Comparative Medicine
Copyright 2017
by the American Association for Laboratory Animal Science

Vol 67, No 3
June 2017
Pages 281–292

Abstracts of Scientific Papers

AISAL Symposium

Rome, 10-11 October 2016

Main lectures and oral presentations

State-of-the-Art, Validated Alternative Methods and Future Challenges

Dal Negro Gianni
GlaxoSmithKline, Londra
gianni.a.dal-negro@gsk.com

The 3Rs principle and alternative methods are increasingly important in the national and international political agendas. Nowadays, the 3Rs principle is firmly anchored to the European legislation in different sectors (Dir. 2010/63/EU, Regulation 1223/2009, REACH). The increasing attention and commitment to the implementation of Alternative Methods have generated a number of initiatives and considerable investments for the development of non-animal methods. This presentation is aimed to provide the state of the art of alternative methods officially validated and embedded in international guidelines. An overview on the most important European initiatives and investments in the development of laboratory animal-replacing methods will be also provided as well as an analysis of existing hurdles to full replacement of laboratory animals in relevant application fields.

The Good, the Bad and the Alternative: Relevance and Limitations of the 3Rs Principle

Vitale Augusto
Istituto Superiore di Sanità, Roma
augusto.vitale@iss.it

The Principle of the 3Rs, introduced in 1959 by Russel and Burch, has been one of the most important theoretical achievements in the field of animal welfare of the past decades. It still inspires a *forma mentis* which allows to think about animal experiments in a more ethical and "humane" way. From a more practical perspective, the Russel and Burch's Principle is the backbone of the Directive 2010/63/EU, with dedicated articles and specific mandatory requirements for personnel dealing with animals, who are required to show knowledge of the 3Rs and their application. However, the Principle has to be also contextualized and understood in its flexibility and real application. In this presentation, I will firstly introduce the Principle of the 3Rs, underlining its fundamental importance, both in terms of alternative to and in animal experimentation. I will then discuss the legislative aspects of the 3Rs, and what the law requires. In the second part of my talk, I will present some practical examples, with the aim of discussing for each R cases in which their application is beneficial to both animal welfare and quality of research, and cases in which the Principle show some limitations, and cannot yet fully implemented. As well as the term "animal experiment," so the term "alternative" cannot be understood as a sort conceptual and practical "monolith," and a discussion on the pro and cons of the 3Rs can be useful to discuss a more flexible approach to these issues.

Experimentation, History, and Communication: Suggestions for the Near Future

Grignolio Andrea
Sapienza—Università di Roma
andrea.grignolio@uniroma1.it

Thalidomide, Bendectin, and TGN 1412 are three important events in the history of animal testing that could offer some suggestions of how to manage social resistance on this topic. The history of thalidomide recounts a tragedy of the past that today represents a typical case of public misunderstanding of the pivotal role of animal experimentation for pharmaceutical development. The history of Bendectin explains how to avoid the scientific authority in favor of empirical evidences in the public discussion of animal testing, with specific concerns in relation to legal trials. Last but not least, the recent history of TGN 1412, a humanized monoclonal antibody—an immunomodulatory drug intended to treat B cell chronic lymphocytic leukemia and rheumatoid arthritis—withdrawn from development after inducing severe inflammatory reactions in the first-in-man study (phase 0) at Parexel in London in March 2006, recently started to casts doubts on testing guidelines, especially concerning the animal to man "transferability." In the light of the future biomedical research, focused on monoclonal antibody therapy with an exquisite, almost individual, specificity, the latter case should be taken as a case study of how to prevent social criticism and address correct public understanding.

Animals and Public Debate

Lalli Chiara
Bioeticista
chiara.lalli@gmail.com

Is it morally admissible to cause distress, pain or death to ani-

mals? Is it acceptable to use them for our benefit? Are humans more important than all animals? What is the adequate role of regulation for research involving animals? And what about the justification of other uses, such as food production? In other words, can any use of animals by humans be justified?

In order to answer these questions, we need to define the moral status of humans and animals. Is there a hierarchical scale with humans at the top and the animals at the bottom (from the simplest to the most complex)? Or should we consider humans and animals morally equal? Do animal have rights? The debate about research involving animals and other uses is often burdened with emotional issues. How can we reduce the existing disagreement? Why is it so difficult to think straight and to talk about animals?

Animal Models of Neurological Diseases

Mercuri Nicola Biagio
Università degli Studi di Roma "Tor Vergata"; IRCCS Fondazione Santa Lucia, Roma
mercurin@med.uniroma2.it

There is a need to simulate and model neurological and psychiatric disorders in animals in order to ascertain the pathological processes and to test medical devices, vaccines, and drugs. Therefore, animals are used to simulate traumatic brain and spinal cord injuries, stroke, epilepsy, neurodegenerative diseases, multiple sclerosis, muscular dystrophy, drug abuse and many other neuropsychiatric diseases. Researchers have used snails and fishes to discover the molecular bases of learning, memory and the circuits of motion. They also use genetically modified flies and worms. However, most commonly they use vertebrates (mice and rats), which can be genetically manipulated to generate behavioural changes that model human disorders. To more faithfully mimic human diseases, it should be realised that, in selected few cases, non-human primates are still necessary. Moreover, the research on animals can lead to the discovery of new effective therapies for pathologies such as diabetes type 2 and obesity that are apparently unrelated to brain dysfunctions. Given the fact that human genetics is ultimately an observational, rather than an experimental, science, and given the ethical and practical limitations to human experimental biology, animal models will almost certainly be a necessary aspect of progress in both pathophysiology and treatment development of human diseases. I will briefly discuss the relative utility of animal models in which a heritable or non-heritable (acquired) manipulation has been used to model a specified trait of a human neurological and psychiatric disorder. In the perspective of translational neuroscience, particular attention will be paid to preclinical studies that are related to the development of experimental therapeutics.

Autism Spectrum Disorders: What Can We Learn from an Animal Model?

Cherubini Enrico
Fondazione EBRI—Rita Levi Montalcini, Roma; Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste
e.cherubini@ebri.it

Autism Spectrum Disorders (ASDs) comprise a group of heterogeneous neuro-developmental disorders, characterized by impaired social interactions, communications deficits and stereotyped behaviors. These disorders affect 1/150 children, particularly under the age of 3. In a few cases, ASDs have been found to be associated with single mutations in genes involved in synaptic function. One of these mutations (R451C), identified in a family with children affected by autism, involves *Nlgn3* a gene encoding for NL3. This is a postsynaptic adhesion molecule that, by binding to its presynaptic partner neurexin (NRX), ensures a corrected cross-talk between post and presynaptic specializations and the maintenance of an appropriate E/I balance. An altered E/I balance within selective brain areas accounts for several neuropsychiatric and neuro-developmental disorders including ASDs that can be considered "synapthopathies." Transgenic mice carrying the human NL3 R451C mutation constitute an ideal animal model for studying ASDs since these animals exhibit impaired social interactions reminiscent of those present in autistic patients. Here, I will provide evidence that mice carrying the R451C mutation of NL3 present from birth deficits in GABAergic signaling and synaptic plasticity processes in the hippocampus and somato-sensory cortex. These alterations may lead to impairment of cognitive functions. I will briefly discuss how animal models of ASDs cannot be easily replaced by alternative methods as for instance organoids that can mimic very well some aspects of development but they need to be ultimately validated in animals.

Ex Vivo Models in the Framework of the 3Rs: Organotypic Slices of Central Neurons

Lossi Laura
Università degli Studi di Torino
laura.lossi@unito.it

Understanding how neuronal circuities are shaped and how they operate is one of the major challenges in modern Neuroscience. Animal experiments aiming to address such a challenge are often associated with a more or less severe degree of sufferance as they generally require the perturbation of these circuits by chemical or physical injury. Therefore, these experiments are all *regulated procedures* as defined by current EU regulations on animal welfare. Here I describe the preparation, the characteristics, and the practical use of acute and organotypically cultured CNS slices obtained from postnatal and young adult mice. Cultures can be easily subjected to a surrogate challenge of diverse nature and monitored by various procedures, such as ELISA or immunocytochemistry, bulk-load calcium imaging or path-clamp electrophysiological recordings. Slices can be further manipulated by genetic engineering using a physical transfection method (gene-gun) that - at least in part - avoids the need to generate novel transgenic strains. They are also amenable to pharmacological high throughput screening to test the physiological relevance of molecules of interest. The primary Rs of this approach are:

Replacement: The procedure includes the use of tissues taken from animals killed solely for this purpose (i.e. *not* having been subject to a regulated procedure)

Reduction: The number of animals used per experiment/study is significantly minimized, as several slices can be successfully obtained from an individual and used in different sets of experiments.

Examples of application are given with emphasis on the regulation of cell death in cerebellum.

