning the surgical gown. Biodots were assessed at the end of the operation as soon as the gown was removed. The colour of dots was agreed upon both by the investigator and the surgeon and compared to the colour chart provided (Figure 1). If the Biodots showed differing colours, the dot showing the highest level of stress was taken. We also recorded the operation type, and whether the patient was an adult or child. We collected data involving nine consultant and four registrar-grade neurosurgeons during 35 operations, over a four-week period at University Hospital of Wales. We used the paired T test to determine statistical significance.

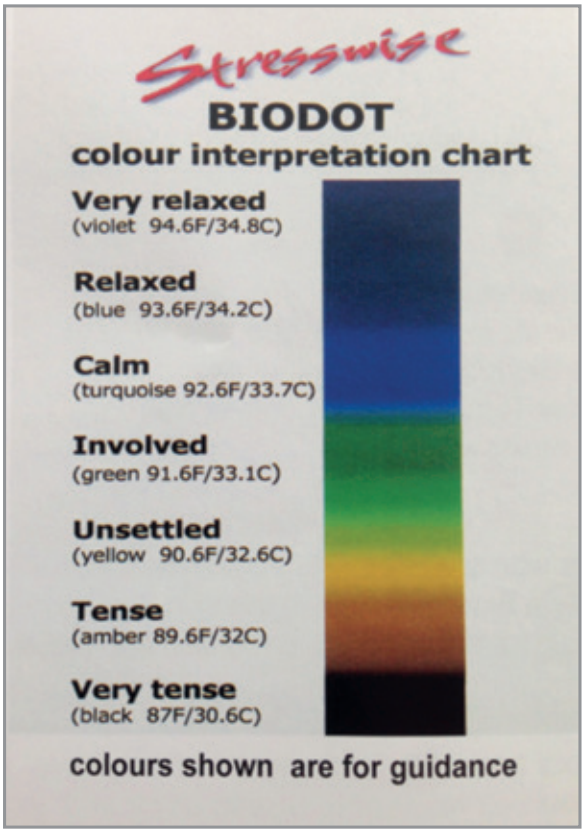

Figure 1. The chart used to interpret the colour of each Biodot

Results

We observed skin temperatures ranging from 30.6°C to 33.7°C pre-operatively, and 33.7°C to 34.2°C post-operatively. Skin temperatures were higher at the end of operations than at the beginning (Table 1). Mean temperatures for consultants and registrars were 31.9°C and 33.0 °C pre-operatively and 33.7°C and 33.9°C post-operatively, respectively. Consultant post-operative temperatures were significantly higher than pre-operative temperatures ($P<0.0001$) but did not significantly differ in registrars ($P>0.05$). Pre-operative temperatures were significantly lower in consultants than registrars ($P<0.001$), although post-operative temperatures were similar.

Overall, pre- and post-operative skin temperatures differed significantly, and were higher post-operatively ($p<0.0001$, 95% confidence interval -2.088 to -1.461). We can reject the null hypothesis that there is no difference between the skin temperature of neurosurgeons pre-operatively and post-operatively.

	Overall	Consultants	Registrars	Craniotomy	Other operations
Mean skin temperature pre-operatively (Celsius)	32.0	31.9	33.0	32.0	32.0
Mean skin temperature post-operatively (Celsius)	33.8	33.7	33.9	33.7	33.8
Difference in mean skin temperature (Celsius)	1.8	1.8	0.9	1.7	1.8

Table 1. The mean skin temperatures pre-operatively and post-operatively, and the difference in mean skin temperature in each group.

The mean pre-operative skin temperature for all participants corresponded to a stress level between "very tense" and "tense", whereas post-operatively corresponded to a stress level between "calm" and "relaxed". However, registrars displayed a mean pre-operative skin temperature corresponding to an "involved" stress level, below "tense", and a post-operative stress level of between "calm and relaxed". In contrast, consultants showed a pre-operative skin temperature indicating that they were "very tense" or "tense", and a post-operative skin temperature indicating "calm".

There was no significant difference between pre- and post-operative skin temperatures during craniotomy operations versus non-craniotomy operations (Figure 2).

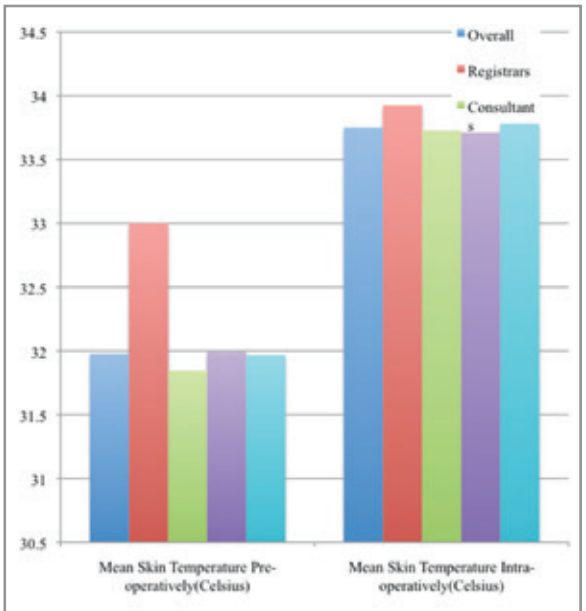

Figure 2 Comparison of pre-operative and post-operative stress among surgeons overall group, and between registrars and consultants.

Discussion

These data demonstrate that neurosurgeons experience higher stress levels prior to surgery than post-operatively. Green et al (2005) also found higher pre-operative stress levels, but linked this to administrative time pressures rather than anticipation of the surgery, because the surgeons reported their perceived stress to be lower whilst operating.[3] Alternatively, their lower levels of stress during surgery may have been due to the familiarity of the procedure if performed many times. The heightened pre-operative stress that we observed may have been due to the use of imaging and navigation tools in craniotomies, which required programming prior to the operation, adding to the surgeon's workload, and potentially causing additional stress.

Our data also demonstrate that registrars experienced less stress than consultants both pre- and post-operatively. This may be because ultimate responsibility for the patient lies not with the registrar, but with the consultant. Additionally, each registrar in our study was assisted by a consultant surgeon. The presence of senior support appears to have provided reassurance and alleviate stress for the registrars, reducing both their stress and of the surgeon.

In contrast to previous studies, we did not find craniotomies to be more stressful to perform than other types of surgery.

Our study was limited by both sample size, due to the limited time frame available for the project, and available technology with which to compare readings obtained from Biodots. The reliance of Biodots on temperature changes to determine stress levels is also a potential limitation, given the potential warming effect of medical clothing and lights used in operating theatres.[14] Theatre temperature is constant throughout the day, and so the time of operation is not likely to be a factor affecting the Biodots. Future studies might address these limitations by comparing operations performed under the same lighting, in the same gowns, although the short time available may pose a challenge. The subjective nature of reading Biodots also limits the replicability of results. Our sample size, although small, was larger than most previous studies.[4,5,6,15]

Conclusion

Our pilot study demonstrates that Biodots are a potentially useful non-invasive method for assessing differences in skin temperature pre- and post-operatively among surgeons, as a potential indicator of changes in stress levels. We found that skin temperature, and thus stress levels, to be significantly higher ($P<0.0001$) pre-operatively than post-operatively. Relative to consultants, registrars were significantly less stressed pre-operatively ($P<0.05$), with no significant post-operative differences observed. There was no significant difference in stress levels during the performance of craniotomies compared to other types of neurosurgical procedures. Small sample size, analysis of only neurosurgeons, and potential measurement subjectivity, limit the validity of these results. Future research should focus on larger samples and a wider variety of operations.

"The reliance of Biodots on temperature changes to determine stress levels is also a potential limitation, given the potential warming effect of medical clothing and lights used in operating theatres"

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Characterisation and functional assessment of mouse embryonic stem cell derived exosomes in vascular regeneration

Vacation Studentship Report

By Andrea Cordaro
Year 4, Medicine, University of Bristol

"I spent my third year as an intercalating medical student on the Master of Research (MRes) programme. I am grateful to the medical school for allowing students to experience research and high quality clinical teaching at the same time - a true advantage that can only make us better doctors, and appreciate how we can help people. I wrote this article to inspire students to undertake a summer research project. There are challenges along the way, but in the end it's so rewarding."

