Injecting the future into toxicology

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Toxicology developed as the study of overt pathological changes caused by natural products and exposure to chemicals. Subsequently, with the development of sophisticated drugs and pesticides, toxicology has become a multilayered process to select products that satisfy safety legislation; toxicologists also try to assess subtle risks to humans from very low chronic environmental and dietary exposures to natural and synthetic chemicals. Understanding relevant novel fundamental mechanisms pertinent to humans, rather than focussing only on the toxicity of classes of drugs and chemicals, is paramount to true assessment of risk. To achieve this goal, it is a priority to attract and train a new generation of scientists cognisant with both fundamental biology as well as toxicological concepts.

The origin of toxicology as a discipline lies way back in history when poisoning by animal and plant toxins and minerals were often intentional and fatal. Italians were particularly well versed in the application of these skills! Eventually, a systematic and measured approach showed that it could be difficult to distinguish whether a chemical was a poison or perhaps beneficial to health[1]. For many drugs, toxicology is at the opposite end to the beneficial pharmacological effects in the spectrum of chemical-biology interactions. The benefits and disadvantages of alcohol are debated. For centuries weak beer was drunk with meals, as it was a lot safer than the local water supply, and red wine at low consumption may be a protectant against cardiovascular disease. On the other hand, alcohol induced liver disease is an increasing health problem even in the young. It is all a matter of dose. Additionally, linear extrapolation of toxicity from high doses, does not necessarily predict incidence and outcome at very low doses.

The rapid growth of the drug, pesticide and chemical industries during the latter half of the 20th century was concomitant with greatly increased safety assessment legislation and in the consideration of risks from contaminants in the environment, food and water.

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Endpoints of concern have changed from overt toxicity to more subtle effects such as possible foetal endocrine disruption, neurodevelopment and compromise of the immune system. These have been much more difficult to study by animal experimentation than the traditional endpoints such as cancer but are also dogged by the same problems as to whether they are relevant to human exposure at treatment doses or at environmental levels of exposure.

Although a reductive approach to identify the chemistry and the genes and their regulation involved in traditional toxicology pathways, has provided immense benefits for understanding mechanisms of known clear toxic mechanisms in rodents, such as those mediated by cytochrome P450 forms and oxidant responses, it is still often difficult to understand the incidences of toxicity observed in drug development and inter-patient variation in adverse drug reactions. 'Knockout' mice and siRNA in vitro approaches have been useful models but are not the same as the polymorphic variations seen in patients. Many toxic sequelae are probably the consequence of complex disturbances between gene products and processes of which we know very little, if anything. The ultimate toxic agents are often endogenous

chemicals such as reactive oxygen species, Ca^{2+} or degraded eicosanoids mediating disruption of cell signalling and cell death rather than the drug or chemical themselves[2].

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Where do we want to go?

Clearly, toxicity can be very complex and it is not easy to extrapolate from cellular and animal studies to humans. This is of importance not only to the drug industry but also to those assessing risk from food contaminants and air pollution.
A decreasing number of drugs are reaching the market because of safety concerns and public perception of food safety is fragile due to the presence of trace contaminants.
Some of the endpoints require detailed mechanistic investigation of novel fundamental biochemical processes. The introduction of genomic and proteomic techniques have led to remarkable findings in new pathways and protein structure variants and modifications that are induced by drugs, but may not always be directly related with phenotype.
Perhaps of even greater significance is the usage of metabolomics which has the advantage of revealing disturbances in the fluctuations of unsuspected endogenous metabolites, and hence pathways, caused by xenobiotics, hormones and diet and their inter-relationship[3].
These technologies need to be integrated, which given the huge amount of information generated is an immense challenge; sometimes the data gathered and processed are devoid of a working hypothesis.