Modeling Safety and Efficacy of Gene therapy

Surace Enrico Maria
Università degli Studi di Napoli "Federico II"; TIGEM, Pozzuoli—Napoli
surface@tigem.it

Gene therapy is the discipline aims at developing therapies by transferring nucleic acids to diseased cells. In the last decade the enthusiasm over successes in clinical trials promoted the expansion of gene therapy-based experimental studies, arising interests from both research funding agencies as well as biopharmaceutical companies. Indeed, funds assigned for translational studies based on gene therapy are increasingly expanding. My laboratory took part to both research and clinical studies based on gene therapy. Based on these experiences my presentation will focus on the role of modeling the transition between research studies and clinical studies. In addition, bioinformatic approaches employed in my laboratory also aimed at tackling the critical issues of animal vs non-animal modeling will be also discussed.

Addiction and Laboratory Animals: Models for Treatment and Prevention of Behavioral Disorders

Chiamulera Cristiano
Università degli Studi di Verona
cristiano.chiamulera@univr.it

Compulsive behaviours, including those for drugs of abuse and palatable food, have a tremendous global impact at both health and socioeconomic levels. These disorders derive from an abnormal functioning of motivational processes and behaviours as a consequence of repeated substance use, unhealthy diet and, in general, of bad life styles: the powerful hedonic value of these unnatural rewards eventually substitute for the natural ones. Motivation and reward-seeking are evolutionary processes common to several animal phyla, and the underlying neurobiological mechanisms have been extensively investigated in laboratory animals. Integrated experimental approaches (ranging from neurobiology to psychology) have shown that animal models own high face, construct and predictive validity for the human condition. In the last few years, animal research allowed to increase basic knowledge, provide mechanistic targets for therapy, and protocols for psychosocial intervention. Data from these animal models are also requested by regulatory agencies for marketing novel drugs with potential abuse liability. Although the complexity of these disorders could not totally mimicked in animal models, in-vitro assays that substitute for the in-vivo assessment are not however viable, at the moment. In the meantime, scientists are developing novel protocol designs in order to improve animal care and welfare, and to further reduce sample size. An interdisciplinary scientific community is designing novel combined approaches that could offer valid alternatives as correlates of the in-vivo data, as for example experimental medicine and biomarkers.

Regeneration and Repair: Moving Towards Patients

De Coppi Paolo
Stem Cells and Regenerative Medicine Section, DBC, UCL Institute of Child Health and Great Ormond Street Hospital, Londra
paolo.decoppi@gosh.nhs.uk

Congenital or acquired surgical conditions are associated to high morbidity and mortality and most of the time functional replacement of the missing or damaged organ remains an unmet clinical need. Tissue engineering led by advances in two specific fields, cell biology and material science, have combined to create the perfect biological substitution. Our team has focused on deriving matrices from organs and tissues through decellularization. Using detergents and enzymatic solutions it is possible to derive natural scaffolds, which are not immunogenic but still maintain many of the characteristic of the tissue of origin. In 2010, using this process we were able to engineer a trachea, which was successful transplanted in a child. Following the clinical success we have focused on various other organs and preliminary data on bladder, kidney, oesophagus, intestine, lung, liver, pancreas and skeletal tissues have been obtained using similar methodologies. Decellularised matrices and cellular product could be combined in vitro and bioreactors could be used to plan *in vivo* translation. Moreover, there is the opportunity to treat congenital malformation during the gestation or in the neonatal period. In particular, it has become evident that is possible to collect the stem cells at diagnosis, even before birth. Human amniotic fluid stem cells (AFSC) can be isolated during fetal development, and have intermediate characteristics between embryonic and adult stem cells. AFSC cells have shown to be effective in various animal models of disease, and could represent an innovative tool for therapeutic application in the future of congenital malformation.

The Therapeutic Plasticity of Neural Stem Cells

Martino Gianvito
IRCCS San Raffaele Hospital, Milano
martino.gianvito@hsr.it

Recent evidence consistently challenges the sole and limited view that neural stem/precursor cells (NPCs) may protect the central nervous system (CNS) from inflammatory damage leading to neurodegeneration exclusively throughout cell replacement. As a matter of fact, NPC transplantation may also promote CNS repair via intrinsic neuroprotective 'bystander' capacities, mainly exerted by undifferentiated stem cells releasing, at the site of tissue damage, a milieu of neuroprotective molecules whose in situ release is temporally and spatially orchestrated by environmental needs. This milieu contains molecules (e.g. neurotrophic growth factors, stem cell regulators, immunomodulatory substances), which are constitutively expressed by NPCs for maintaining tissue homeostasis either during development or adult life. The intrinsic nature (pleiotropism and redundancy) of these molecules as well as their 'constitutive' characteristics, may also reconcile data showing that other sources of somatic stem cells (e.g. mesenchymal stem cells), with very low capabilities of neural (trans) differentiation, may efficiently promote CNS repair. Thus, the concept of 'therapeutic plasticity' of stem cells is now emerging as the capacity of both embryonic-like and somatic stem cells to adapt their fate and therapeutic function(s) to specific environmental needs occurring as a result of different pathological conditions. The challenging ability of transplanted NPCs to protect the brain from several types of injuries using different and/or articulated bystander strategies is of pivotal importance for the future of stem cell based therapeutic approaches.

Regenerative Medicine for the Nervous System: Appropriateness of the Design of the Preclinical Studies as a Tool for the 3R Policies

Calzà Laura
Alma Mater Studiorum, Università di Bologna
laura.calza@unibo.it

The poor therapeutic repertory for degenerative diseases and acute injuries of the nervous system represents a primary medical and economical issue for the society. Regenerative medicine

is recognized as a new frontier for these conditions, generating a great expectation and pressure on the scientific community from patients and families. A major issue in the field is how to establish appropriate scientific standards for preclinical studies aimed to proof-of-concept and efficacy studies. This discussion also coincides with the increasing concern of the scientific community on how to increase data reproducibility for preclinical data. In fact, the poor reproducibility in preclinical studies is producing substantial dis-investments of big-pharma in the field, and is also impacting on the reliability of translational neuroscience. Starting from lab experience and literature on two widely used preclinical model for testing regenerative medicine therapies, i.e. experimental allergic encephalomyelitis for multiple sclerosis, and spinal impact for spinal cord injury, the following points will be discussed:

1. how to establish the appropriate number of animals to be included in a study and how to establish the appropriate primary and secondary end-points;
2. animal observation: direct observation vs computerized videotracking techniques;
3. multiple site studies, data repository and data sharing;
4. Standard Operating Procedures: something helpful?

The 3R rules will be the pivot of the discussion, as follows. Replacement: how to establish the appropriate end-points for in vivo studies? Reduction: not one more, not one less. Refinement: standardization of lesion models, the intra- e inter-labs variability and reproducibility of complex animal models.

The Use of Animal Models for the Testing of New Immunotherapeutic Approaches in Oncology

Santoni Angela*, Bernardini Giovanni
Sapienza—Università di Roma
*Corresponding author: angela.santoni@uniroma1.it

It is well documented that the immune system is able both to inhibit and to promote the onset, growth and spread of tumors. Recent advances in understanding the mechanisms of activation and regulation of the immune response against tumors have led to the development of several drugs that can overcome the inhibition provided by key molecules involved in immune tolerance (referred as immune check-point inhibitors) or boost the immune response through co-stimulatory molecules. In addition, increasing evidence show that also chemotherapy may act by stimulating the immune system in some conditions, suggesting that the outcome of therapy is dependent not only on the effect of drug on tumor cells, but is also mediated by immunomodulatory effects, verifiable only by considering the whole body. The success of these drugs is therefore largely attributable to the use of appropriate animal models of tumors that has led to the identifications of key molecules and cells controlling tumor immunity. Finally, the new approaches in adoptive immunotherapy, based on the infusion of in vitro activated or expanded immune cell populations, require in vivo validation, which at present only the use of animal models can provide.

Imaging of Systemic Proliferation as a Novel Approach for the Identification of Clinically Useful Tumor Biomarkers

Manni Isabella[1], De Latouliere Luisa[1], Iacobini Carla[2], Pugliese Giuseppe[2], Menini Stefano[2], Piaggio Giulia[1*]
[1]Istituto Nazionale Tumori, Regina Elena, Roma; [2]Sapienza—Università di Roma
*Corresponding author: giulia.piaggio@ifo.gov.it

In the entire body the organ fates are determined by cellular and molecular interplay between tissues, micro-environment, and macro-environment. While how the micro-environment influences tumor evolution has been extensively characterized, the study on the host macro-environment as an important determinant of early steps of tumor progression is still in its infancy. We recently developed a mouse model (MITO-Luc) engineered to express the luciferase reporter gene in cells undergoing active proliferation. We used MITO-Luc mice with mammary or pancreatic carcinogenesis to measure cell proliferation in longitudinal studies by in vivo bioluminescence imaging. We noticed that in the hematopoietic system of these mice invariably, photon emission increases several weeks before tumor appearance. Based on these considerations we hypothesize that systemic proliferation events occur in the animal macro-environment at early stages, before tumor appearance.