Abstract

Embryonic stem cells (ESCs) are pluripotent cells that can promote angiogenesis, but their potential for clinical use raises ethical issues. Recently, ESC-derived cell-free components known as exosomes have been developed for promoting endogenous repair. This study explores the possibility of using mouse ESC-derived exosomes in vascular regeneration. Mouse ESCs were cultured either in the presence or absence of feeder cells. Exosomes were extracted from the ESC cultures and from control mouse embryonic fibroblast cultures and added to human umbilical vein endothelial cells (HUVECs) before being assessed by immunocytochemistry. ESC exosomes of 82nm and 100nm peaked at $3.8x10^8$ and $3.6x10^8$ particles/ml, respectively. 90nm MEF-derived exosomes peaked at $4.7x10^8$ particles/ml. HUVECs took up more ESC exosomes than MEF exosomes. Tube formation in HUVECS was inhibited more by ESC exosomes than by MEF exosomes. Both ESC and MEF exosomes were incorporated perinuclearly into HUVECs with ambiguous effects on angiogenesis.

Introduction

Embryonic stem cells (ESCs) hold great promise for vascular regeneration but their use is subject to various ethical concerns. They have been associated with teratoma formation and adverse immune-reactivity due to unavailable autologous cells, and their moral status as 'human' remains equivocal. Recently, beneficial effects of stem cells have been connected to exosome secretion.[1] Exosomes are cell-free components carrying micro-RNA and proteins that can act on target cells in an analogous way to the ESCs from which they are derived.[2] ESCs have the ability to produce exosomes but their effect in the context of vascular regeneration is unknown.[3] This study aimed to: (1) establish mouse ESC cultures in 'feeder-dependent' (cultivation on feeder cells) and 'feeder-free' (cultivation in the absence of feeder cells) conditions; (2) assess the pluripotency of cultured ESC; (3) extract exosomes from stem cell cultures; (4) assess the incorporation of ESC-derived exosomes in target cells; (5) assess the influence of stem cell-derived exosomes in tube formation in human umbilical vein endothelial cells (HUVECs).

Methods

Mouse ESCs were cultured as feeder-dependent (on mouse embryonic fibroblasts, MEFs) and eventually feeder-free (on MEF-conditioned medium) conditions. Immunocytochemistry was performed to confirm the pluripotency of the cultured stem cells using octamer-binding transcription factor ¾ (OCT ¾) and stage-specific embryonic antigen 1 (SSEA-1). Exosomes were extracted from the ESC cultures using commercially available Exospin midi columns, and particle concentration measured using Nanosight. The bicinchoninic acid assay (BCA) was used to measure protein concentration. ESC-derived exosomes were fluorescently labelled and their uptake into target cells (HUVECs) assessed by immunofluorescence microscopy using 4',6-diamidino-2-phenylindole (DAPI) for nuclei, Phalloidin for cytoskeleton and Exo-Green for exosomes. The influence of ESC-derived exosomes on tube formation in HUVECs was assessed by Matrigel-based tube formation assay.

Results

1. Exosomes extracted from ESCs and MEF

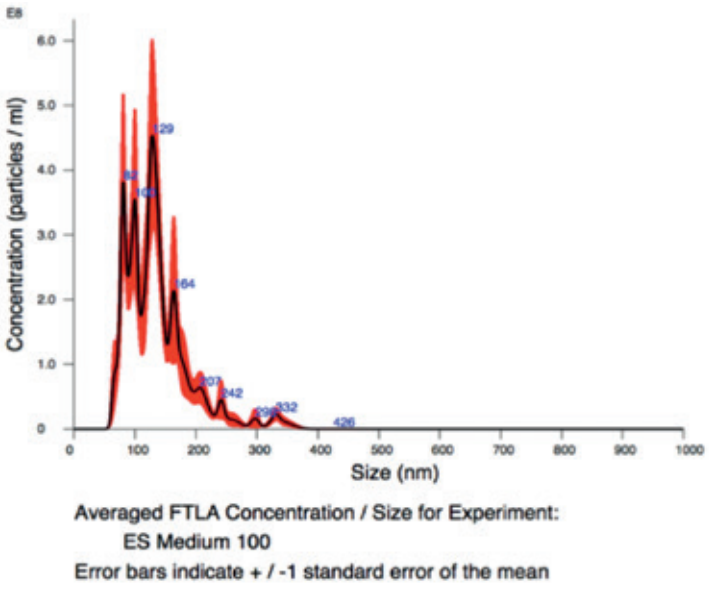
1 (i) Characterisation of exosomes: concentration using BCA assay

	OD Average-Blank (y)	Concentration (µg/µl)	Volume (µl)	Total Amount (µg)
ES-D3 Exosome	0.6905	1.19	300	355.56
MEF Exosome	0.3125	0.41	300	121.87

Table. 1 Protein concentrations for ESC and MEF exosomes using the BCA-assay standard regression equation

1 (ii) Characterisation of exosomes: concentration using Nanosight

ES-D3 Exosomes



MEF Exosomes

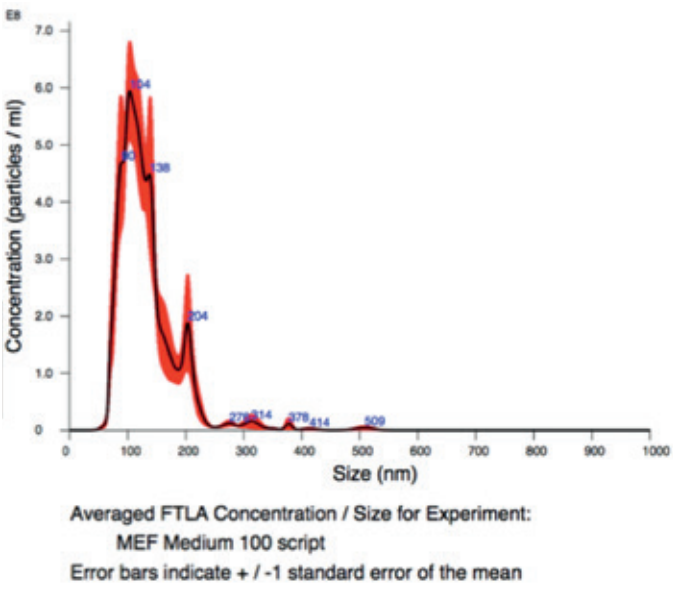


Fig. 1 Nanosight examination of exosomes from ES-D3 and MEF cell culture medium using NanoSight 3000 (Malvern). Black lines represent the average concentration of particles of different sizes while the red area shows the error bars (mean ± standard deviation).