Most importantly for drug development are initiatives in which toxicology can be used as a tool to probe novel biology, rather than only for assessment of individual chemical safety. Although not a new philosophy, regeneration of this approach with modern tools should provide insights into targets for therapy of which no one has yet dreamed, so that toxicology is emphasised as a creative rather than a negative discipline[4]. New imaginative approaches are required and clinical and nonclinical scientists should be attracted from other fields, for example neurobiology, immunology, chemistry, imaging and systems biology to provide fresh minds and insights into toxicology focussing on known human diseases and feedback into therapies at the same time appreciating basic toxicology concepts such as the importance of age, nutrition, length of exposure and dose.

Examples of fundamental biology influencing toxicology

In brain ischemia failure to clear glutamate from synapses and other sites triggers excitotoxicity, deregulation of calcium homeostasis leading to Ca^{2+} overload and neuronal loss. This can occur in stroke and in the relatively slow demise of neurons in neurodegenerative diseases.
Studies of the cleavage, by calpain proteases, of the sodium-calcium exchanger NCX3 isoform 3 extruding Ca^{2+} from neurons have shown Ca^{2+} toxicity linked to neuronal demise.
Inhibition of calpain by the endogenous inhibitor calpastatin delayed NCX3 cleavage, Ca^{2+} overload and neuron loss[5]. Exploitation of the disruption of these pathways may be new approaches for pharmacological intervention in stroke and chronic neuronal degeneration therapies.

Fundamental studies of mitochondria dysfunction are showing important findings for a wide range of human pathologies including topics traditionally considered to be of toxicological concern. Somatic mutations in mitochondrial DNA malignant cells have been found to lead to dysfunction of respiratory complex I, elevated reactive oxygen species and anti-apoptotic factors. Scavengers of free radicals lower metastatic potential and offer routes of chemotherapy[6].

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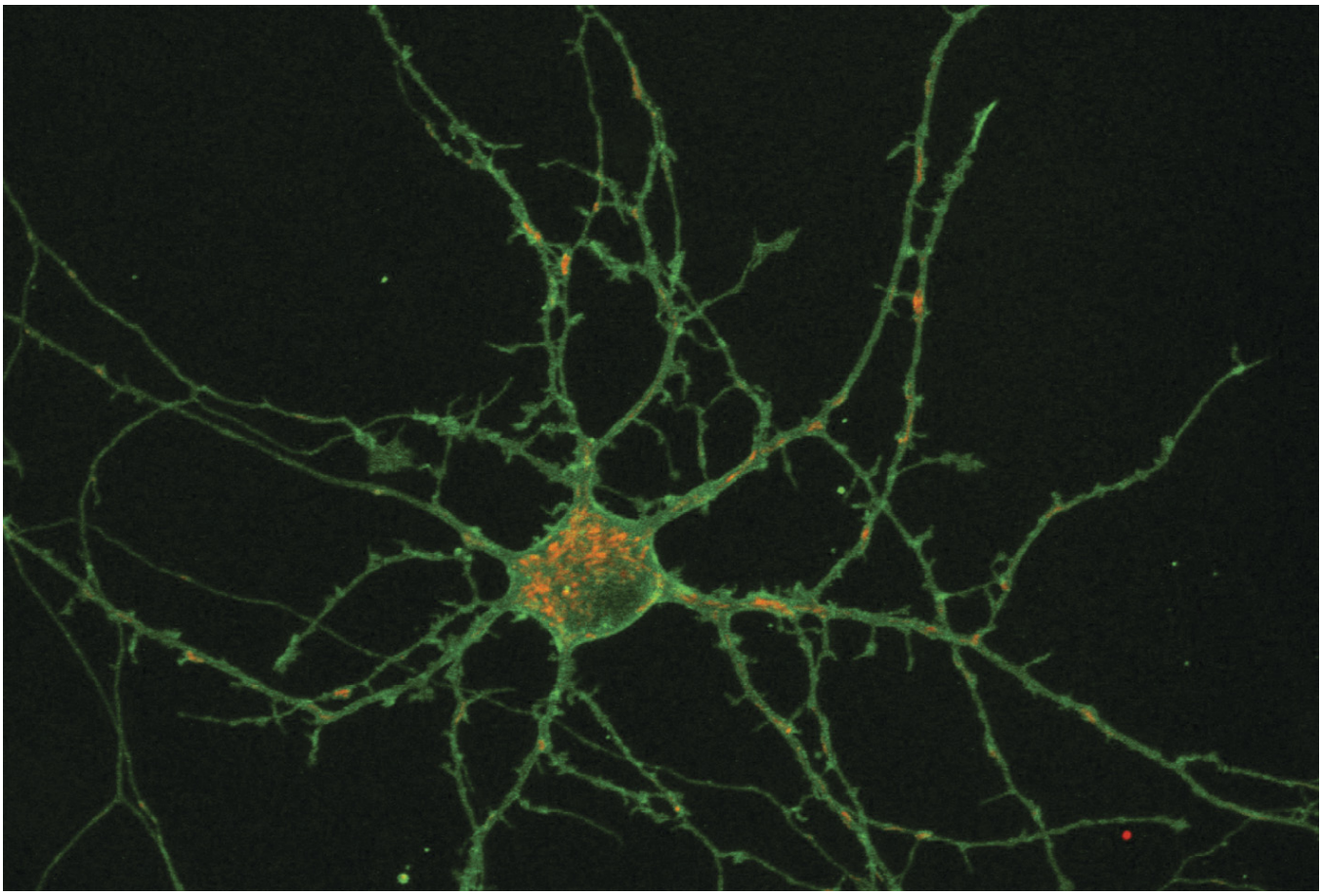
Many laboratories have dissected the molecular pathways involved in controlling apoptotic cell death in cancer progressions as in B-cell lymphocytic leukemias especially over expression of the anti-apoptotic protein BCL-2 leading to failure of the cancer cells to die. Unfortunately, some patients rapidly develop chemotherapy-resistant disease. Detailed knowledge of BCL-2, related