Role and Contribution of Italian High Institute of Health (IHIH) for Correct Welfare Preservation of Animals Used for Research Purposes

Lorenzini Rodolfo Nello
Istituto Superiore di Sanità (IHBH), Roma
rodolfo.lorenzini@iss.it

The presentation deals with innovative aspects introduced by the European Directive and *Decreto Legislativo* 26/2014 to protect the welfare of animals used for research purposes. In particular the activities carried out by IHIH, according to article 31 of and *Decreto Legislativo* 26/2014, for the technical and scientific evaluation (VTS) of research projects. In the first part logistic and organizational aspects of VTS are described as well as the competence of evaluators from IHIH. In the second part the presentation critically analyses the main problems encountered by IHIH. In particular cultural diversities emerging from the composition of Italian Animal Welfare Bodies (OPBA), their various operating methods, and the balance of power of different professionals that on one side perform research on animals and on the other are in charge of protecting their welfare. Last but not least the presentation analyses the criteria adopted by various OPBA to evaluate the level of animal suffering and the way OPBA elaborates a "justified approval" about the project. The third part deals with the cultural and training activity IHIH is carrying out to provide OPBA and Investigators with correct and standardised education. This part describes educational paths adopted by IHIH to train evaluators and to spread among researchers a culture of responsibility. With regard to training a comparison is also made among different educational paths and various competence levels of professionals involved in animal research.

Designing Health Monitoring Schemes for Rodents

Foà Massimo
IDEXX BioResearch
massimo-foa@idexx.com

Micro-isolation cage systems (mainly IVCs and FTCs) are widely used nowadays, with the aim of protecting animals and operators. Health monitoring of these units has always been problematic because each cage is in reality an independent microbiological unit. Traditionally these units are monitored with the use of a variable number of immunocompetent sentinels that have been exposed to dirty bedding, tested via serology, bacteriology and parasitology. This procedure relies upon the transmission (not always efficient) of agents in addition to other uncontrollable variables such as preva-

lence of disease; dose of agents that are shed by resident animals; frequency and amount of bedding transferred in addition to the susceptibility and receptivity of the sentinels. The hypothesis is that modern technologies allow sampling points other than sentinels to make the screening more sensitive. We analysed and compared results obtained in different sampling points (sentinels, principal animals, and environment) and different methodologies (mainly serology, PCR and parasitology) for agents believed to transmit inefficiently to sentinels via dirty bedding. Apparently a better health monitoring is obtainable by combining tests conducted on different sampling point depending on the agent of interest.

ICM^{TM}, an Innovative Web-Based Management of Genetically Altered Research Models

Galbiati Francesca*, Gentile Stefania
Charles River Laboratories Inc.
*Corresponding author: francesca.galbiati@crl.com

Managing GA animal models is a complex, labor-intensive process. There are thousands of GA models and defined microbiological status strains used in research today. In 2012 Charles River developed an innovative web-based tool to efficiently track the high volumes of data generated through the colony management process; we call this system "Internet Colony Management." Recording thousands of data using tablet computers and RFID, ICMTM prioritizes tasks and drives day-to-day activities. The data collected are instantly made available through an on-line portal to technicians, project managers and customers helping them to immediately determine goal achievement. The "inactive" colonies can be identified in real-time and strategic decisions can be taken avoiding the production of unnecessary animals. 24-hour access to review real-time data and direct action within your colony from everywhere is guaranteed. The main features of ICMTM are two-way, real-time communication, secure online storage of project information, extensive sorting features to quickly find data, and a user-friendly interface. Animal welfare is of the utmost importance. While working on the strain, the technician can record clinical signs and changes in the phenotype: this generates an automated notification to the veterinarian and project manager. Promptly, veterinarians can take a decision in case of severe health issues. ICMTM allows us to manage GA colonies more efficiently and effectively, reducing also the number of mice that the clients need to reach their research goal.

Osteoarthritis and Chronic Pain (PREMIO AISAL 2015)

Vacca Valentina[1,2,3,4*], Joao De Sousa Valente[1], Marinelli Sara[1,2,4], Pavone Flaminia[1,2], Malcangio Marzia[3]
[1]Wolfson Centre for Age-Related Diseases, King's College Londra; [2]Istituto di Biologia Cellulare e Neurobiologia, Roma; [3]IRCCS Fondazione Santa Lucia; 4YAP Young Against Pain group—SIMPAR (Study In Multidisciplinary Pain Research)
*Corresponding author: lale_84@libero.it

Osteoarthritis (OA) is a common chronic degenerative disease characterized by a worn and damaged joint structure with loss of articular cartilage components and subchondral bone. Although pain is a major symptom of OA that leads to considerable disability among older adults, its pathophysiology is largely unknown. Accumulating clinical evidence indicates that subchondral bone plays a role in generating joint pain. The cell bodies of primary sensory neurons innervating subchondral bone are located in the dorsal root ganglia (DRG) and are calcitonin gene-related peptide (CGRP) - and tyrosine receptor kinase A (TrkA)– immunoreactive; for this reason they are highly sensitive to nociceptive stimuli and inflammatory processes. The present study aimed to investigate the neurological involvement of GCRP in joint pain in greater detail using a mouse OA model, examining pain-related behavior and macroscopicalprogression. OA was developed in C57BL/6 mice by intra-articular injection of monoiodoacetate. Weight distribution, mechanical allodynia and immunohistochemistry analysis were assessed. OA mice showed a significant mechanical allodynia anda reduction in weight bearing. Gliosis andsprouting of CGRP positive fibreswas observed in L4 dorsal horns ipsilateral to the OA bone. In addition, the percentage of L4 DRG neurons positive for CGRP andTrkA were increased in OA mice compared to sham mice. These data indicate that the pathological sprouting of peripheral CGRP-positive sensory fibres in the OA bone is associated with higher CGRP content in DRG cell bodies. Furthermore, the peripheral CGRP increase is mirrored by high levels of CGRP centrally, which facilitate neuronal pain signalling, possibly through glia activation. The result of the present study indicates the possible association of CGRP in OA pain development. This animal model demonstrates a clear inter-relationship between cartilage damage and subchondral bone change and could be used to study the role of subchondral bone in the progression of OA.

Posters

Oxaliplatin Neurotoxicity Is Related to SLC4A1 Expression in BALB/C Mice Dorsal Root Ganglia

Ballarini E.[1], Pozzi E.[1], Riva B.[2], Genazzani A.[2], Cavaletti G.[1], Marmiroli P.[1], Carozzi V.[1,3]
[1]Università di Milano-Bicocca, Monza (MB); [2]Università del Piemonte Orientale, Novara; [3]Young Against Pain Group

Oxaliplatin induced peripheral neurotoxicity (OIPN) is one of the most dose limiting factor in the treatment of colorectal cancer but unfortunately still today OIPN pathogenesis has not been completely understood. The identification of molecular targets linked to Oxaliplatin (OHP) neurotoxicity could lead to new OIPN treatments development.There are evidence that support a possible role of solute carrier super-families 22 (i.e.SLC22A, OCTs) in the transport, accumulation and toxicity of OHP in dorsal root ganglia (DRG). In order to clarify the role of the SLCs in OHP neurotoxicity, we created an animal model of OIPN in BALB/c mice able to accurately reproduce the clinical features of the pathology. Mice showed neurophysiological impairments, decreased cold and mechanical sensory thresholds, decreased density of the small unmyelinated fibers in skin biopsies and neuropathological abnormalities in peripheral nervous system target sites. Secondly, by gene-arrays analysis, we tested the expression of all slc*s* in the DRG of treated and untreated animals (954 genes evaluated). Only slc4a1, a chloride and bicarbonate exchanger involved in chloride intracellular concentration, PH and cell volume regulation, was significantly overexpressed in the DRG of OHP-treated animals. The validation with RT-PCR demonstrated an 8-fold increase of slc4a1 in treated animals compared to the untreated. Immunohistochemical analysis evidenced that SLC4A1 was expressed in the cytoplasm of DRG sensory neurons with a perinuclear spotting distribution. This work demonstrated a key role of SLC4A1 in the development of OIPN pointing out new neuroprotective as well as analgesic strategies.

Supported by AIRC and Cariplo Foundation.

The Challenge in Establishing HEP in Laboratory Swine

Barone F.[1], Elmi A.[1], Ventrella D.[1], Bacci M.L.[1]
[1]Alma Mater Studiorum, Università di Bologna

In 1998 from the International Conference on Humane Endpoints (HEP) in animal experiments for biomedical research emerged that HEPs are part of a dynamic process, influenced by scientific developments as well as by animal welfare concerns as they evolve with time. Nowadays there are several publications regarding criteria, definition and evaluation of HEPs, but mainly related to the most common laboratory animals such as mouse and rats. In the last years the role of swine as laboratory animal is increasing due to its translational value and relatively lighter ethical implications when compared to the other large animal models. The need for a specific approach for each protocol, and the limited records available, are probably two of the main reasons behind the lack of dedicated HEP guidelines for the laboratory swine. Despite the above-mentioned issues, the Directive 2010/63/EU requires specific HEPs for each submitted protocol, thus the need for more in-depth knowledge regarding pain assessment. A humane endpoint can be considered as a possible refinement alternative for those experiments that involve pain and discomfort to the animals leading to higher quality research without compromising welfare itself. The task of veterinary medicine is to find new biomarkers and behavioral patterns in order to provide researchers with accurate tools that allow for a better understanding of objective swine welfare in each experimental condition. Our aim, as veterinary physiologists, is to establish reliable guidelines for an early detection of para-physiological and pathological stress and pain patterns in the laboratory swine. In this abstract we will present our experience collected within the last years regarding different experimental protocols with the swine as laboratory animal.