2. Uptake of exosomes into HUVECs

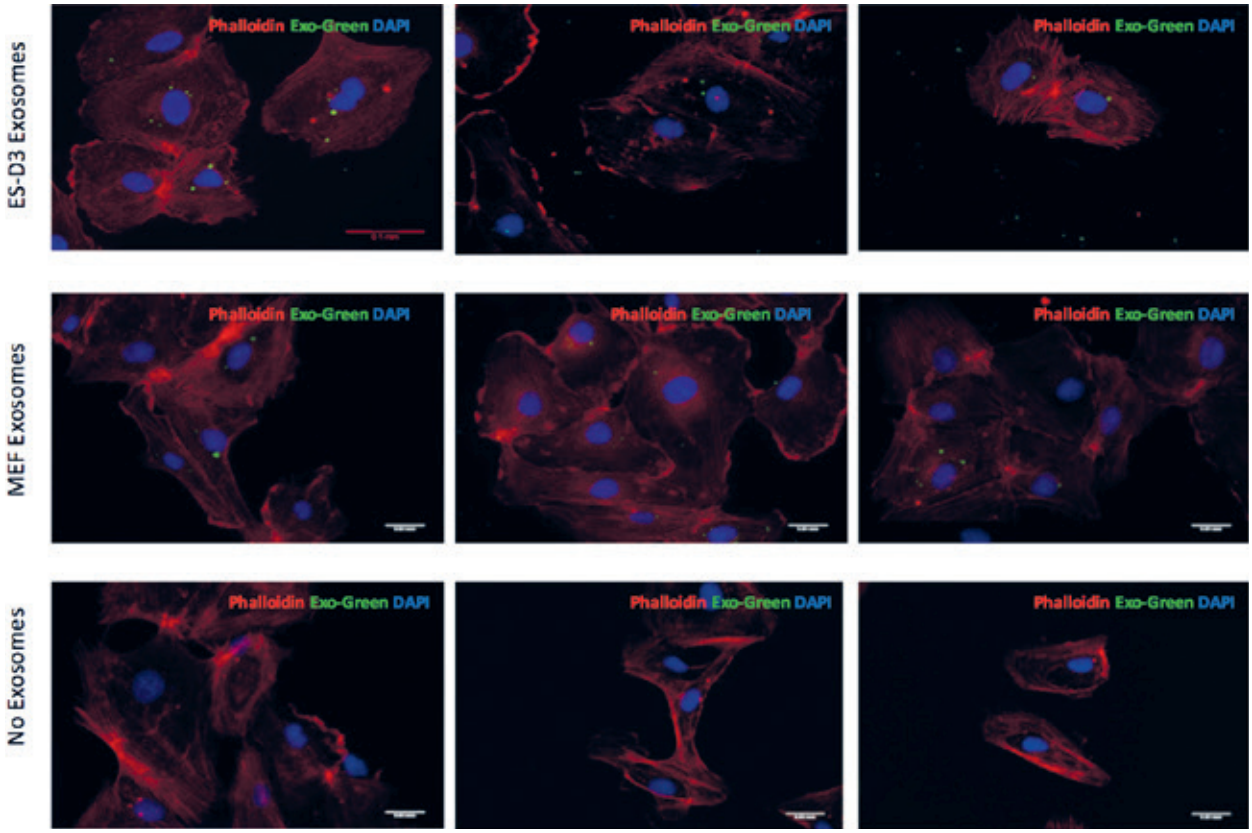


Figure 2. Uptake of exosomes into HUVECs. The HUVECs attached to the well bottom overnight in normoxia and then incubated with Exo-Green-stained exosomes in hypoxia for 24 hours. They were then stained with the nuclear stain DAPI and cytoskeletal antibody Phalloidin. 20x magnification using Olympus BX40 with the camera Photometrics CoolSNAP and Image-Pro Plus software.

3. Effect of ESC exosomes on HUVEC tube formation assay

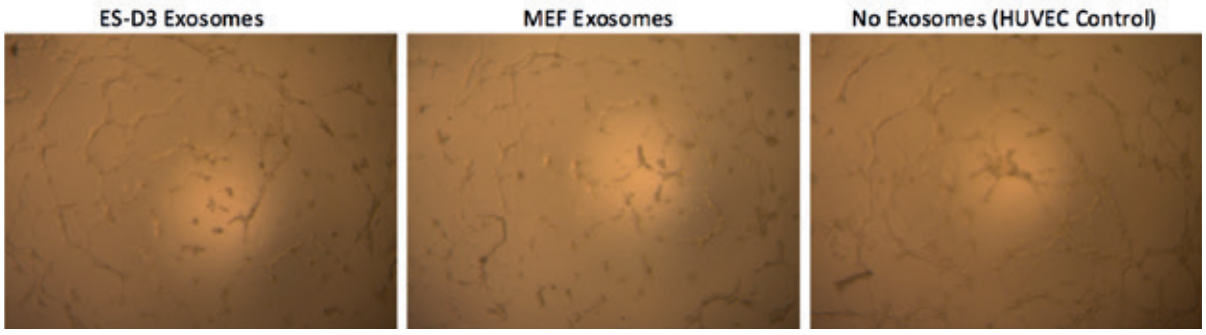


Figure 3. Effect of ESC exosomes on HUVEC tube formation. Tube formation was carried out in a 96-well plate. Pictures were taken 6 hours post-incubation in normoxia after seeding HUVECs co-cultured with exosomes, using 4x magnification with microscope Zeiss-Primo Vert and AxioCam ERc 5s.

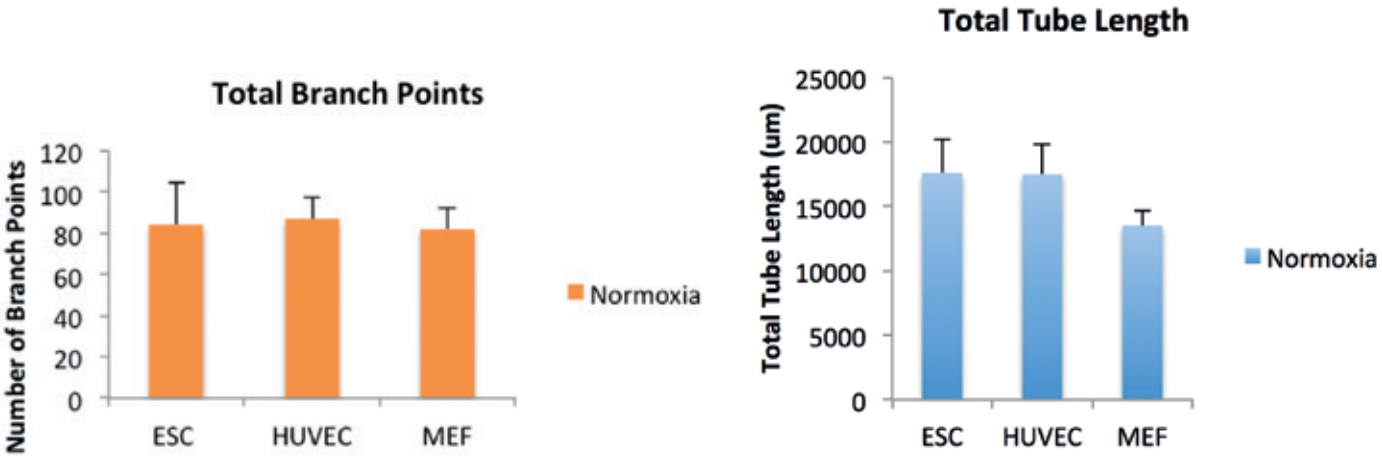


Figure 4. Graphs showing measurement of total tube length and total branch points from Matrigel tube formation assays.

Results and discussion

In this study we used mouse ESCs as a preclinical study and to allow for future embryoid body formation experiments, for which our lab has an established mouse ESC protocol. As in previous studies,[1] human cells (HUVECs) were used to enable examination of the effects of exosomes on target cells – a first step in testing the potential for using exosomes as an angiogenic treatment in humans. Figure 1 shows the exosome-sized particles (30-100nm) in both ES-D3 and MEF samples. For the ESC exosomes, the concentration of the 82nm and 100nm exosomes peaked at $3.8x10^8$ and $3.6x10^8$ particles/ml, respectively. For the MEF exosomes, 90nm exosomes peaked at $4.7x10^8$ particles/ml (Table 1). Although the BCA assay and Nanosight do not allow for the exclusive identification of exosomes, as the sample can contain other unrelated proteins, the peak concentration of particles within the exosomal size range points to an exosome-containing sample.

The functional effects of exosomes on angiogenesis were explored using immunofluorescent and tube formation assays. Immunocytochemistry revealed that there was a greater uptake perinuclearly by HUVECs of ESC exosomes than of control MEF exosomes (Figure 2). In future, it would be better to examine ESC incorporation by confocal microscopy in order to exclude the possibility that the exosomes were attached to the HUVEC cell membrane. The nuclei of HUVECs incubated with exosomes were visible under green light, which could signify that the exosomal membrane had fused with the nucleus and released its micro-RNA content into the nuclei. This in turn could potentially regulate gene expression. We do not know what type of micro-RNAs the exosomes contained. Micro-RNA analysis is a future experiment.

