News & Views

Potential Grapefruit–Drug Interactions

A review article that summarises the current evidence on the potential adverse effects from interactions between grapefruit and certain drug mechanisms has been published. The authors, who first discovered the association more than 20 years ago, believe that the evidence in the paper will increase awareness within the healthcare community of the possibility of serious adverse effects when grapefruit is consumed with certain prescription drugs.

More than 85 drugs can interact with grapefruit, and for 43 of those, this can lead to serious side-effects, including renal toxicity, respiratory failure and gastrointestinal bleeding. The active ingredients responsible, the furanocoumarins, irreversibly inhibit CYP3A4, which is the enzyme responsible for inactivating about 50% of all medication. According to one of the authors of the paper, Dr David Bailey, "Between 2008 and 2012, the number of medications with the potential to interact with grapefruit and cause serious adverse effects… has increased from 17 to 43, representing an average rate of increase exceeding 6 drugs per year. This increase is a result of the introduction of new chemical entities and formulations."

[1] Barnhardt, K. (2012). *Grapefruit–medication interactions increasing*. [EurekAlert, 26.11.12]. Available at: http://www.eurekalert.org/pub_releases/2012-11/cmaj-gii112012.php (Accessed 04.12.12).

Cost and Benefits of Animal Experiments

Few ethical issues create as much controversy as invasive experiments on animals. Some scientists claim that they are essential for combating major human diseases or detecting chemicals that are toxic to humans. Others claim the contrary, backed by thousands of patients harmed by pharmaceuticals developed by using animal tests. Some claim that all the experiments involved are conducted humanely, to high scientific standards. Yet a wealth of studies have recently revealed that laboratory animals suffer significant stress, which may distort experimental results. In a new book,[1] the bioethicist and veterinarian, Andrew Knight, presents more than a decade of groundbreaking scientific research, analysis and experience to provide evidence-based answers to the key question: Is animal experimentation ethically justifiable?

[1] Knight, A. (2011). *The Cost and Benefits of Animal Experiments*, 272pp. Basingstoke, UK: Palgrave Macmillan.

Human Serum Replaces Bovine Serum in Stem Cell Cultures

Scientists from the University of Udine, in Italy, have successfully grown dental pulp stem cells (DPSCs) *in vitro,* with a low percentage of human serum as a replacement for fetal bovine serum.[1]

The standard cell culture media for the isolation and expansion of stem cells include animal sera, which raises the possibility of infections and immune reactions. For these reasons, regulatory authorities discourage or prohibit the use of animal sera and other components for the production of biological products for human use. In order to safely produce DPSCs for clinical applications, the researchers developed an isolation and proliferation medium with human serum, and reduced the amount of serum required by adding specific cytokines and growth factors, in order to obtain a well-defined composition. They then tested the isolation, proliferation, morphology, phenotype and osteoblastic differentiation potential of DPSCs in the new medium and in a medium with 10% fetal bovine serum. The human serum-based medium was found to be comparable to a medium with a higher amount of FBS, in its ability to isolate a highly proliferative population of DPSCs, which expressed embryonic as well as mesenchymal stem cell markers and osteoblastic differentiation capacity.

[1] Ferro, F., Spelat, R., Beltrami, A.P., Cesselli, D. & Curcio, F. (2012). Isolation and characterization of human dental pulp derived stem cells by using media containing low human serum percentage as clinical grade substitutes for bovine serum. *PLoS One* **7**, e48945.

Low-level Organophosphate Exposure and Impaired Cognition

A meta-analysis that was carried out to determine the neurotoxic effects of long-term exposure to low levels of organophosphates (OPs) in

occupational settings, has found a significant association between exposure and impaired neurological and cognitive function.[1]

OPs are widely used for industrial, domestic and agricultural purposes, and are one of the most toxic pesticides to vertebrates. Although their toxic effects at high levels are acknowledged, doubt remains with regard to the consequences of long-term, low-level exposure. To clarify the issue, researchers at University College London and the Open University have performed a quantitative evaluation of data from 14 studies and more than 1600 participants. The lead author, Dr Sarah Mackenzie Ross said: "The analysis reveals that the majority of well-designed studies undertaken over the last 20 years find a significant association between low-level exposure to organophosphates and impaired cognitive function."[2] These impairments in neurobehavioural function are small-to-moderate, and are primarily related to psychomotor speed, executive function, visuospatial ability, and working and visual memory. The researchers hope their findings will be of interest to Government advisory committees and departments who are currently reviewing the neurotoxicity of low-level exposure to OPs, particularly as more individuals are at risk of low-level exposure than acute poisoning.