Variations of Behavioral Phenotype, Parenting Style, and Their Influences on Pups

Berretta E.[1,2], D. Laricchiuta[1,3], M. Pesoli[1,2], G. Pasqualini[1], L. Petrosini[1,2]
[1]IRCCS Fondazione Santa Lucia, Roma; [2]Dipartimento di Psicologia, Sapienza—Università di Roma; [3]Dipartimento di Psicologia Dinamica e Clinica, Sapienza—Università di Roma

The disposition to approach or avoid particular classes of salient stimuli constitutes the basis of motivated behavior of organisms. Such a critical feature of behavior seems to be biologically rooted and normally distributed, representing the primary reaction to rewarding or aversive stimuli. The purpose of this study is to investigate if a particular advancing or withdrawing phenotype in response to salient stimuli could affect parental relationship, parental care and finally offspring behavior. An animal model engaging C57BL/6J inbred mice is a particularly suitable tool to identify different approaching/avoidance (A/A) tendencies. By using behavioral test such as A/A Y-Maze it was possible to characterize male and female mice, classifying them into three main categories. Animals were defined Balancing (BA) when they reacted with balanced responses to a conflicting stimuli, Approaching (AP) or Avoiding (AV) when they reacted, respectively, with advancing or withdrawing responses to the stimulus. In order to assess the influence of parental phenotype on pups behavior, AV/BA/AP animals of both sexes were selected. AV/BA/AP males and females were tested in the Open Field Test with novel object (OF) and Elevated Plus Maze (EPM) and then mated, to assemble parenting couples of double (both AV or AP parents), single (one AV or AP parent and the other BA) and control (both BA parents) composition. The different couples obtained were kept in exclusive relationship throughout pregnancy, delivery and even up to the offspring weaning. To investigate the influence of phenotype on parenting style, an undisturbed parental care 30-min observation was performed at pup post-natal day (pnd) 3. Male and female offspring was likewise tested in the A/A Y-Maze (pnd 35-38), OF and EPM (pnd 55-60) for assessing offspring behavior. Results indicate that offspring varied according to maternal/paternal phenotype as well as to parental care, with significant differences between males and females.

The Interplay between IFNγ, Synaptic Alterations and Mood Disturbance in a Mouse Model of Multiple Sclerosis

S. Bullitta[1,2], A. Musella[1], A. Gentile[1,2], D. Fresegna[1,2], F. De Vito[1,2], H. Sepman[2], G. Mandolesi[1], D. Centonze[2,3]
[1]IRCCS Fondazione Santa Lucia-CERC, 00143 Roma; [2]Unità Operativa Sclerosi Multipla, Università degli Studi di Roma "Tor Vergata"; [3]IRCCS Istituto Neurologico Mediterraneo (INM) Neuromed, Pozzilli (IS)

Anxiety and depression are often diagnosed in patients with MS and its animal model, experimental autoimmune encephalomyelitis (EAE), independently of motor disability. A growing body of evidence suggests that mood disturbances reflect specific effects of proinflammatory cytokines on neuronal activity. Here we investigated whether interferon gamma (IFNγ), which is heavily involved in MS pathophysiology, could exert a role in the anxiety-like behavior observed in mice with EAE, and in the modulation of type-1 cannabinoid receptor (CB1R), which has already been shown to regulate mood in response to environmental stress. We performed behavioral and electrophysiological recordings in corticostriatal slices prepared from mice with EAE or subjected to IFNγ intracerebroventricular administration. EAE-induced anxiety-like behavior was associated with increased expression of IFNγ in the striatum. In parallel, we observed the downregulation of CB1R-mediated control of striatal GABAergic transmission even in the presymptomatic phase of EAE. Incubation of EAE slices with a IFNγ receptor antagonist rescued striatal CB1R sensitivity. Furthermore, bath application of IFNγ on control corticostriatal slices was able to completely block the reduction of inhibitory currents mediated by the CB1R agonist HU210, in a way reminiscent of the effect of EAE. In parallel, mice treated with intracerebroventricular IFNγ showed an anxiety-like phenotype. Overall these results indicate that IFNγ modulates CB1R function in the striatum, and that this effect plays a role in mood abnormalities in EAE.

Prelimbic α1-Adrenergic Receptors Modulate Extinction of Amphetamine-Induced Conditioned Place Preference

Chiacchierini G.[1], Latagliata E.C.[2], Sancandi M.[1], Campus P.[1], Puglisi-Allegra S.[1,2]
[1]Dipartimento di Psicologia Centro "Daniel Bovet", Sapienza—Università di Roma; [2]IRCCS Fondazione Santa Lucia, Roma

Norepinephrine (NE) in medial prefrontal cortex (mpFC) is critical for the acquisition of conditioned place preference (CPP) based on different addictive drugs or natural rewards (Ventura et al., 2003, 2007). Moreover, it has been demonstrated the involvement of prefrontal NE also in the modulation of the extinction of conditioned responses, in particular, through its action on β-adrenergic receptors. However, recent results have suggested a potential

contribution on the extinction of appetitive conditioned response also of α1-adrenergic receptors although their role in mpFC has not yet been fully clarified. Here, we assessed whether and how α1-adrenoceptor in the prelimbic (PL) cortex are involved in modulation of the persistence of drug-associated cue memories. We investigated the effects of the α1-adrenoreceptor antagonist Prazosin infusion in PL mpFC on extinction of acquired CPP to amphetamine and on expression of c-Fos levels in Nucleus Accumbens (NAc) core and shell of C57BL/6J mice in a no-confined extinction paradigm (subsequent daily testing sessions). In Prazosin treated mice the place preference is no more evident from the day 5 of extinction, while in the Vehicle group the preference persists for further 10 days. Moreover, Prazosin treated mice showed a lower c-Fos expression in both NAc core and shell region in comparison with Vehicle group. These results indicate that prefrontal NE contributes to delay extinction of drug associated memories, modulating NAc activity, through action on α1 receptors in PL cortex.

Animal Welfare and Health Can Be Achieved through Pharmacological Treatment, Safe Housing, and Management Procedures: A Case of Eradication of *Klebsiella* Spp from Mice Colonies in a Conventional Animal Facility

Paolo Coluccio[1], Annarita Wirz[2], M. Cristina Riviello[3]
Libero Professionista, IRCCS Fondazione Santa Lucia Foundation[2], CNR—Istituto di Biologia Cellulare e Neurobiologia[3,2]

Animal welfare includes also physical health. This entails a strict control over the potential diffusion of opportunistic pathogens that may cause health issues, especially when immunocompromised animals are used. Our study presents the results of the containment, in a conventional facility, of commensal pathogens as *Klebsiella*, a potential issue for immunocompromised animals, through pharmacological treatment with *Enrofloxacin* and appropriate working and sanitization procedures. From February 2012 to December 2015 we received, from different animal facilities, 254 mice of different strains positive to *Klebsiella* spp. All the animals were allocated in quarantine using ventilated cabinets in conventional cages with filter top and environmental enrichment. Of those 254 mice, 141 have been pharmacologically treated with Enrofloxacin (0,2mg/ml of drinking water for 2 weeks), while 113 have not been treated for experimental reasons. Check-up tests have been performed on biological samples from each cage at the end of the 7-days washout period after the pharmacological treatment. They consisted in cultural tests on pharyngeal swabs or on pools of fecal pellets. Once the tests resulted negative for *Klebsiella* spp., the mice went back to the normal routine of health check-ups, consisting of quarterly tests on sentinel animals. During the study period, routine tests never showed positive results for *Klebsiella*. This confirms that the enforced procedures—pharmacological, sanitization and working procedures—have been successful in containing bacteria. A good control can only be achieved through effective and complete communication about health status among animal facilities that share animals. The compiling of comprehensive reports perfectly fits into the "refinement" concept, understood not only as the improvement of procedures directly aimed at the animal but also as the management of everything that regulates the life of the animal and therefore its use.