Despite the visual incorporation of exosomes, the Matrigel assay showed that there was no significant difference in tube length or branching points between exosome-treated HUVECs and control HUVECs (Figure 3 and Figure 4). However, previous results (not shown) showed that ES-D3 exosomes inhibited angiogenesis. The inhibitory effect could be explained by the nuclear incorporation, which seems to revert HUVECs back to an undifferentiated state. A fluorescent image of HUVECs co-cultured with ES exosomes revealed two OCT ¾ positive cells (not shown), indicating "stemness" and thus a less differentiated state. This does not imply that exosomes are not beneficial in angiogenesis. Indeed the next step is to explore the stage of differentiation from which these exosomes have been produced, which may influence their effect on angiogenesis.

> "Despite the visual incorporation of exosomes, the Matrigel assay showed that there was no significant difference in tube length or branching points between exosome-treated HUVECs and control HUVECs"

Conclusion

In conclusion, both ESC and MEF exosomes incorporated perinuclearly in HUVECs. However, this appeared either to have no effect or to inhibit tube formation as a first step in angiogenesis. Future studies using exosomes from ESC at different stages of differentiation may reveal if they can have different effects on angiogenesis depending on the stage of ESC differentiation. If a stage can be identified that promotes angiogenesis, then potentially exosomes could be delivered as a therapy for vascular and ischaemic conditions.

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Developing a human proximal tubular cell model for cystinuria kidney disease

Vacation Studentship Report

Timothy Knight
Year 4, Medicine, University of Bristol

"I am in the Bristol Graduate Entry Medical course, having previously studied neuroscience at Leeds University. It was there that I gained a passion for research, leading to me to partake in this successful INSPIRE summer research project. My report outlines the success of the project and its potential future implications for research into cystinuria kidney disease."

Abstract

Cystinuria is a debilitating hereditary disorder for which an effective, targeted treatment strategy has yet to be established. It is defined by an inability to absorb the amino acid cystine from the proximal tubular cells of the kidney, resulting in the formation of urinary tract cystine stones.

Normal functional cystine transporters consist of the protein subunits rBAT and $b^{0,+}$AT (BAT), coded for by the genes SLC3A1 and SLC7A9 respectively, which together form the functional $b^{0,+}$ heterodimer. We have identified through i) mutant creation, transformation and cellular transfection, ii) biotinylation experiments, and iii) western blotting, that the recently identified T471R rBAT mutant and the novel G458R rBAT mutant[1] show impeded trafficking to the cell membrane when compared to Wild Type (WT) rBAT cellular migration. The inability of these mutant forms to be expressed at the cell surface is likely to account for the functional deficit in the cystine transporter channel and thus the manifestation of cystinuria. We were able to reverse this trafficking impedement by co-transfection with the WT BAT subunit, highlighting the therapeutic potential of restoring BAT function in order to reverse rBAT mutant trafficking defects.

Introduction

Cystinuria is a metabolic, inherited condition and is often highly symptomatic, with sufferers experiencing severe episodes of renal colic due to regular stone formation. The condition occurs in one in 7000 neonates globally, and treatment is limited to managing the consequences of the condition rather than the cause, through maintaining alkaline, dilute urine, and using chelating medicines to reduce stone size. If this approach fails the main treatment is surgical intervention.

Mutation of the SLC3A1 gene results in the inability of the transporter to be ferried to the apical membrane, whereas mutation of the SLC7A9 gene leads to loss of normal transporter activity.[2] This study contributes to improving the knowledge of specific rBAT mutant variants through the analysis of the novel G458R mutation in the SLC3A1 gene.

Methods

Mutant creation, transformation and transfection

Mutant DNA was designed using specific primers and incorporated this into a WT backbone using Polymerase Chain Reaction (PCR). The PCR product was run on a gel to confirm size, treated with the restriction endonuclease DPN1 to remove template contamination, transformed into E.coli and incubated overnight. A resultant colony sample was taken, grown overnight and then mini-prepped to extract the mutant DNA. Nanodrop was used to measure DNA concentration, and the use of sequencing confirmed the desired mutant DNA formation. Mutant DNA was transfected into Human Embryonic Kidney (HEK) Cells.

Biotinylation experiments

Combinations of i) WT rBAT and WT BAT co-transfections, ii) mutant rBAT transfection alone, and iii) mutant rBAT and WT BAT, were biotinylated in non-permeabilised cells to label only the membrane-expressed subunits.

Western blotting

Samples of total protein and membrane protein were taken and, following the western blot protocol, immersed in both primary and secondary antibody solutions to allow for imaging (see Results below). rBAT was labelled with the mCherry red fluorescent protein and detected using anti-rabbit secondary antibody.

Results

Sequencing

The Thr471Arg mutant and the novel mutant Gly458Arg,[1] were successfully sequenced and used to carry out further biotinylation and western blotting experiments.

Biotinylation and western blotting

An initial western blot, using a specific antibody against the labelled WT rBAT, showed that the normal WT rBAT subunit is expressed at the cell surface of transfected HEK cells (Figure 1). This is also the case when cotransfected with the BAT subunit. A western blot of an untransfected HEK cell confirmed that both subunits are not normally expressed in these cells. Further western blotting showed that both rBAT mutant variants alter the ability of rBAT to be expressed at the cell surface. Figure 2 shows that for both mutant types, the rBAT subunit is present in the cell (A), is unable to get to the cell surface alone (B), is present in the cell when cotransfected with BAT (C), and is able to get to the cell surface when cotransfected with BAT (D).

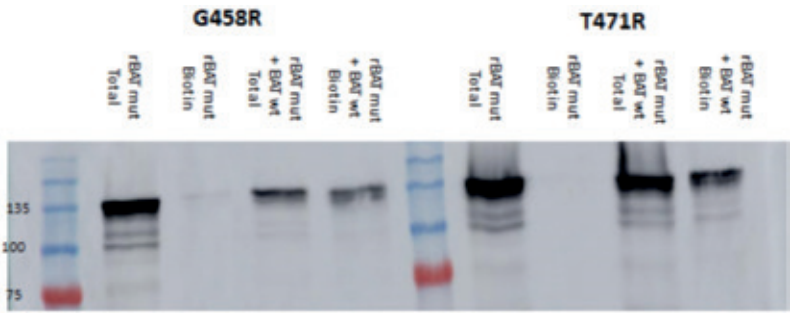

Figure 1: Western blot of both total and biotinylated mCherry-labelled Rbat WT.

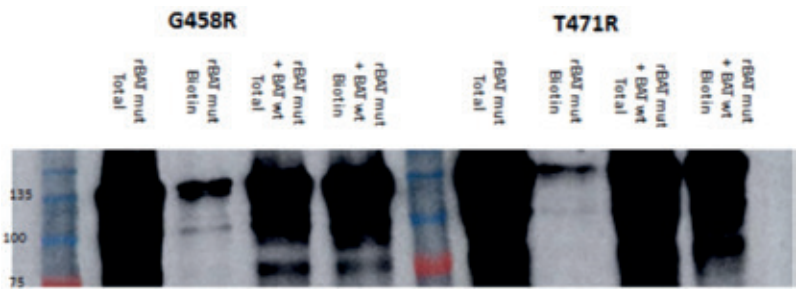

Figure 2: Western blot of both total and biotinylated mCherry-labelled G458R and T471R rBAT mutants, alone and co-expressed with WT BAT.

Discussion

It was important to confirm that WT rBAT is normally expressed at the cell surface so that comparisons with mutant forms could be made. Based on western blot analysis (Figures 1 and 2) and our initial demonstration that the HEK cell type used does not normally express either rBAT or BAT, we conclude that the subunits were successfully expressed in transfected cells.

