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Abstracts of Scientific Papers

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Posters

A New Severe Immunocompromised Hemophilia A Mouse Model and Its Application in Cell Therapy

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Hemophilia A (HA) is an X-linked bleeding disorder occurring in 1:5000 males caused by a lack or reduced activity of coagulation Factor VIII (FVIII). Based on residual activity there are 3 forms of disease: severe (<1%), mild (1-5%) and moderate (up to 40%). The current therapy is the administration of blood derived or recombinant FVIII. However, this therapy is not definitive and 20-40% of severe patients develop neutralizing antibodies impairing efficacy of replacement therapy. Being a monogenic disease it is a good candidate for gene and cell therapy approach since a stable FVIII activity >1% is sufficient to improve patient's quality of life. To study the correction of the bleeding phenotype by cell therapy or ex-vivo gene therapy using human cells in mouse we generated a new severe immunocompromised and hemophilic strain by crossing NOD-SCID γ-null, the most used model for xenotransplantation, with NOD-SCID-HA mice obtaining a double knockout mouse model combining the hemophilic phenotype with the severe immunodeficency (NSG-HA). These mice showed both features from parental strain as demonstrated by bleeding assay and immunophenotype. Since we had demonstrated that total bone marrow transplantation from healthy donor mice to hemophilic recipients corrected the hemophilic phenotype, we investigated whether human hematopoietic stem cells (hHSC) transplantation and engraftment in NSG-HA mice can increase FVIII levels, shortening the bleeding time of transplanted mice. Three months after CD34+ cells xenotransplantation, human chimerism was assessed up to 60% in blood, spleen and bone marrow of injected NSG-HA mice. Moreover, most of them showed FVIII levels higher than 3%, sufficient to improve the bleeding time, since 75% of mice survived to tail clip assay. All these results showed that NSG-HA is a suitable mouse model to achieve long-term engraftment and to study new therapeutic approaches for HA based on human cells transplantation.

Support Care to Minimize Animal Suffering in Mice Models of Neuroinflammation

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Experimental autoimmune encephalomyelitis (EAE) is the most used model for studying Multiple Sclerosis (MS) in laboratory animals, primarily miming the autoimmune component of MS. The EAE, with all its limitations, is still the only tool available for the development and testing of novel treatments for MS. EAE-associated pain behaviors develop prior to the onset of clinical signs and in the absence of significant demyelination. Aim of this work is to standardize a protocol to minimize the suffering of animals during the development of EAE. Ten SJL/J female mice 6 weeks old were immunized subcutaneously with PLP139–151 peptide in complete Freund's adjuvant. Pertussis toxin was injected intraperitoneally (the same day of immunization and 1 day later). Once a day, as soon as mice began to show the first symptoms of dehydration and weight loss, they were injected subcutaneously with a solution of 5% glucose, vitamin B12 and essential aminoacids. Furthermore a gel diet was put in mice cages. Clinical signs in EAE mice were scored from 1 to 4 point. In EAE mice we succeeded in visualizing 4 different pathological scores and we performed preclinical imaging to monitor in vivo the development of the neuroinflammatory disease from the onset of symptoms. All the animals remitted completely from clinical signs after 14 days from EAE induction and they survived in good state of health till the fourth month of observation. These results suggest that the use of appropriate support therapies may be useful in order to prolong animal life span and well-being (according to the refine principle), and it also allows to study EAE mice for a longer time even when performing preclinical imaging. Thanks to appropriate treatments we can prolong animal survival from the onset of clinical signs.

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Newborn Piglets as Experimental Model for Human Neonates: Blood and Cerebrospinal Fluid Analyses towards the Standardization of the Model