Immunophenotypic Characterization of Ulcerative Dermatitis in HMGA1L6 and HMGA1L7 Transgenic Mice

De Biase D.[1], Esposito F.[2], Palma G.[3], Arra C.[3], Fusco A.[2], Paciello O.[1]
[1]Dipartimento di Medicina Veterinaria e Produzione Animale, Università degli Studi di Napoli "Federico II"; [2]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II"; [3]IRCCS Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G. Pascale", Napoli

Ulcerative dermatitis (UD) is an idiopathic, spontaneous, and progressive disease typically affecting aged mice on a C57BL/6 background. This condition is clinically characterized by intense pruritus and scratching leading to chronic ulceration with marked dermal fibrosis. The etiopathogenesis of UD is still largely unknown, so every attempt to treatment has been so far ineffective. Here, we report a severe ulcerative dermatitis observed in 20 out of 60 (33,3%) HMGA1L7 and HMGA1L6 transgenic mice. The average age of onset was 16 months with no predilection for sex or coat color. Lesions varied from coalescing crusts to irregularly shaped areas of ulceration extending mostly to the dorsal cervical region, dorsal and ventral thorax and in one case to hind limbs. Histological examination of formalin-fixed and paraffin embedded 4-mm sections of affected skin revealed extensive areas of ulceration with marked hyperplasia of the adjacent epidermis. A diffuse, severe and mixed inflammatory infiltrate consisting mostly of neutrophils, fibroblast, lymphocytes, macrophages, and mast cells was observed in the dermis, often reaching deep underlying structures. In one case, a mild, non-necrotizing, lymphocytic vasculitis was also found in a subcutaneous small vessel. Immunohistochemical analysis was performed to further characterize the lymphocytic infiltrate, showing a predominant CD3+ population with a fewer number of CD79+ cells. Moreover, a MHC class II immunoreaction of dermal and subcutaneous endothelial cells as well as macrophages, B and T cells was also observed. Taken together, these preliminary data suggest an immunomediated response and that dermal and subcutaneous endothelial cells can express MHC class II antigens and may contribute to the initiation of the immune response. A better explanation of the pathogenesis of this disease is necessary in order to improve the development of new and possibly useful treatment options for veterinarians and investigators.

Challenge for Life Scientists in Animal Care and Welfare

Di Cosmo A.[1], Chiandetti C.[2], Galligioni V.[3], Zarattini P.[2], Giulianini P.[2]
[1]Università degli Studi di Napoli "Federico II"; [2]Università degli Studi si Trieste; [3]FNUSA-ICRC, Brno, Repubblica Ceca

A great variety of crustaceans, and in particular decapods, is widely used in basic research ranging from studies on physiology, immunology, pathology, neuroethology to that on cognition. It is now well established that decapods possess elaborate multisensory perceptual abilities, have long- and short-term memory systems, exhibit complex behaviors and both basic and secondary emotions, such fear, pain and anxiety. Recently, cephalopods have been included in laboratory animal legislation (Directive 2010/63/EU) because these mollusks are considered "advanced invertebrates" for their cognitive capabilities, as learning and memory, and sophisticated behavior such as problem solving and play-like abilities. They can experience pain, suffering, distress, and lasting harm. We argue that decapods' housing and maintenance should be considered under the FELASA recommendations. Indeed, some countries, for instance Norway and New Zealand, have already included them in animal welfare regulation. Here we present a first attempt to develop guidelines for the care and welfare of the species currently used in laboratory. We summarize the main methods of capture,

transport, husbandry, and euthanasia, focusing on the environment (water, light, temperature and noise) and its control, the health/illness assessment and the housing procedures (stocking, feeding, handling, marking) with particular regard to the species *Procambarus clarkii*. Due to its high market value and consumer demand *P. clarkii* is one of the most commonly farmed crayfish and has been recently recognized as an invasive alien species by the UE regulation 2016/1141 and its management has to be improved. Waiting for an EU legal protection including harm-benefit assessment and adherence to the 3Rs principles (Replacement, Refinement and Reduction), it would be advisable to share knowledge and data on decapod crustaceans among researchers, facility managers and animal care staff with the aim to improve both the animal care and the experimental procedures.

Actions of the Antihistaminergic Clemastine on Presymptomatic SOD1-G93A Mice Ameliorate ALS Disease Progression

Fabbrizio P.[1,2], Apolloni S.[1,3], Amadio S.[1,3], Volontè C.[1,3]
[1]IRCCS Fondazione Santa Lucia; [2]Università degli Studi di Roma "Tor Vergata"; [3]CNR - Istituto di Biologia Cellulare e Neurobiologia, Roma

Amyotrophic lateral sclerosis (ALS) is a disease with a strong neuroinflammatory component sustained by activated microglia contributing to motor neuron death. However, how to successfully balance neuroprotective versus neurotoxic actions by the use of antinflammatory agents is still under scrutiny. We have recently shown that the antihistamine clemastine, an FDA-approved drug, can influence the M1/M2 switch occurring in SOD1-G93A ALS microglia. Here we have chronically treated female SOD1-G93A mice with clemastine, evaluated disease progression and performed mice lumbar spinal cord analysis atsymptomatic and end stage of the disease. Moreover, we have studied the mechanism of action of clemastine in primary adult spinal SOD1-G93A microglia cultures and inNSC-G93A motor neuron-like cells. We found that a short treatment with clemastine (50 mg/kg) from asymptomatic (postnatal day 40) to symptomatic phase (postnatal day 120) significantly delayed disease onset and extended the survival of SOD1-G93A mice by about 10%. Under these conditions, clemastine induced protection of motor neurons, modulation of inflammatory parameters, reduction of SOD1 protein levels, and SQSTM1/p62 autophagic marker, when analyzed immediately at the end of the treatment (postnatal day 120). A long treatment with clemastine (from asymptomatic until end stage) instead failed to ameliorate ALS disease progression. At end stage of the disease, we found that clemastine short treatment decreased microgliosis and SOD1 protein, and increased LC3-II autophagic marker, while the long treatment produced opposite effects. Finally, in spinal microglia cultures from symptomatic SOD1-G93A mice clemastine activated inflammatory parameters, stimulated autophagic flux via the mTOR-signalling pathway and decreased SOD1 levels. Modulation of autophagy was also demonstrated in NSC34 SOD1-G93A motor neuronlike cells.

By gaining insights into the ameliorating actions of an antihistamine rgiccompound in ALS disease, our findings might represent an exploitable therapeutic approach for familial forms of ALS.

Mice Over-Expressing Placenta Growth Factor Exhibit Increased Vascularization and Vessel Permeability Independently of Keratinocyte-Derived VEGF-A

Failla C.M.[1], De Luca N.[2], Zaccaria M.L.[2], Avitabile S.[1], Tatangelo L.[3], Strinati G.[2], Rossiter H.[4], Tschachler E.[4], Odorisio T.[2]
[1]Laboratorio di Immunologia Sperimentale, IRCCS Istituto Dermopatico dell'Immacolata, Roma; [2]Laboratorio di Biologia Molecolare e Cellulare, IRCCS Istituto Dermopatico dell'Immacolata, Roma; [3]Centro Servizio Topi Transgenici, IRCCS Istituto Nazionale Tumori Regina Elena, Roma; [4]Dipartimento di Dermatologia, Università di Vienna

Placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, manifests strong pro-angiogenic properties. In the skin, where keratinocytes represent the major source of both VEGF-A and PlGF, these two factors are contemporaneously induced in physiological and pathological events associated with active angiogenesis. While several lines of evidence indicate that PlGF acts by enhancing VEGF-A-driven angiogenesis, it has not been clearly demonstrated that PlGF would exert a biological effect independently from synergism with VEGF-A. To address this issue in the skin context, mice over-expressing PlGF in basal keratinocytes and lacking keratinocyte-derived VEGF-A were generated. PlGF Tg/VEGF-A- mice show a reddish skin color, indicative of increased skin vascularization, as compared to wild-type littermates, similarly to what observed in PlGF Tg mice. Large vessels were visible in the ear skin of both PlGF Tg and PlGF Tg/VEGF-A- mice, but not in wild-type and VEGF-A- littermates. Computer-assisted morphometric analysis of ear preparations confirmed significant increase in vessel size and reduction distance between vessel branches in both PlGF Tg/VEGF-A- and PlGF Tg mice compared with wild-type and VEGF-A- littermates. To analyze vessel permeability, PlGF Tg/VEGF-A- and control mice were injected with Evans blue dye into the tail vein and monitored for dye extravasation. Spectrophotometric quantitation of the dye confirmed an augmented leakage in PlGF Tg mice compared with wild-type and VEGF-A- animals. Interestingly, extravasated dye concentration is comparable in PlGF Tg and PlGF Tg/VEGF-A- mice. This data suggests that PlGF over-expression in keratinocytes is sufficient *per se* in inducing skin vessel permeability.

In conclusion, our analysis of the PlGF Tg/VEGF-A- mouse model confirms the important role played by PlGF in promoting skin angiogenesis and indicates that PlGF is able to physiologically exert such a pro-angiogenic effect independently of keratinocyte-derived VEGF-A.