It is intriguing that both the G458R and T471R rBAT mutant forms impede the trafficking of the mutant subunit alone to the cell surface, but both forms are expressed at the cell surface when co-transfected with the BAT subunit. Deficient rBAT trafficking of mutant forms to the cell surface is consistent with the published literature. Pineda et al (2004),[3] highlighted that a mutation in rBAT hinders its translocation to the cell membrane and alters the functional properties of the whole b0,+ heterodimer cystine uptake system. The fact that both rBAT mutants are expressed when co-transfected with WT BAT implies that BAT can enable recovery of trafficking defects in the rBAT mutation. However, cystine uptake analysis would be needed in order to establish whether recovery of functional uptake of cysteine also occurs. We suggest that it may be possible to develop a new treatment approach for cystinuria based on the pharmacological enhancement of rBAT mutant cellular migration, as has been successfully accomplished in cystic fibrosis.[4]

Total internal reflection fluorescence (TIRF) microscopy would be a useful method for visualising the trafficking properties of both the mutant and WT rBAT subunits, alone and in conjunction with the BAT subunit. If this was also done in Human Proximal Tubular Epithelial Cells it would relate to the human condition, and may help to shed light on the underlying pathophysiology. In turn, this may lead ultimately to a pharmacological route to reversing the trafficking defect seen in cystinuria.

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Preferential assembly of a novel small-conductance, calcium-activated potassium channel

Intercalation Project Report

James Higham
Year 4, Medicine, University of Bristol

"I am interested in research on potassium channels, memory and Alzheimer's disease and chose to write the two following reports because although the methods are vital to understanding current research, few people are familiar with them."

Abstract

Small-conductance calcium-activated potassium (SK, or KCa_2) channels are key to the proper function of heart and brain cells and, as such, are poised as a therapeutic target of the future. A good understanding of their assembly and structure is a prerequisite to targeting them. This article explores the assembly of these channels by investigating the pharmacology of the channel formed from the SK2 and SK3-ex4 subunits. It shows for the first time that these subunits can form a functional channel, and gives clues to its interesting yet still mysterious structure.

Introduction

SK channels are of vital importance to the regulation of cellular excitability of the heart and brain. Their cardiac atrial function is controversial and is fast becoming an area of extreme interest and debate.[1,2] Research groups disagree over the exact SK channel type responsible for atrial repolarisation, and its contribution. SK channels may offer a novel drug target for atrial fibrillation (AF), allowing repolarisation to be prolonged and blocking re-entrant electrical circuits that contribute to disordered atrial function, but more research is needed in this area.

SK channels consist of four subunits, derived from different combinations of three isoforms SK1, 2 and 3, in either homomeric or heteromeric assemblies.[3] There is also a splice variant of SK3 called SK3-ex4, which is also present in atrial myocytes,[2,4] but little is known about its assembly and function. This project aimed to determine if SK3-ex4 can assemble with the SK2 subunit in human embryonic kidney (HEK 293) cells. For the first time, our results suggest that these subunits can form functional channels and that they may preferentially assemble with one another. This result and the channel pharmacology raise interesting questions about its structure and its potential as a therapeutic target.

Methods

Cell culture

HEK 293 cells were transfected using polyethyleneimine with SK channel subunit plasmids and enhanced green fluorescent protein (eGFP) plasmids as described previously.[5] In co-transfected cells, the ratio of SK channel subunit plasmids was 1:1. Following transfection, cells were incubated in a serum- and antibiotic-free medium at 37°C and 5% CO_2 for 24 hours.

Electrophysiology

Whole-cell currents were recorded from eGFP-expressing cells that were bathed in a solution containing (in mM): 97 KAspartate, 30 KCl, 10 HEPES, 10 EGTA, 6.19 $CaCl_2$ (60 nM free Ca^{2+}) and 1.44 $MgCl_2$ (1 mM free Mg^{2+}). Recording electrodes were pulled from borosilicate glass, fire-polished and had a resistance of 2-5 MΩ when filled with (in mM): 97 KAspartate, 20 KCl, 10 HEPES, 10 EGTA, 9.65 $CaCl_2$ (1 μM free Ca2+), 3 $MgCl_2$ (1 mM free Mg^{2+}) and 1.5 Na_2ATP.

SK currents were activated by the free calcium in the pipette solution and revealed using one-second voltage ramps from -100 to +100 mV. In all subsequent figures, I-V plots show current derived from this protocol. The block of current

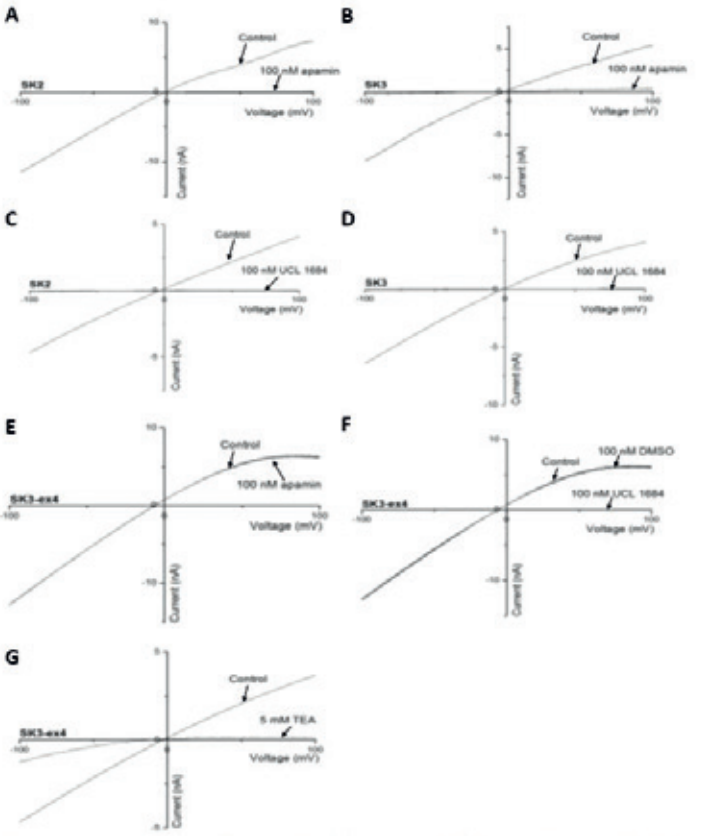

Figure 1: Representative current traces showing the sensitivity of homomeric channels to 100 nM apamin and UCL 1684. (A-D) SK2 and SK3 homomers are blocked by both apamin and UCL 1684. (E-F) SK3-ex4 homomers are insensitive to blocked by apamin and UCL 1684. DMSO (F) is the solvent in which UCL 1684 is dissolved. (G) SK3-ex4 is blocked by 5 mM TEA, showing no significant change in pore shape.

by a toxin (100 nM – supramaximal concentration) was measured at 100 mV. We used apamin and UCL 1684 as toxins with a well-characterised blocking mechanism.[6,7] Data were analysed on GraphPad using non-parametric statistical tests due to low n. Figure 2C illustrates the use of a Friedman's test with Dunn's pairwise post-hoc to establish significance of block (** represents $p < 0.01$ and N.S. represents $p > 0.05$).