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Complete and accurate knowledge of any preclinical model represents the first and most important step to high-quality research aimed to produce strong and reliable results. The comprehension of the physiology of the model is key to fulfill both reduction and refinement standards. Nowadays biological fluids are the most investigated specimens since their collection can be performed easily and is not as invasive as other sampling techniques e.g. biopsy. Blood testing give important information about our model and analytic methods are highly standardized. On the other hand, interpretation of the results can be difficult due to the lack of specific reference intervals for newborn piglets. Cerebrospinal Fluid can give precious information about Central Nervous System, but even in this case literature lacks of in-depth physiological data. The aim of our study is to standardize and acquire new knowledge about physiological biological fluids for newborn piglets. We developed age-related reference intervals for hematologic and biochemistry variables and analyzed CSF normal composition. We analyzed 150 hybrids piglets (50% 5 days old; 50% 30 days old) blood samples performing Complete Blood Count and Clinical Biochemistry. Twenty five days old piglets were sampled and CSF sent to 1H NMR spectroscopy for qualitative and quantitative assessment. All animals were enrolled in various experimental protocols in our department. Blood results were robust and reliable, allowing us to create accurate Reference Intervals; some parameters related to hematocrit and Iron profile showed statistical differences between the 2 age group, opening us new perspectives about iron supplementation and metabolism in the pig. For CSF, on each of the analyzed sample, 27 molecules could be observed above their limit of detection. Our analyses showed glucose and lactate among the most concentrated metabolites, mirroring what has already been described in human CSF. Composition may vary in elder animals due to Blood Brain Barrier maturation.

Can Different Mice Strains Be Differently Susceptible to Oxaliplatin-Induced Peripheral Neurotoxicity?

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Peripheral neurotoxicity is a limiting problem during oxaliplatin (OHP) chemotherapy so that most of cancer survivors have to deal with a compromised quality of life. OHP treatment produces an acute neuropathy with allodynia, dysaesthesia and burning neuropathic-like pain and a chronic sensory syndrome with enduring characteristics. OHP-induced peripheral neurotoxicity (OIPN) occurs in up to 80% of OHP-treated patients even if avariability in its severity was observed despite the identical treatment schedules. In this work we tested the sensitivity to OHP of 6 widely employed mice strains in order to identify a genetic hypothesis of OIPN. We performed a multimodal characterization of OIPN phenotype in genetically different mice strains (BALB C, CD1, FVB, DBA, C57BL6, AJ). After a 4-week treatment period with OHP 3.5 mg/Kg, OIPN features were assessed: the neuropathic pain was measured through the dynamic aesthesiometer and cold plate tests and by a quantitative measure of the electrical activity of spinal dorsal horn (SDH) neurons. Peripheral nerve and dorsal root ganglia integrity and functionality were assessed by nerve conduction velocity and by morphometric analysis. The unmyelinated nerve fibers degeneration was evaluated through the Intra-Epidermal Nerve Fibers density quantification. Our results demonstrated that all strains generally well tolerated OHP treatment. Mechanical allodynia was present, even if at a different extent, in all the strains while the cold hyperalgesia only in BALB C, AJ and FVB. BALB C and FVB showed strongly compromised NCV; CD1, AJ, DBA and C57Bl6 had only mild damages, while no alterations were evident in AJ. Neuronal atrophy was evident in BALB C and FVB. The electrical activity of SDH was significantly increased In AJ. These results demonstrated that a different genetic background can have a key role in determine the response to OHP-induced peripheral damages.

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Aging and Peripheral Nervous System: An in Vivo Study in C57BL/6 Mice Model

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Several studies support the hypotheses that aging is able to influence different functional and structural characteristics of peripheral nerves but the role of these changes in the damage/repair mechanisms involved in the onset of peripheral neuropathies is still unclear. For that reasons a multimodal, long-term evaluation in a mice model of changes induced by aging, that would represent an optimal tool to reproduce experimental neuropathy studies investigating the role of aging, was performed. In this study we used 66 4-week-old C57BL/6 mice and we followed-up them for twenty-six months. We performed the analysis of nerve conduction velocity (NCV) in the caudal and digital nerves every two months. At the same timing, four animals were sacrificed to collect caudal and sciatic nerve, dorsal root ganglia (DRG) and skin biopsies; these samples were processed to perform morphological and morphometrical analysis. The measure of NCV showed a remarkable increase of caudal nerve conduction velocity until the age of 7 months and it remained unchanged until the age of 19 months, when it started to decrease. Morphometrical analysis of caudal and sciatic nerves showed a decrease in fibers density while an increase in axon and fibers diameters.