Adversity in Childhood and Psychiatric Disorders: From Mice to Humans

Lo Iacono L.[1], Valzania A.[1], Viscomi M.T.[2], Chiurchiu V.[2], Catale C.[3], Bussone S.[1], Aricò E.[4], Casella P.[5], Troisi A.[6], Puglisi-Allegra S.[1], Carola V.[2]
[1]Dipartimento di Psicologia, Sapienza – Università di Roma; [2]IRCSS Fondazione Santa Lucia, Roma; [3]Dipartimento di Biologia e Biotecnologie, Sapienza – Università di Roma; [4]Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Istituto Superiore di Sanità, Roma; [5]U.O.C. Dipendenze, ASL RM1, Roma; [6]Departimento di Medicina dei Sistemi, Università degli Studi di Roma "Tor Vergata"

Child maltreatment frequently confers risk for multiple psychiatric diagnoses. The molecular and neurobiological substrates engaged during early traumatic events and mediating the risk for psychiatric diseases are poorly understood. Our research aims to: 1. Model the exposure to early life stress (ELS) in preclinical models and evaluate the behavioral effects in adulthood; 2. Identify the long-term molecular and neurobiological consequences of ELS in

mouse brain and blood; and 3. Translate our findings to clinical research. In one recent study we investigated the link between ELS and depression. Adult depression-like behavior was induced in mice by juvenile isolation stress, and was associated with a reduced level of Sirtuin1 deacetylase in both brain and blood. In humans, the blood levels of Sirtuin1 correlated significantly with the severity of symptoms in major depression patients, especially in those who received poor parental care during childhood. In a second study, we modeled childhood maltreatment by exposing juvenile mice to a threatening social experience (SS). This experience increased the levels of cocaine-seeking behavior in adulthood. The analysis of blood lymphocytes transcriptome, revealed greater blood coagulation and inflammation associated with this behavioral phenotype. Furthermore, treatment with an anticoagulant/anti-inflammatory agent during cocaine withdrawal abolished the susceptibility to relapse in SS mice. Similar to our preclinical model, in cocaine-abstinent individuals we found increased level of inflammation, with a higher extent in patients who experienced childhood maltreatment. Overall these findings demonstrated the possibility of successfully translating results from preclinical models to clinical research in the context of the study of the psychopathologies.

Pharmacokinetics of IVIg in Wistar Rats

Monza L.[1], Fumagalli G.[2], Alberti P.[2,3], Oggioni N.[2], Dondio M.[4], Spinoni N.[5], Galliani C.[5], Brivio R.[5], Marjanovic I.[6], Scali C.[6], Meregalli C.[2]
[1]Medicina Traslazionale e Molecolare - DIMET, Università di Milano-Bicocca, Monza (MB); [2]Unità di Neurologia Sperimentale, Università di Milano-Bicocca, Monza (MB); [3]PhD in Neuroscience, Università di Milano-Bicocca, Monza (MB); [4]Aphad S.r.l., Buccinasco (MI); [5]Laboratorio di Analisi, A. O. S Gerardo, Monza (MB); [6]Kedrion S.p.A., Castelvecchio Pascoli (Barga, LU)

Intravenous Immunoglobulin (IVIg) are therapeutic polyspecific human IgG derived from plasma pools of thousands of healthy donors. Although there are studies in literature evaluating their effectiveness in animal models, no data about their pharmacokinetics in rodents are available. Here, the pharmacokinetics parameters of human IVIg (Ig VENA 50 g/l solution for infusion; Kedrion SpA) were determined in 10 female Wistar rats randomized in two groups: group A (n=5) was treated with IVIg on 2 consecutive days, while group B (n=5) received 1 daily dose every 2 weeks. Both groups were treated with IVIg 1 gr/Kg/day with an infusion time of 10 minutes. For the determination of IVIg concentrations, serum samples were collected at days 1, 2, 3, 7, 14, 21 and 28 and analyzed through automatic ECLIA method (Elecsys systems Immunoassay; Roche diagnostics, Mannheim, Germany). IVIg serum concentration-time data were further evaluated by means of non-compartmental analysis using the PK software AnalystTM 6.1 (Applied BioSystems). The pharmacokinetic parameters Cmax, Tmax, MRT 0-inf and AUC (0-t) (area under the concentrations-time curve from the start of infusion/injection until time post-dose) were calculated. Our results demonstrated that the animals generally well tolerate the IVIg administration. No remarkable difference was observed between the two infusion schedules: the Cmax and AUC values were similar between the groups of treatment. While the t1/2 was duplicated ($p<0.001$) in group A compared to group B. This results demonstrated that the concentrations of IVIg can be detected and monitored in the serum of rats in a chronic setting.

This work was supported by Kedrion SpA.

Administration of a Lentiviral Vector Expressing Decorin Improves Survival and Clinical Manifestations of a Mouse Model of Recessive Dystrophic Epidermolysis Bullosa

Cianfarani F.[1], Castiglia D.[1], Mittapalli V.R.[2], Bruckner-Tuderman L.[2], Zambruno G.[3], Odorisio T.[1]
[1]Laboratorio di Biologia Molecolare e Cellulare, IRCCS Istituto Dermopatico dell'Immacolata, Roma; [2]Departimento di dermatologia, University Medical Center, Freiburg, Germany; [3]Unità di Dermatologia, IRCCS Ospedale Pediatrico Bambino Gesù, Roma

Recessive dystrophic epidermolysis bullosa (RDEB) is an invalidating skin blistering disease due to mutations in the gene COL7A1 coding for type VII collagen (C7). Hallmarks of RDEB are trauma induced blisters associated with inflammation and fibrosis, and, in the more severe cases, syndactyly, mitten deformities and aggressive skin cancer development. Identical COL7A1 mutations can result in significant clinical variability. By studying a phenotypically discordant RDEB human monozygotic twin pair, we found that activation of the TGF-β signaling can be crucial in determining disease clinical outcome. We identified the proteoglycan decorin (DCN), a biological inhibitor of the TGF-β pathway, as an agent able to counteract the profibrotic and proinflammatory phenotype of fibroblasts from RDEB patients. Here, we evaluated the therapeutic potential of human DCN in a mouse model of severe RDEB (C7-hypomorphic mice). Levels of endogenous decorin were found to be reduced by 50% in the skin of untreated Col7 hypomorphic mice as compared to wild type littermates. The reduced expression of decorin confirms data obtained in RDEB patients and represents the rationale for evaluating the therapeutic potential of this molecule in lessening fibrosis and inflammation in the mouse model. Decorin was delivered in the skin by intraperitoneal administration of a recombinant lentivirus (LV-DCN). Hypomorphic mice were divided into 3 groups: 1) mice treated with LV-DCN; 2) mice treated with a control lentivirus (LV-CTRL); 3) mice treated with saline solution. Moreover, mice were classified into four subgroups, according to disease severity, and distributed homogeneously among the three experimental groups. Results indicate an increased percentage of LV-DCN treated mice (n=27) surviving up to 12 weeks of age (38.7%; survival average time: 31 days), as compared with LV-CTRL-treated mice (n=31) (21.1%; survival average time, 11 days; p value 0.04 vs LV-DCN-treated mice) or saline (n=25) (34.6%; survival average time, 13 days). Moreover, LV-DCN administration significantly increases digit length and reduces digit loss of C7-hypomorphic mice. Our data support a therapeutic role for decorin in mitigating disease severity in RDEB.

The Antiepileptic Drug Lamotrigine, Through Foxo3a, Inhibits Breast Cancer Growth and Development both In Vitro and In Vivo

Pellegrino M.[1], Rizza P.[1], Giordano F.[1], Spina E.[1], Ceraldi R.[*], Andò S.[1,2], Morelli C.[1], Sisci D.[1]
[1]Dipartimento di Farmacia, Università della Calabria, Arcavacata di Rende (CS); [2]Centro Sanitario, Università della Calabria, Arcavacata di Rende (CS); [*]Dipartimento di DiBEST, Università della Calabria, Arcavacata di Rende (CS)

Several antiepileptic drugs (AEDs) show anti-cancer activity as an off-label effect in different cancer cell types, including breast. Here we investigate the influence of lamotrigine (LTG), an anticonvulsant drug, on the growth and progression of human breast cancer models. The effects of LTG on cell viability and on anchor-

age-dependent and -independent cell growth were evaluated in both breast cancer (BC) and normal breast epithelial cell lines. The expression of cell cycle and survival genes was examined by Western blot and RT-PCR. The effects of LTG on the development of breast carcinomas were also evaluated in mice xenografts. LTG significantly decreased BC cells viability, leading cancer cells to apoptosis by modifying the expression of several proteins related to apoptosis, cell cycle and survival (p-Akt, AKT, Bax, p27Kip1, p21Waf1/cip1, cyclin D1, cyclin E), including FoxO3a. LTG inhibited anchorage-dependent and independent cell growth in BC cells. In in vivo xenografts model LTGconfirmed antineoplastic action. LTG, through the activation of FoxO3a, induces apoptosis of BC cells and inhibits tumor growth in vivo. These effects are independent of ERá/PR status. Therefore, LTG represents a promising candidate as an adjuvant antitumor therapeutic agent, for BC.