Results

Homomeric channels made up of only SK2, SK3 or SK3-ex4 subunits have been characterised extensively in the past.[3,6,8,9] 100 nM apamin blocked both SK2 homomers (99.52 ± 0.001%, $p < 0.05$, $n = 2$; **Figure 1A**) and SK3 homomers (99.04 ± 0.08%, $p < 0.01$, $n = 2$; **Figure 1B**). Both of these homomers are sensitive to block by 100 nM UCL 1684 (SK2: 97.57% and SK3: 98.03%; **Figure 1C-D**). SK3-ex4 homomers are insensitive to block by both of these toxins (**Figure 1E-F**). SK3-ex4 channels are, however, blocked by

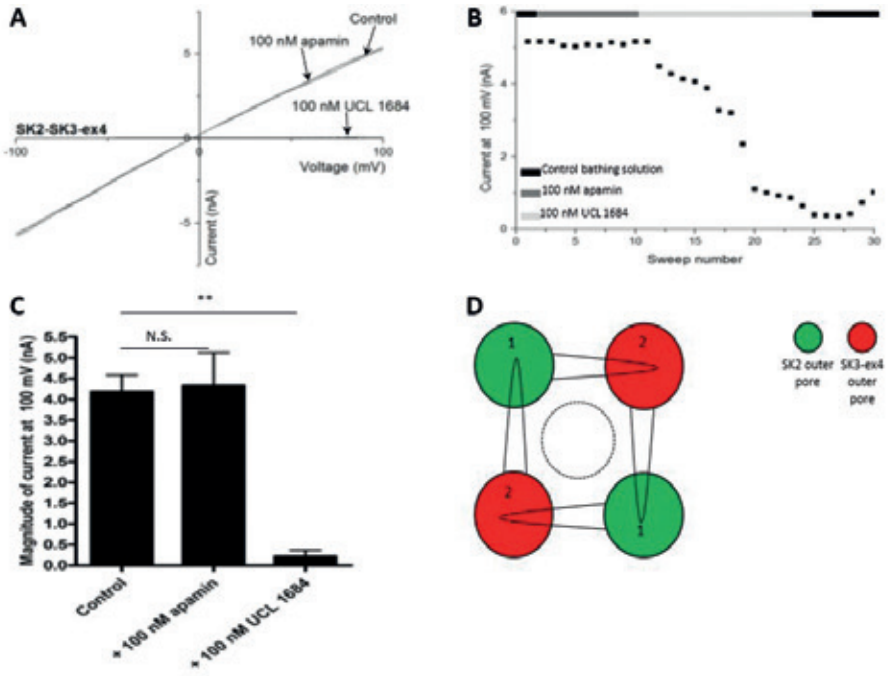

Figure 2: SK2 and SK3-ex4 subunits form heteromeric channels which are sensitive to block by UCL 1684 but not apamin. (A) Representative whole-cell current races from HEK 293 cells co-transfected with SK2 and SK3-ex4 subunit DNA. The time-course and magnitude of block by the two toxins are shown in (B) and (C), respectively. (D) Schematic demonstrating the potential binding sites for UCL 1684 and apamin. (D1) shows the proposed binding sites for UCL 1684 at the outer pore of the SK2 subunit. Apamin cannot bind to the site shown by (D2) because it is unable to interact with the SK3-ex4 outer pore. It is not clear why apamin cannot bind to site (D1); it may be due to more subtle interactions between the subunits.

the pore-blocking agent tetraethyl ammonium (TEA), suggesting there is no significant change in the pore shape of this channel (**Figure 1G**).[6]

Whole-cell currents recorded from cells co-expressing SK2 and SK3-ex4 subunits were completely blocked (98.99 ± 2.93%, $p < 0.01$, $n = 3$) by 100 nM UCL 1684, but were insensitive to 100 nM apamin (0.085 ± 4.39% block, $p > 0.05$, $n = 4$) (**Figure 2A-C**).

Discussion

Although this project is limited by small sample sizes, it does raise interesting questions about the assembly of SK channels. The sensitivity of SK2-SK3-ex4 channels to UCL 1684 can be explained by the presence of the SK2 subunit outer pore, to which this toxin can bind (Figure 2D).[8] However, apamin does not block these channels, despite the fact there appears to be a binding site for it where the SK3-ex4 S3-S4 loop overlaps the SK2 outer pore (Figure 2D).[6,8] The reason underlying this channel's insensitivity to apamin is not known but could be due to subunit interactions changing the shape of the extracellular domain of the channel.[10] We cannot resolve whether or not apamin binds to the SK2-SK3-ex4 channel; perhaps it can bind to, but not block, the channel. Understanding the channel's structure in future is of great importance for rational drug design.

The pharmacology of the SK2-SK3-ex4 currents also suggests that all the channels formed are heteromers, because SK2 homomers would have been blocked by apamin and SK3-ex4 homomers would not have been blocked by UCL 1684. These subunits may, therefore, preferentially assemble as found with other subunits[2,5] and this may occur in native tissue. Further, their preferential assembly may contribute significantly to atrial repolarisation if they make up a major proportion of the channel population.

If found to be present *in vivo*, the channel described here could appeal as a target for AF treatment: the limited co-expression pattern of SK2 and SK3-ex4 subunits[4] potentially would lead to fewer of the side-effects caused by channel blockade in non-cardiac tissue. Further investigations on atrial myocytes are needed in order to identify and understand the role of this channel *in vivo* and its pharmacological potential.

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Measuring learning in the fruit fly, *Drosophila*: a research method

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Introduction

The fruit fly, *Drosophila melanogaster*, is a commonly used animal model for various physiological and pathological processes. It is an extremely good model organism because of its easily-manipulated genetics, short life-span (around forty days) and simple husbandry. This is in contrast to mice, which live much longer and have more complex living requirements.

Drosophila has become the principal animal model for the study of learning and memory and associated defects such as Alzheimer's disease. One can, with relative ease, study the gene products and neurons involved in memory formation in *Drosophila* using a genetic expression system called GAL4-UAS.[1] It is an incredibly powerful tool, developed in the early 1990s, allowing the experimenter to express or knock-out any gene in any subset of neurons in the fly brain.[2] For example, it is possible to knock-out a gene thought to be involved in memory formation and compare memory retention between these and wild-type flies.[3] Alternatively, the role of particular neural circuits in memory can be investigated by over-expressing a potassium channel in cells of that circuit, in order to hyper-polarise and 'silence' them,[4] and see the effect on learning ability.

An association assay can be used to test fruit fly memory, by conditioning the flies to associate a particular odour with an electric shock[5] and then remember this initial conditioning.

Method

This assay is carried out under dim red light because *Drosophila* exhibit positive phototaxis (in other words, they would normally move towards a white light source) which could bias their choices.[6] Each group of flies is, in turn, transferred into a 'shock vial' which is placed at the top of a plastic escalator system.[5] The flies acclimatise to the vial for 90 seconds (s). During this time, the vial is connected, via crocodile clips, to a 60 V electrical supply. The flies are then exposed to the first of two odours – either 3-octanol (OCT) or 4-methyl-cyclohexanol (MCH) – and shocked with this odour for 60 s. After this time, the odour and shock were removed and the flies are given a 30 s break. The odour is then switched and the flies are exposed to this new odour for 60 s without shock.

The flies are then transferred back to their holding vials and left in normal conditions for sixty minutes. After this time, the flies are put into a T-maze and allowed to acclimatise for 90 s. The T-maze has two possible pathways: one exposed to the odour with which they were shocked, and one exposed to the odour with which they received no shock. The flies are given 120 s to choose one of the two paths.

To avoid any bias towards one particular odour, this assay was carried out in tandem: one set-up shocked with OCT while the other shocked with MCH, and the results averaged.

The number of flies choosing each pathway is counted and these numbers are used to calculate a performance index (PI). The flies choosing the pathway exposed to the odour with which they received no shock were said to have made the correct decision.

The performance index is calculated using the following formula[7]

$$PI = Ncorrect - NincorrectNtotal$$

A PI of 1.0 would indicate 100% learning, where all of the flies in the maze learned to avoid the shock-paired odour. A 50:50 split between the two choices would result in a PI of 0, showing that the flies had not learned.

An alternative to using a shock-paired odour would be to use a reward-paired odour; for example, giving sugar with one odour but not the other. This assay would still assess the ability of the flies to learn, but it uses reward rather than p ositive punishment.

This assay, combined with the genetic techniques applicable to *Drosophila*, forms an excellent technique for screening the proteins and neurons involved in learning and memory. It is relatively straight-forward and fast, and allows for complex analysis of protein interactions which take place during learning (using protein mutants or knock-outs). Therapeutic strategies for treating memory disorders can also be tested in *Drosophila* to elucidate their mechanism of action and to test their efficacy.