[1] Ross, S.M., McManus, I.C., Harrison, V. & Mason, O. (2012). Neurobehavioral problems following low-level exposure to organophosphate pesticides: A systematic and meta-analytic review. *Critical Reviews in Toxicology*. [E-pub ahead of print.]
[2] Weston, D. (2012). *Brain and nervous system damaged by low-level exposure to pesticides*. [UCL News, 03.12.11]. Available at: http://www.ucl.ac.uk/news/news-articles/1212/031212-Brain-and-nervous-system-damaged-by-rganophosphate-pesticides-MacKenzie-Ross (Accessed 04.12.12).

Gender Differences in Alzheimer's Disease

According to a study presented at the Annual Meeting of the Radiological Society of North America,[1] the loss of grey matter in Alzheimer's disease (AD) patients is significantly different between men and women.

The study spanned five years and involved 109 patients (60 men and 49 women). Over that time period, each of the individuals progressed from amnesic mild cognitive impairment to AD. The researchers assessed changes to the patients' grey matter by comparing magnetic resonance images that had been taken 12 months before AD diagnosis, at diagnosis, and 12 months after. They found that, 12 months prior to diagnosis and at diagnosis, the women had lost more grey matter than the men. In addition, as the disease progressed, both men and women lost grey matter from different areas of the brain. Lead researcher Dr Maria Vittoria Spampinato said that "the gender differences in atrophy patterns have important implications for the development of therapies… and should be taken into account when testing new drugs in clinical trials".

[1] Brooks, L. (2012). *Researchers discover gender-based differences in Alzheimer's disease*. [Eurek Alert, 26.11.12]. Available at: http://www. eurekalert.org/pub_releases/2012-11/rson-rdg111612.php (Accessed 04.12.12).

C. elegans Study Aids Neurodegeneration Research

In certain neurodegenerative disorders, such as Huntington's disease and Alzheimer's disease, the inappropriate accumulation of proteins might contribute to the pathology. Researchers at the University of Montreal, Canada, have used the nematode worm, *Caenorhabditis elegans*, to identify a potential means of protecting neurons from the toxic effects of such protein aggregates.[1]

Dr J. Alex Parker's team set out to investigate whether two proteins that accumulate in Huntington's disease patients — TAR DNA-binding protein 43 (TDP-43) and fused-in-sarcoma (FUS) — contributed to the neurodegeneration caused by the mutant huntingtin protein. In *C. elegans*, the loss of function mutations for the nematode orthologues of TDP-43 or FUS were shown to reduce the behavioural defects and neurodegeneration caused by mutant huntingtin. In addition, the researchers found that, in both *C. elegans* and mammalian cells, TDP-43 interacts with the survival factor, progranulin (PGRN), to regulate polyglutamine toxicity. According to Dr Parker, "removing progranulin from either worms or mammalian cells enhanced huntingtin toxicity, but increasing progranulin reduced cell death in mammalian neurons".[2]

[1] Tauffenberger, A., Chitramuthu, B.P., Bateman, A., Bennett, H.P. & Parker, J.A. (2012). Reduction of polyglutamine toxicity by TDP-43, FUS and progranulin in Huntington's disease models. *Human Molecular Genetics*. [E-pub ahead of print.]
[2] Raillant-Clark, W. (2012). *Researchers find chemical 'switches' for neurodegenerative diseases*. [EurekAlert, 27.11.12]. Available at: http://www.eurekalert.org/pub_releases/2012-11/uom-rfc112612.php (Accessed 03.12.12).

Coronary Artery Disease Genetic Study

In the largest genetic study of coronary artery disease to date, 15 genetic regions were found to be associated with the disease.[1] In addition, 104 independent genetic variants were also found that were likely to be associated with coronary artery disease (CAD).

The consortium of over 180 researchers worldwide analysed DNA from 60,000 people with CAD and from 130,000 apparently unaffected individuals. The genetic data were integrated into a network analysis to uncover biological pathways that underlie the disease — lipid metabolism and inflammation were identified as the most and second most prominent pathways, respectively.

According to Dr Panos Deloukas, who is the co-lead author from the Wellcome Trust Sanger Institute (Cambridge, UK), "Our research strengthens the argument that, for most of us, genetic risk to CAD is defined by many genetic variants, each of which has a modest effect".

[1]Anon. (2012). *Insights into the genetic causes of coronary artery disease and heart attacks*. [Wellcome Trust Sanger Institute, 02.12.12]. Available at: http://www.sanger.ac.uk/about/press/2012/121202.html (Accessed 03.12.12).