A New Drug Blocker Topoisomerase Enzyme, Generates a Potent Inhibitor of Proliferation and Migration of Cancer Cells in Experiment in Vitro and in Vivo

Rea D.[1] and G. Palma G.[1]; Mariconda A.[2], Luciano A.[1], Falco M.[1], Caporale A.[3], Barbieri A.[1], Del Vecchio V.[1], De Biase D.[1], Sinicropi M.S.[4], Longo P.[3], Saturnino C.[3], Arra C.[1]
[1]AnimalFacility, Istituto Nazionale Tumori IRCCS – Fondazione "G. Pascale", Napoli; [2]Dipartmento di Chimica e Biologia, Università di Salerno; [3]Dipartimento di Farmacia, Università di Salerno; [4]Dipartmento di Farmacia, Università della Calabria, Arcavacata di Rende (CS)

Recently, any authors reported that the topoisomerase enzymes such as TOPO II is more expressed in aggressive subset of tumors (for example breast tumor that overexpresses HER-2/neu). This enzyme is essential for DNA metabolism, as it reduces the number of supercoils in DNA by transient breaks in the double stranded DNA polymer, generating DNA topological isomers; for this reason it play a key role in the cellular processes of transcription and replication. TOPO II are largely confined in eukaryotic cells in the proliferation therefore are targets of choice for anti-cancer drugs. Several TOPOII inhibitors/drugs have been approved by Food and Drug Administration, (doxorubicin, daunorubicin, epirubicin and idarubicin), but their therapeutic efficacy is associated with high toxicity. Based on these evidences recently our group has synthesized a new molecule (SC4) which inhibits the TOPOII, reducing the proliferation and acts negatively on the cellular migration; also has SC4 shown in vivo a reduction of toxic effects. We have tested SC4 on two cancer cell lines, that representing the principal "Big Killer" of the oncology world: pancreatic cancer human cell (MIA-PACA2) and breast cancer triple negative human cell (MDA.MB231). In vitro cytotoxic assays they have demonstrated the ability of SC4 to restrict cell proliferation. The MTT assay showing the 45% inhibition of proliferation on MDA.MB231, and 40% inhibition on MIA-PACA2 versus the control, at concentration of 20µM. The FACs assay results have confirmed this data, showing a dose-dependent cytotoxic activity, similar to doxorubicin. The Colony Assay results support the inhibition of proliferation, observed a decreased number of colonies of five-fold in SC4, 20 µM treated samples versus control in both cellular lines. To test the ability to inhibit the cell migration was carried out Wound Healing assay, the results showed that SC4 cause non-closure of the wound made on monolayers of both cancer cell lines, after 48h of treatment. Moreover we performed two experimental in vivo for evaluated the SC4 inhibitory activity on proliferation and migration, and to assess the degree of safety. Orthotopic cancer xenograft mouse models they are obtained by injection of both cell lines used for the in vitro experiments, in a number of 2X106 cells/100µl PBS on the right flank of the mice. SC4 it was administered by intraperitoneally injection at 1mg/kg for 12 days. SC4 reduces the rate of tumor growth of 50 % with a p<0,001 SC4 vs CTR. Twice a week controls of body weight show that there is no weight loss compared to mice treated with doxorubicin, and the echocardiography, performed on mice at three time, does not show cardio-toxic effects than the group treated with doxorubicin. In vivo models of secondary localization (Llung Colonization), performed by injected via tail vein (5x105) B16F10 cells, showed the ability of SC4 to reduced number of metastasis of 70% respect to control non treatment p<0,001. SC4 inhibits proliferation and migration of human tumor cell lines by bloking the activity of TOPO II, and may be considered as a potent inhibitor of tumor growth and invasion in mouse model; for these reasons could be considered a valid prototype for the development of new antineoplastic therapies.

Animal Welfare and 3Rs: E-Learning Course on Laboratory Animal Science

Riviello M.C.[1,] Casale F.[2], Macchioni S.[3], Pagani S.[2], Uliana F.[2], Wirz A.[4], Fusco S.[3]
[1]Istituto di Biologia Cellulare e Neurobiologia - CNR, Roma; [2]SPR Reti e Sistemi Informativi - CNR, Roma; [3]Ufficio Formazione della Direzione Centrale Gestione delle Risorse Umane – CNR, Roma; 4IRCCS Fondazione Santa Lucia, Roma

The European Directive (2010/63/UE) and the new Italian law (DL 26/2014) highlight the importance of the relation between animal welfare and the education and training of those involved in animal research. We strongly believe in this relation and, since 2007—long before those regulations were in place—we have been organizing training courses in Laboratory Animal Science, followed in 2010 by training courses in Statistics, with the objective of improving the knowledge of Laboratory Animal Science and the skills of all those involved with the animal model. Lately, CNR and Santa Lucia Foundation are cooperating to expand their training programme with new E-learning courses. Such an innovative course typology has several advantages. On one hand it increases the flexibility and effectiveness of education, as it offers the possibility to monitor learning progress and to reach a wider audience. On the other hand, it offers tangible advantages in terms of management, including a decrease in costs, the possibility to reuse and expand the course content over time and the direct accessibility of online learning material. Appropriate competence of the personnel involved in the care and use of laboratory animals is required in order to guarantee both research quality and animal welfare, in compliance with the 3Rs principles. For this reason, it is necessary to encourage scientific institutions to offer appropriate training. In this respect, we believe that E-learning courses can be a good strategy to ensure continuing professional development in the field of Laboratory Animal Science.

In Vitro Test as Alternatives Methods to Animal Use in the Efficacy Control of BTV8 Inactivated Vaccines

Rossi E.[1], Ronchi G.F.[1], Luciani M.[1], Di Febo T.[1], Di Pancrazio C.[1], Sacchini F.[2], Ulisse S.[1], Salini R.[1], Teodori L.[1], Podaliri Vulpiani M.[1], Tittarelli M.[1], Di Ventura M.[1]
[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo; [2]International Livestock Research Institute (ILRI)

Animal tests play a crucial role in routine quality control of veterinary vaccines. It includes in vivo potency tests, such as challenge for batches released of inactivated vaccines. The application of the 3Rs concept (replacement, reduction and refinement) is nowadays demanded, but regulatory and technical issues mainly related to in vivo tests still limit its implementation. The consistency approach implies the use of a set of parameters to constitute a product profile, which is monitored throughout production to guarantee that each lot released is similar to a manufacturer-specific vaccine of proven clinical efficacy and safety. It is based on direct methods such as serology instead of challenge, antigen quantification tests and systematic application of Good Manufacturing Practice (GMP). Two inactivated Bluetongue virus serotype 8 (BTV8) vaccines (12X-concentrated and diluted) were produced, submitted to quality controls and tested on sheep in comparison with the Merial ®BTVPUR AlSapTM 8 inactivated vaccine. Body temperature, antibody titers and viremia of vaccinated sheep and controls were determined. In order to extend the number of serological parameters to check, also interleukin-4 (IL4) and interferon gamma (IFNγ) in sera and in vitro (stimulation of blood cells) were quantified. Results obtained indicates that the in lab evaluation of cell-mediated (IFNγ quantification) and humoral (serum neutralization) immune response could be useful to predict the efficacy of BTV inactivated vaccines, allowing to avoid the challenge phase. An ELISA capture was setup to quantify VP2-BTV8 protein in the inactivated vaccines by using a BTV8-VP2 specific MAb and purified BTV8 specific sheep IgG. Our data show that the quantification of VP2-BTV8 protein by capture ELISA is possible and could be used for the quality control of different batches of BTV8 inactivated vaccines, produced with the same method of production and the same adjuvants.

OTC2 Transporter Involvement in Development of Cisplatin Toxicity

Semperboni S.[1,2], Chiorazzi A.[2], Ceresa C.[2], Canta A.[2], Nicolini G.[2], Cavaletti G.[2], Marmiroli P.[2]
[1]PhD in Neuroscienze, Università di Milano-Bicocca, Monza (MB); [2]Unità di Neurologia Sperimentale, Università di Milano-Bicocca, Monza (MB)

Cisplatin (CDDP) is a chemotherapeutic drug widely employed in the treatment of several solid tumors, but its clinical use is limited by severe side effects. CDDP can enter cells through membrane transporters among which Organic Cation Transporter (OCT) 2. Furthermore OCT2 specific organ distribution may explain CDDP selective toxicity. We used OCT2 (OCT2-/-) knockout mice (KO) to investigate the possible role of the transporter in CDDP peripheral neurotoxicity onset. CDDP was intraperitoneally administered 4 mg/kg 2qvx4w in C57BL6 wild type (WT) and KO mice. At the end of treatment caudal and digital Nerve Conduction Velocity (NCV) was measured and dynamic and plantar test were performed to evaluate peripheral neurotoxicity. Dorsal root ganglia (DRG) were used for morphological and morphometrical examination, kidney for hystopathological analysis while hematological and hematochemical studies were performed on blood samples. Our results showed the insurgence of allodynia only in WT mice treated with CDDP; moreover only in this group digital NCV is slightly reduced by CDDP treatment. We also investigated *in vitro* the cell survival of DRG neurons primary culture obtained from WT and OCT2-/- mice, untreated and treated with CDDP, in order to evaluate a possible protection due to OCT2 KO. *In vitro* results do not demonstrate any difference in CDDP response between WT and KO derived cells. In future we will study the CDDP effect in WT and OCT2-/- KO derived satellite cells that, tightly enveloping sensitive neurons in DRG, could contribute to the response to the drug.