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I would like to thank Dr James Hodge for supervision.

Pocket Haematomas: defining the spectrum

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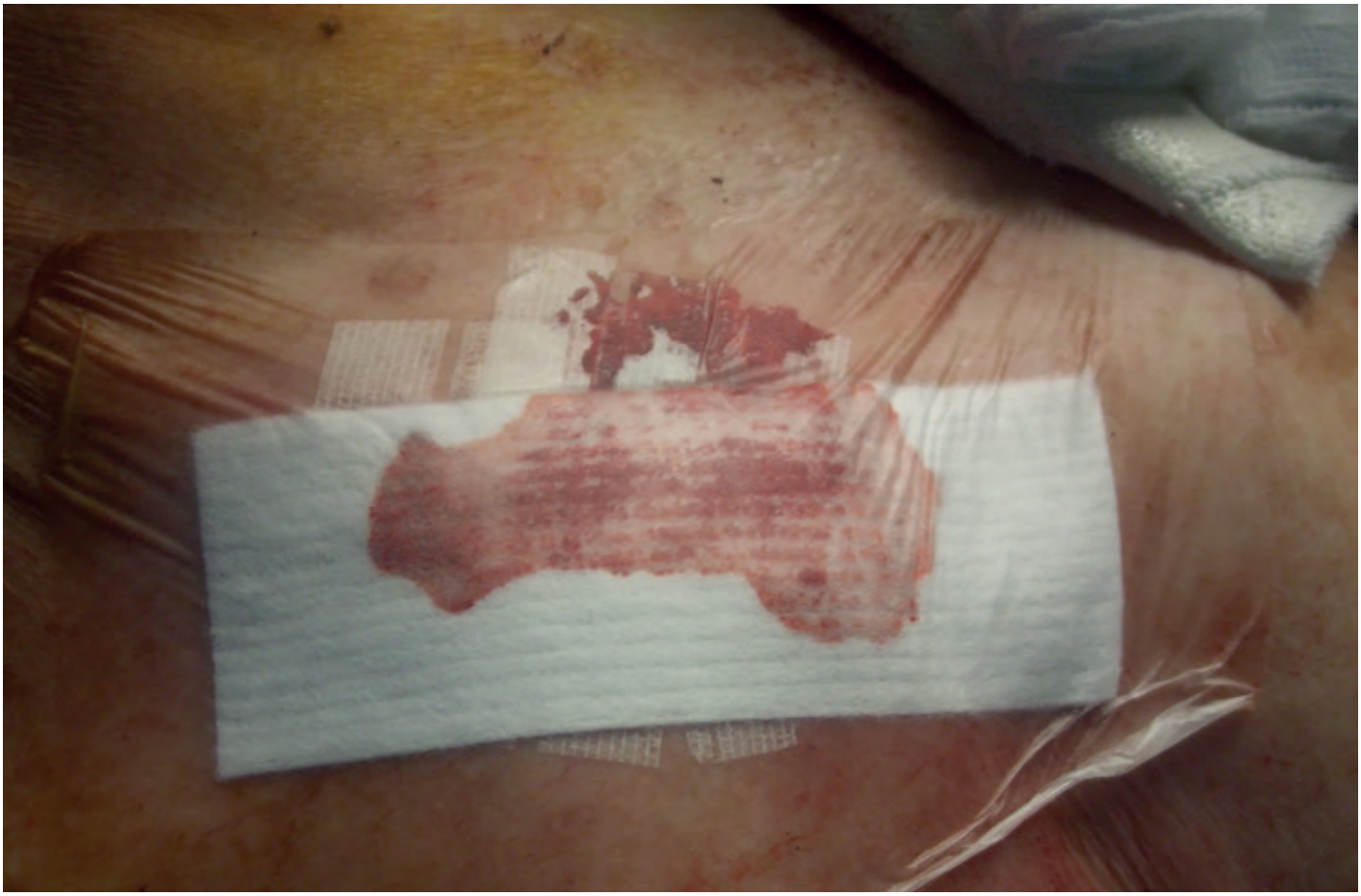


Abstract

Pocket haematomas are a common bleeding complication which occur frequently after cardiac implantable electronic device (CIED) procedures and may lead to further complications. This pilot study has developed and evaluated an easily accessible and clinically directive grading scale for classification of pocket haematomas at the Bristol Heart Institute (BHI). 30 patients who had undergone CIED procedures at the BHI between 2014-5 were analysed and had their pocket haematoma image captured following the procedure, with written consent. This formed the basis of appropriate haematoma definition and severity grading. BHI haematoma incidence was consistent with that found in other centres at 20%, with 3.3% severe cases. Neither the route of lead entry nor operator experience was strongly predictive of haematoma severity. Unanimous definition is required for both centre comparison and also correct clinical management.

Introduction

Cardiac implantable electronic device (CIED) procedures commonly produce pocket haematomas, in which bleeding into the device pocket causes visible swelling. This painful complication is associated with further problems including infection,[1] pocket erosion[2] and poor in-hospital outcomes.[3] Indeed, it is probably exacerbated by perioperative anticoagulation therapy[4] and accounts for at least 14% of early reoperations.[5,6] Therefore, correct and careful management is a necessity.

However, there is huge variability in the severity of pocket haematomas, which is linked to further complication risk. Moreover, no gold standard grading of pocket haematoma severity is established, making between-centre comparison difficult. We conducted a pilot study at the Bristol Heart Institute (BHI) using photographic evidence to assess the feasibility of a clinically relevant grading scale and further developed categories.

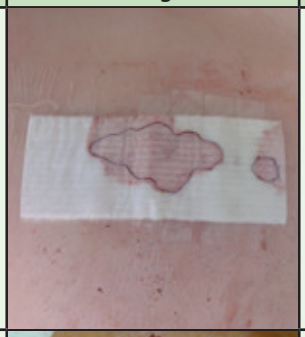
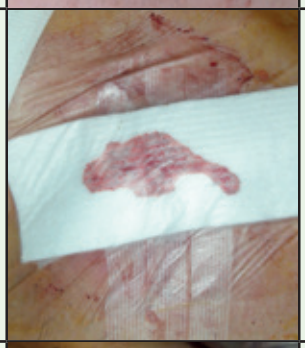
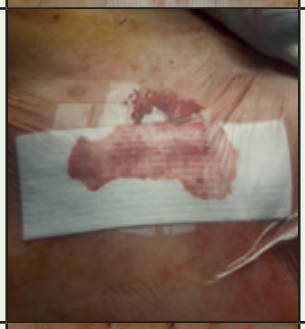
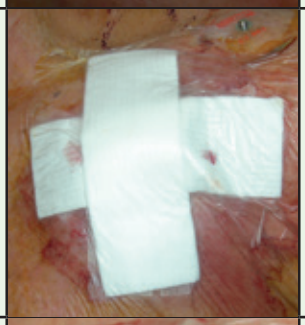
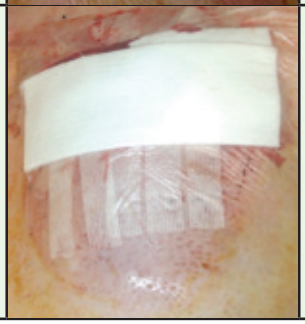
Grade	Image	Definition
0		**Grade 0: No haematoma** There may be bleeding present
1		**Grade 1: Fullness around the implanted device** Recheck in one hour. Should not delay discharge if not increasing in size.
2		**Grade 2: Mild haematoma (Small < 5 cm)** Pressure bandage for two hours then recheck. Should not delay discharge if no size increase two hours after the pressure bandage is removed.
3		**Grade 3: Moderate haematoma (> 5 cm - < 15 cm)** 10 minutes manual pressure. Outline the bleeding with a skin marker, pressure bandage for four hours. Senior review and keep overnight for two hourly observation.
4		**Grade 4: Severe haematoma - Any of the following:** (Large > 15 cm/ tense/ tender/ increasing in size/dehiscence of the suture line/ substantial ongoing bleeding) Urgent senior review for pocket revision.