Table 1: Some areas of research supported by ITTP studentships

Cannabinoid receptors in developing brain
Susceptibility to interference in mRNA processing
Human stem cells in toxicology
Antiapoptosis and cytoprotection through regulation of intracellular redox status.
Allergenic sensitisation to chemicals
Antiapoptotic proteins in neurotoxic cell death
Mitochondrial DNA damage in endothelial cell aging
Mechanisms of drug-induced liver injury
Health effects of transition metals in particulate air pollution
Nanotoxicology
Metabonomics and epidemiology of outcomes of contamination of water supply



proteins and its functions have led to the design by Abbott of inhibitors like ABT-737 and its orally active analog ABT-263 for treatment of B-cell malignancies. Understanding the biology also enabled studies showing that resistance could develop due to de novo synthesis of anti-apoptotic proteins which might develop in lymph nodes in vivo but that treatment strategies targeting multiple anti-apoptotic proteins simultaneously may have synergistic activity[7].

For over thirty years molecular toxicologists have identified and studied the aryl hydrocarbon receptor that acts as a ligand-binding transcription factor for carcinogenic aromatic chemicals such as benzo[a]pyrene in cigarette smoke and 'dioxin' the chlorinated agent highly toxic for animals but is less clear for humans at environmental levels. The predominant endogenous ligands for the receptor are still not definitively identified but the transcription factor is clearly involved in aspects of liver development and immunology as well as in the well established control of drug metabolism and in the toxicity of air and food contaminants. Recently, immunologists from non toxicology backgrounds have discovered that it is this transcription factor binding endogenous ligands that modulates a T cell subset (T_H17) differentiation programme, production of interleukin-22 and implication in aspects of autoimmunity[8,9]. This may explain the contribution of some environmental exposures to autoimmune diseases. Intervention in such pathways could provide new therapeutic targets for diseases like rheumatoid arthritis.