In Silico Analysis Shines Light on Prion Disease Development

The development of prion diseases is poorly understood, but the activation of immune cells leading to sustained brain inflammation is thought to be one of the earliest events. In an attempt to understand how prion proteins accumulate and eventually destroy neurons, scientists from the University of Luxembourg[1] carried out a computational network analysis based on known gene expression data of prion-infected and uninfected mouse brain tissue.

Their *in silico* approach identified a core set of 16 immune response-related genes, which are capable of activating a further 58 genes that are differentially expressed in infected *versus* uninfected tissues. These genes determine the stability of the network and are the critical element between the different pathological processes (disease-causing prion replication and accumulation, immune response and neuronal cell death). The team hypothesises that two stable states exist — inactive (healthy) and active (diseased). Perturbation analysis of the regulatory core genes showed that each gene can trigger the transition between two stable states in the larger network — from an inactive (healthy) to an active (diseased) state. However, once a new active state is reached, the regulatory core becomes very stable and the network remains locked in the diseased state. This locking might be the cause of the sustained immune response observed in prion diseases, which eventually leads to neuronal death and clinical symptoms.

[1] Crespo, I., Roomp, K., Jurkowski, W., Kitano, H. & Del Sol, A. (2012). Gene regulatory network analysis supports inflammation as a key neurodegeneration process in prion disease. *BMC Systems Biology* **6**, 132.

Environmental Exposure to be Monitored in Europeans

The European Commission will fund two projects to monitor the chemicals to which individuals are exposed everyday. The funding, a total of €17.3 million, is expected to ultimately benefit citizens, as it is aimed at determining the effects of environmental exposures on health — i.e. the 'exposome'.

Genome-wide association studies, which attempt to establish links between genetic variants and disease, have not been able to fully explain individual susceptibility to certain chronic diseases, partly because environmental factors are also involved. Hence, the worldwide interest in exposomics (i.e. environmental exposure).

The €8.7 million Exposomics project, led by Professor Paolo Vineis at Imperial College London, will give thousands of people smartphones equipped with sensors to record the chemicals to which they are exposed. In addition, since the goal of the project is to identify changes to blood biomarkers in individuals walking through areas of low and high air pollution in order to increase understanding of the environmental triggers of heart disease, asthma and lung cancer, the participants' blood will be monitored.

The second project — the Human Early-life Exposome, which is headed by Dr Martine Vrijheid at the Centre for Research in Environmental Epidemiology in Barcelona, Spain — will focus on children and pregnant women, because the exposure of developing organs might have different consequences.

These four-year projects will generate large amounts of data, so data-sharing policies are being drafted to ensure that other researchers can use them.

[1] Callaway, E. (2012). *Daily dose of toxics to be tracked*. [Nature News, 27.11.12]. Available at:

http://www.nature.com/news/daily-dose-of-toxics-to-be-tracked-1.11901 (Accessed 27.11.12).

InterNICHE Wins Prize

InterNICHE, the International Network for Humane Education, has won a Lush prize of £25,000.

The annual £250,000 Lush Prize is designed to help speed up the replacement of animal experiments for product testing and was established by the cosmetics company, Lush. The prize money was split between five categories — science, training, young researcher, public awareness, and lobbying. InterNICHE jointly won in the training category, sharing the £50,000 prize with the Institute for In Vitro Sciences (IIVS), Gaithersburg, MD, USA.

InterNICHE Co-ordinator, Nick Jukes, said "This is a validation of the InterNICHE vision of 100% replacement in education and training, and of the practical achievements by InterNICHE National Contacts, Partners and other volunteers in making this vision real. It is also a recognition of the crucial role of humane education and its impact on individuals, on society and on science. Graduates who have been trained with innovative, humane methods, and whose critical thinking and compassion have not been dulled by animal experimentation, can provide the skills necessary to help bring science fully into the 21st century — and to replace animal testing for good."

[1] Anon. (2012). *InterNICHE wins Lush Prize*. [InterNICHE, 27.11.12]. Available at: http://www.interniche.org/en/news/interniche-wins-lush-prize (Accessed 27.11.12).