Figure 1: Grading scale for pocket haematoma severity. Grade definition includes both descriptors and clinical response. A pressure bandage is applied over the left shoulder and under the right axilla, whereas manual pressure is applied directly by hand.

Methods

Between 2014 and 2015, we retrospectively analysed data from 30 patients chosen randomly at the BHI. All were within three hours of having undergone CIED procedures consisting of pre-pectoral device implantations, lead revisions or generator replacements. Pocket haematoma severity was measured using the grading scale shown in Figure 1 and images of the pocket taken for illustration, with appropriate written consent for research. The categorical Fisher's exact test was used to analyse severity between entry route and severity between operators.

Results

Using this scale six haematomas were recorded – an incidence of 20% (grade 2 = 10%, grade 3 = 6.7%, grade 4 = 3.3%). Severe haematomas constituted 3.3% of all haematomas (Figure 2).

> "Providing a consistent definition of haematoma severity will also enable further investigation of the predisposing risks and consequences of pocket haematomas"

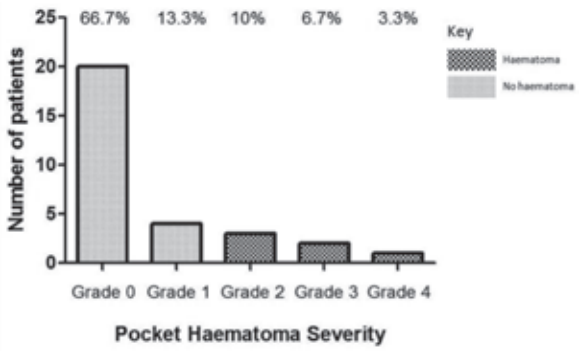


Figure 2. Pocket Haematoma severity incidence. Haematoma incidence and severity found at the Bristol Heart Institute 2014 – 2015 (n = 30). Grade 2 – 4 is consistent with definitions of haematoma used by previous studies.[7]

The patients in our study showed a mean (± standard deviation) patient age of 70 ± 21 years, with a range of between 22 – 95 years; 50% were male. There was no significant difference ($P > 0.6$; Fisher's Exact test) in haematoma severity between the different sites of lead entry - the subclavian vein (n = 10) or the cephalic vein (n = 11). Equally, there appeared to be a marginal difference in the mean haematoma severity between 11 operators comprising consultants and registrars, with operator 3 as an outlier (Table 1; n = 28), although this was not significant ($P > 0.1$; Fisher's Exact test).

Table 1.											
Operator	1	2	3	4	5	6	7	8	9	10	11
Total number of procedures	13	5	4	2	2	2	1	1	1	1	1
Mean Haematoma severity	1.46	1.40	2.75	2.00	1.00	1.00	2.00	2.00	1.00	1.00	1.00
Standard Error of the Mean	0.25	0.40	0.48	1.00	0	0	-	-	-	-	-

Table 1: Haematoma severity in relation to the number of recorded procedures for each operator.

Patient operational characteristics are summarised in Table 2. There were no major differences in the type of procedures each operator undertook. Five procedures had two operators.

Table 2.		
Device	First implantation	Generator replacement/ lead revision/ lead extraction
Pacemaker	22	2
Single-chamber	12	2
Dual-chamber	10	0
Defibrillator	2	3
Single-chamber	1	3
Dual-chamber	1	0
Loop recorder	1	0

Table 2. Characteristics of implanted devices for which information on pocket haematomas was recorded.

Effect of Anticoagulation on pocket haematoma severity

Low-Molecular-Weight Heparin (LMWH) such as enoxaparin or tinzaparin is thought to increase haematoma risk,[4] We compared the pocket haematoma incidence in patients taking LMWH or aspirin alone and found the ratio of haematoma incidence in patients taking LMWH versus oral anticoagulants (aspirin) to be 2.0. One patient on warfarin therapy had a haematoma, consistent with no increased complication risk.[4]

Discussion

The variability of pocket haematoma incidences reported by different studies highlights the need for a standardised definition of this complication. A large randomised multicenter trial defined a haematoma as any palpable mass or ecchymosis, and found an incidence of up to 33%,[7] whereas a prospective study found a severe haematoma incidence of 2%, and defined it as requiring reoperation.[8] Our data are consistent with these reports when considering grades 2 – 4 as haematoma, and grade 4 as severe haematoma. Our LMWH versus oral anticoagulants ratio of 2.0 was also consistent with an expected ratio of 3.7.[9]

Conclusion

This novel grading scale is currently being evaluated for use in classifying haematomas following device implantation at the BHI. Improving haematoma identification will help facilitate correct clinical management steps. Providing a consistent definition of haematoma severity will also enable further investigation of the predisposing risks and consequences of pocket haematomas.

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The senior editors at the INSPIRE Student Health Sciences Research Journal

Praveena Deekonda

I am a 4th year medical student at the University of Exeter, originally from Toronto. My interests include clinical neuroscience, medical education and all things surgical. My motivation for being involved in the INSPIRE journal stemmed from my own INSPIRE summer studentship after my 1st year, which led on to various research opportunities. I hope to become a clinician-scientist in the future. Outside of medicine, I enjoy baking and reading.

Catarina Dores Fernandes Ferreira

Having studied an International School in Lisbon, Portugal, I moved to Bristol for university after spending a gap year volunteering in Mozambique. I am now a 4th year medical student at the University of Bristol. It was while intercalating in Anatomical Sciences (BSc) that I developed an interest in research. I have since developed a special interest in plastic and orthopaedic surgery. I have been involved in a number of studies and audits in these fields.

Amy Hough

I am a 4th year medical student at the University of Exeter and I am currently studying in Truro, Cornwall. I have been involved with the INSPIRE programme since my second year and enjoyed many opportunities through it. I am also part of the national committee for the charity Sexpression:UK which is an organization that sends university students into schools to deliver sex and relationships educational workshops. I have a keen interest in genitourinary medicine, and obstetrics and gynaecology.

Jenny McKeon

I have just completed my medical degree Cardiff Medical School. I am interested in accident and emergency medicine and obstetrics and gynaecology. I have a passion for travelling and during my 5th year in medicine I conducted my elective in Samoa. I started as an F1 in Swindon in August, where I am enjoying finally putting my training into practice!

James Russell

I am currently a 4th year medical student at Plymouth University Peninsula Schools of Medicine and Dentistry. I have taken a particular interest in research since conducting my own summer project on neurofibromatosis type II, as part of the INSPIRE scheme. Since then I have participated in clinical research projects on acute coronary syndrome and aortic dissection, as well as audit projects in general and spinal surgery.

Izabella Smolicz

I undertook the role of senior editor during my Intercalated BSc in Health Sciences at the University of Bristol. My research interests are in neonatology and paediatrics, neuroscience and anaesthesia. In the summer of 2015, I enjoyed an INSPIRE Summer Vacation Studentship in vascular medicine. I would like to become a clinical academic in the future.

INSPIRE is a national initiative coordinated by the Academy of Medical Sciences.

University of Bristol

http://www.bristol.ac.uk/medical-school/whybristol/inspire

Leads: Tony Pickering, Reader in Neuroscience and Honorary Consultant Anaesthetist, and Elizabeth Coulthard, Consultant Senior Lecturer in Dementia Neurology

University of Cardiff

http://cures.cardiff.ac.uk/inspire

Lead: Colin Dayan, Professor of Clinical Diabetes and Metabolism

University of Exeter

http://medicine.exeter.ac.uk/bmbs/inspire

Leads: Prof Tamsin Ford, Professor of Child and Adolescent Psychiatry, and Joanna Tarr, Lecturer, Medical Sciences

Plymouth University Peninsula School of Medicine and Dentistry

https://www.plymouth.ac.uk/your-university/about-us/university-structure/faculties/medicine-dentistry/inspire

Leads: Vehid Salih, Reader in Oral & Dental Health Research, and David Parkinson, Professor of Neuroscience.

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