Different Types of Animal Models to Study Hepatocellular Carcinoma and Potential Therapeutic Agents

Tonon F.[1], Kamensek U.[2], Cemazar M.[2,3], Trotta F.[4], Ferrari C.[5], Dapas B.[6], Farra R.[7], Grassi G.[6], Zennaro C.[1]
[1]Università degli Studi di Trieste; [2]Istituto di Oncologia, Ljubljana, Slovenia; [3]Università di Primorska, Polje, Slovenia; [4]UO Chirurgia Generale, Azienda Ospedaliera, Lodi; Universitàdegli Studi di Pavia; [6]Dipartimento Scienze della Vita, Università degli Studi di Trieste; [7]Dipartimento di Ingegneria e Architettura, Università degli Studi di Trieste

For Hepatocellular carcinoma (HCC), the third leading cause of cancer-related death worldwide, the available therapeutic treatments are poorly effective, especially for its advanced forms. Being HCC a type of cancer strictly correlated with the aberrant genes promoter methylation, we explored the effects of de-methylating agents, in HCC in vitro and in vivo models. In different HCC cell lines, de-methylating agents showed remarkable anti-migratory and anti-proliferative effects, acting on different signaling pathways. To confirm the powerful effects observed in vitro, we used different types of animal models, i.e. Zebrafish, mouse and rat. The generation of a xenograft model of HCC in Zebrafish is based on the injection in the larvae yolk of a small amount of human HCC cells, which engraft in very few days. This xenograft model is particularly suitable for studying the angiogenetic processes associated with tumor mass growth and micro-metastasis formation, considering that the larval stage of Zebrafish is nearly transparent and its living tissue can be visualized by using a microscope. The Zebrafish xenograft model of HCC allows evaluating easily the effect of de-methylating agents on tumor mass growth and metastasis formation. We also used a SCID subcutaneous xenograft mouse model of HCC to evaluate the effects of de-methylating agents in term of animal survival and tumor mass growth. Moreover, this type of xenograft model allows us to confirm in vivo the de-regulation of the pathways involved in tumor cell proliferation and migration observed in vitro. Finally, we consider a syngenic and orthotopic rat model of HCC, injecting rat derived HCC cells directly into the rat liver. Among the animal models proposed, the syngenic rat model of HCC represents the closest to the human liver cancer. By using these HCC animal models, we could deepen liver tumor cell behavior and de-methylating agent effectiveness in the treatment of HCC.

Behavioral Sex-Related Differences in Neuropathic Mice and Metabolic Perturbations: A Pioneering Strategy for Chronic Pain Management

Vacca V.[1,2,3], De Angelis F.[1,3], Pavone F.[1,2], Marinelli S.[1,2,3]
[1]CNR—Istituto di Biologia Cellulare e Neurobiologia, Roma; [2]IRCCS Fondazione Santa Lucia, Roma; [3]YAP Young Against Pain group—SIMPAR (Study In Multidisciplinary Pain Research);

Epidemiological studies have shown a sex-linked difference in the incidence of various pain diseases; in particular, a higher prevalence of neuropathic pain (NeuP) in women than in men was observed. From an experimental point of view, data confirm that sex can interfere with transmission and control of pain; however, the mechanisms investigating the relation between them are still poorly investigated. Another emerging issue concerns the sex-re-

lated differences in dysmetabolism and its correlation with neuropathic pain. Dysmetabolism is a chronic condition representing a pandemic syndrome in the developed and western countries. The goal of the present study was to investigate the metabolic alteration that occurs after nerve injury and the possiblesex-associated differences. Chronic Costrinction Injury (CCI) of the sciatic nerve was used as neuropathic pain model, in female and male adult CD1 mice. Temporal trend over a long time interval (121 days) of pain-related responses and functional recovery was analyzed. Seven days after CCI, changes in: metabolic parameters (body weight, food intake, triglycerides, cholesterol, glycemia and leptin), immunohistochemical markers in sciatic nerves, and circulating cytokines/chemokines, were evaluated. Data demonstrated important sex-related differences in metabolic alterations induced by NeuP. These differences are correlated to a faster regenerative process in males compared to females as shown by the evaluation of proteins' expression associated with nerve injury, repair and metabolism. NeP produces debilitating consequences in patients and has a large socioeconomic impact. Dietary interventions are attractive option due to low cost and low toxicity. Thus, a deeper comprehension of the involvement of metabolic factors and the demonstration that changes in energy balance can represent a pioneering strategy for chronic pain management with important therapeutic implications. Moreover, this study provides novel pharmacological targets and early biomarkers.

Funded by Italian Ministry of Health Ricerca Finalizzata Giovani Ricercatori GR-2011-02346912

Phenotype Study in a Mouse Model of Progeria (Transgenic G609g Lmna) to Evaluate Drugs Able to Reduce Progerin

Squarzoni S.[1], Barboni C.[3], Sanapo M.[3], Loi M.[2], Rambaldi J.[3], D'Apice M.R.[4], Mecca R.[5]; López-Otín C.[6], Barone F.[3], Zaghini A.[3]
[1]CNR - IGM, Bologna; [2]SC Lab. Biologia Cellulare Muscoloscheletrica, IOR, Bologna; [3]Dipartimento di Scienze Mediche Veterinarie, Università di Bologna; [4]Fondazione Policlinico Tor Vergata - U.O.C. Laboratorio di Genetica, Roma; [5]Lab. di Tecnologia Medica, IOR, Bologna; [6]Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Oncología-IUOPA, Universidad de Oviedo, Spain

The purpose of this research is to study the phenotype of transgenic mice lmna G609G that reproduce the human progeria, i.e. the Hutchinson Gilford Progeria Syndrome (HGPS) and to assess the optimal breeding conditions for these animals. Based on these observations the research will continue with the evaluation of drugs able to reduce or eliminate pathological features. HGPS is a rare autosomal dominant condition caused by is a de novo mutation in chromosome 1q21 exon 11 leading to creation of an abnormal splice donor site that results in expression of a truncated, permanently farnesylated prelamin A (precursor of lamin A/C, a component of nuclear lamina inside the nuclear membrane), called progerin. The disease develops in the first year of life with severe premature senescence involving almost all tissues. Patients suffer from delayed growth, short stature, alopecia, skin thinning, loss of subcutaneous fat, midface hypoplasia, osteolysis, atherosclerosis, cardiac failure, stroke. Life expectancy is about 13.5 years. Thus all effects on reproduction and related problems are completely unknown in humans, but they are of utmost importance in experimental animal breeding, particularly regarding the mouse model of progeria.

Our study represents the first-ever description of G609G-C57BL mice breeding over long periods, with a special attention to reproduction, weaning, growth, sensitivity to common pathologies and specific symptom expression. The observation has been particularly focused on heterozygous animals which reflect the human genotype.

The Renal Phenotype of Allopurinol-Treated Lesch Nyhan Mouse Model

Tonon F.[1], Rastaldi M.P.[2], Liuzzi A.[3], Marchetti M.L.[3], Ronda L. [4], Corbelli A.[5], Zarattini P.[6], Clai M.[1], Carraro M.[1], Bettati S.[4], Percudani R.[3], Zennaro C.[1]
[1]Dipartimento di Scienze Mediche Chirurgiche e della Salute, Università di Trieste; [2]IRCCS Fondazione Ospedale Maggiore Policlinico, Milano; [3]Dipartimento di Bioscienze, Università di Parma; [4]Dipartimento di Neuroscienze, Università di Parma; [5]Unità di Bioimaging, IRCCS Istituto di Ricerche Farmacologiche "Mario Negri" Milano; [6]Dipartimento di Scienze della Vita, Università di Trieste

Lesch-Nyhan disease (LND) is a rare X-linked disease caused by an inborn error of purine metabolism. It is caused by severe deficiency of hypoxantine-guanine phosphoribosyl-tranferase (Hprt), a key enzyme for recycling of purine bases, resulting in overproduction of uric acid, which accumulates in plasma (hyperuricemia) and urine (hyperuricosuria). Because of its low solubility, uric acid tends to precipitate in the joints and kidney, causing juvenile gout and renal failure. Severe Hprt-deficiency has other consequences that have a less clear connection with the protein enzymatic activity; patients are affected by motor disabilities, cognitive impairment, and by self-injurious behavior. Hyperuricemia in LND patients is treated with allopurinol, which inhibits xanthine oxidase, though the treatment does not cure neurological symptoms and paradoxically can cause acute renal failure due to xanthine deposits. The best established Lesch Nyhan mouse model is the Hprt KO mouse, which however fails to reproduce the human disease phenotype. In this model, therefore, we wanted to reproduce the pharmacological condition of patients by early allopurinol administration. Our results show that in absence of Hprt, the blockade of xanthine oxidase by allopurinol causes rapidly developing renal failure due to xanthine accumulation and deposition within the kidney while the drug is well-tolerated by wild type animals and also by the heterozygous Hprt+/- mice. The kidneys show macroscopically a yellowish appearance and are characterized by a diffuse interstitial nephritis; mechanistically, this seems to be due to the activation of adipogenesis by xanthine and consequent epithelial-mesenchymal transition of tubular cells. Interestingly, administration of the drug at much lower doses preserves the kidneys but causes motor coordination defects at the rotarod test, suggesting a potential role of allopurinol in worsening the neuronal phenotype of Lesch Nyhan patients.