Heart Disease in Captive Apes

In 2010, Zoo Atlanta was selected to lead the Great Apes Heart Project from the US federal Institute of Museum and Library Services (IMLS). The multi-institute programme is led by Dr Hayley Murphy, Director of Veterinary Services at Zoo Atlanta. The aim is to identify, diagnose and treat cardiovascular diseases in all four non-human great ape groups, namely, gorillas, orangutans, chimpanzees and bonobos. These diseases are a leading cause of death among apes living in zoological settings. The project now has more than 50 partners in the USA and Europe. In September 2012, Zoo Atlanta received a further IMLS grant of almost $500,000, to continue the project for a further three years.[1]

According to *The Sunday Times*,[2] Zoo Atlanta has found that up to 60% of captive apes are likely to develop heart problems — Dr Murphy said that heart disease is a significant cause of death in 41% of gorillas, 20% of orangutans, 38% of chimpanzees, and 45% of bonobos. By contrast, a study in Rwanda, Uganda and the Democratic Republic of Congo found heart disease in only 4% of the wild apes.

This brings into sharp focus the study reported in the last issue of *ATLA*,[3] when independent pathologists found that "the majority of chimpanzees which died in laboratories had been suffering from significant chronic or incurable disease, and most often had multi-system diseases that should have made them ineligible for future research, on scientific, as well as on ethical grounds".

[1] Anon. (2012). *Great Ape Heart Project Awarded Second Grant*. Atlanta, GA, USA: Zoo Atlanta. Available at: http://www.zooatlanta.org/home/article_content/great_ape_heart_grant2 (Accessed 31.12.12).
[2] Leake, J. (2102). Captive apes in heart alert. *The Sunday Times*, 30 December 2012.
[3] Capaldo, T. & Peppercorn, M. (2012). A review of autopsy reports on chimpanzees in or from US laboratories. *ATLA* **40**, 259–269.

Transposition of *Directive 2010/63/EU*

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes,[1] came into force on 9 November 2010, to replace *Directive 86/609/EEC*. The 27 EU Member States were required to transpose its requirements into their own national legislation, so that they could be implemented by 1 January 2013.

In the UK, the Home Office, the Government department responsible for the use and protection of laboratory animals, embarked on a wide consultation process, which closed on 5 September 2011, and which resulted in responses from 98 organisations and 13,000 individuals. The Home Office published the results of the consultation on 17 May 2012, then circulated proposed amendments to the 1986 Act on 27 July 2012. This carefully-orchestrated process eventually led to debates in the House of Commons[2] and the House of Lords[3] in December 2012, on the *Draft Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012*.

The Government ministers who introduced the two debates, Mark Harper MP and Lord Taylor of Holbeach, both noted the three aims of the new Directive: to rectify wide variations in

the implementation of procedures among the Member States, to strengthen the protection of animals used in scientific procedures, and to promote the Three Rs — strategies that "replace", "reduce" and "refine" the use of animals in scientific procedures. The regulations were approved by both Houses of Parliament, and were signed by Lord Taylor, on behalf of the Government, on 18 December 2012.

The new legislation contains many useful definitions and provisions, including: definitions of the Three Rs; specification of the Named Person with specific responsibilities; the requirement for a project summary in non-technical language; the evaluation of a programme of work; the retrospective evaluation of certain programmes of work; the requirement for education and training; conditions concerning the re-use of protected animals; conditions concerning the use of neuromuscular blocking agents; the definition of alternative strategies; and the retention of additional protection for endangered animals, primates, cats, dogs and *equidae*. At FRAME, we are particularly pleased by the inclusion in the UK legislation of a ban on the use of great apes as laboratory animals.

The Directive clearly affords great opportunities for achieving the second two of the three aims mentioned above, but it is not clear how variation in practice among the Member States will be reduced. The Directive tends to say what should be done, but not how it should be done. In the UK, we are awaiting proposals for Home Office *Guidance* and a Home Office *Code of Practice*, which will eventually be the basis for the detailed interpretation and implementation of the provisions of the Directive in the UK. However, if similar documents are to be produced in the other Member States, it seems inevitable that they will reflect differences in interpretation, experience and culture. What the European Commission will propose as a basis for rectifying wide variations, remains to be seen.

[1] Anon. (2010). *Directive 2010/63/EU* of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* **L276**, 20.10.2010, 33–79.

[2] Anon. (2012). *Draft Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012*. Considered in a General Committee debate on 3 December 2012. Columns 3–22, 17pp. Commons Text for 3 December 2012. London, UK: Parliament UK. Available at: http://www.publications.parliament. uk/pa/cm201213/cmgeneral/deleg6/121203/121203s01.htm (Accessed 04.12.12).

[3] Anon. (2012). *Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012*. Considered in Grand Committee on 13 December 2012. Columns GC379–GC400, 22 pp. Lords Hansard Text for 13 December 2012. London, UK: Parliament UK. Available at: http://www.publications.parliament. uk/pa/ld201213/ldhansrd/text/121213-gc0001. htm#12121339000271 (Accessed 14.12.12).