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Stimulation of toxicology research training

In the UK there has been considerable concern voiced by industry (especially through the ABPI), academia and government organisations that not enough young, highly motivated research toxicologists are being attracted into toxicology and conversant with the input of modern biological concepts pertinent to human diseases. Toxicologists in drug safety, chemical, plant protection and food industries together with environmental health and drug regulatory authorities need to have a wide ranging knowledge of integrative skills from the latest biology to risk assessment criteria. In 2007 the Medical Research Council instigated a funding programme for four year PhD studentships and some career Development Fellowships entitled the Integrative Toxicology Training Partnership (ITTP) and managed by the MRC Toxicology Unit at the University of Leicester with

advice from Steering and Scientific Committees http://www.le.ac.uk/mrctox/MRCTox/ittp.htm. In November 2007 a meeting was held at the Royal Society of Medicine in London to discuss the format of the initiative and the views of interested parties.

The emphasis of the initiative is on aligning modern cell and molecular biology with other fundamental and health-related disciplines to provide an integrative holistic approach in research and training relevant to predicting the toxicity of chemicals and drugs as well as to develop an understanding of the chemical,

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pharmacological and biological processes involved. It is important that applications are truly multidisciplinary. Collaborative partnerships in universities and with other relevant organisations, including industry and Government agencies have been encouraged. Priority is on bringing to the initiative innovative concepts and approaches from fields traditionally not considered to be associated with toxicology. A key feature of the initiative aims to be its training programme which will bring together aspects of established toxicology courses with other relevant sciences and an annual residential meeting exclusively for ITTP PhD students and Fellows and meetings at the British Toxicology Society congress. Students will also benefit from a broad training in generic and transferable research and scientific skills, including good experimental design, in their host establishments.

After intense competition from across the UK, 20 PhD studentships have been funded in 11 different universities starting in October 2008 or 2009 and covering a wide range of research areas and collaborations. A synopsis is shown in Table 1. A Career Fellowship has been awarded to Dr Muireann Coen at Imperial College to study the metabolomics of adverse drug reactions. It is hoped that by boosting fundamental science in toxicology this will lead to advantages not only in better prediction and understanding of risks for humans from side effects of drugs, as well as environmental chemicals, but should strengthen the competitive side of European Drug research.

References

1. *Timbrell JD (2005) The poison paradox; chemicals as friends and foes. Oxford University Press, Oxford.*
2. *West JD and Marnett LJ (2006) Endogenous reactive intermediates as modulators of cell signaling and cell death. Chem Res Toxicol 19(2):173-194.*
3. *Holmes E, Wilson ID and Nicholson JK (2008) Metabolic phenotyping in health and disease. Cell 134(5): 714-717.*
4. *Congreve M, Murray CW and Blundell TL (2005) Structural biology and drug discovery. Drug Discov Today 10(13):895-907.*
5. *Bano D and Nicotera P (2007) Ca2+ signals and neuronal death in brain ischemia. Stroke 38(2 Suppl): 674-676.*
6. *Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y and Hayashi J (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320(5876):661-664.*
7. *Vogler M, Butterworth M, Majid A, Walewska RJ, Sun XM, Dyer MJ and Cohen GM (2009) Concurrent upregulation of BCL-XL and BCL2A1 induces ~1000-fold resistance to ABT-737 in chronic lymphocytic leukemia. Blood. In press*
8. *Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M and Weiner HL (2008) Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature 453(7191):65-71.*
9. *Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC and Stockinger B (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453(7191):106-109.*

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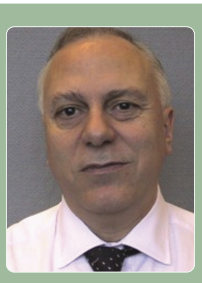

Pierluigi Nicotera

Professor Pierluigi Nicotera is the newly appointed Director of the German Centre for Neurodegenerative Diseases, Bonn and former Director of the MRC Toxicology Unit, University of Leicester and Professor of Molecular Toxicology, University of Konstanz. Pierluigi Nicotera has interests in understanding mechanisms regulating pathways of cell death, injury and survival especially involving the impairment of Ca2+ transport systems in models of human disease, particularly those of stroke and neuronal degeneration.