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Morphometrical analysis of dorsal root ganglia did not evidence significant changes in somatic and nuclear area but showed a mild reduction in nucleolus area in older mice. Moreover, the intraepidermal nerve fiber density analysis showed an age-related reduction. These data can be considered a first step useful to create a background for future studies on the relationship between damage induced on peripheral nervous system and aging.

Anesthesia and Care of Cephalopods Used as Laboratory Animals

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Since 2011, the scientific community worked to produce the guidelines for the care and welfare of cephalopods in research. At the same time were published a number of scientific developments that provide tools for using cephalopods in research. Yet the English legislation has produced and published a code of practice which is included a section dedicated to these species. Current knowledge about the standards for a proper maintenance of cephalopods has been recently summarized based on researchers' experiences that for nearly a century worked with these animals. Anesthesia of cephalopods is discussed more than ever before due to the work in progress for their welfare legislation. Different approaches to anesthesia in cephalopods have been tried by a number of scientists, but in most cases the animals were not truly anesthetized. Several workers have simply used muscle relaxants or simple hypothermia under the name anesthesia. This approach will not be adequate in the future. Inhalational anesthetics such as isoflurane reduce L-type calcium currents and potassium currents in a dose-dependent manner in the pulmonate mollusk *Lymnaea stagnalis* and there is evidence from cell culture that such anesthetics also block excitatory chemical synapses, more effectively than inhibitory synapses. In our recent work we report, for the first time, on the effects of clinical doses of the isoflurane on the behavioral responses of *Octopus vulgaris*. The well-known anesthetic isoflurane was equilibrated into seawater via an air stone to adult *Octopus vulgaris*. We found that different animals of the same size responded with sAfter gradual application of 2% isoflurane (for a maximum of 5 minutes), when all the responses indicated deep anesthesia, the animals recovered within 45 to 60 minutes in fresh aerated sea water similar behavioral changes as the isoflurane concentration was gradually increased.

Development of ex Vivo Models for Translational Studies on Irritable Bowel Syndrome

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The irritable bowel syndrome (IBS) is a functional disease characterized by abdominal pain and changing in bowel habit. The functional nature of this disease makes particularly questionable the existing animal models. In this view, experimental models can be improved through the application of exogenous factors derived from patients. Compare and characterize two neuronal primary culture obtained from porcine and guinea pig myenteric plexus; evaluate the morphologic changes of guinea pig primary culture treated with soluble mediators obtained from biopsies of patients with IBS and healthy controls (HC). Seven pigs between 40 days and 2 months of age were used; a similar procedure was applied to 2 guinea pigs of 3 months of age. Neuronal ganglia were obtained from the longitudinal muscle layer of proximal ileum and seeded in 24 well plates. Immunofluorescence analysis toward HuD (1:100), GFAP (1:200) and Tubulin (1:200) was applied to detect respectively neurons, glial cells and the cytoskeletal structure. The same analysis was performed on guinea pig neurons exposed to IBS and HC supernatants (1:10). Compared to guinea pig neuronal cultures, swine cultures show a lower yield in terms of number of ganglia per well (~ -65%); after 24h from cell plating both guinea pig and swine neuronal ganglia contained a comparable number of neurons and glial cells. The former decreases dramatically after 5 days of culture (~47%); ~ 15% of cells resides still in a cluster structure, with a radial development of the peripheral glial cells, the resting population forming a network-like structure. Neurons exposed to IBS supernatants showed a loose shape with a lower number of cell/ganglion compared to the samples treated with HC supernatants (~41% p<0,01). This experimental evidence suggests the existence of factors favoring intestinal neuroplasticity in the mucosal milieu of patients with IBS.

Effects of EU Directive 2010/63 on Cephalopod Facilities

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Cephalopod mollusks are the sole invertebrates that have been included in the EU Directive 2010/63, therefore requirements for facilities and equipment need to be established, but no indications about cephalopods housing in the Directive 2010/63, Annex II, section B, were species-specific housing information should be reported. The aim of our study was to run one systematic review of papers published before (2010) and after (2013) EU Directive implementation to gather which scientific information were available to prepare our facilities for hosting cephalopods and to track how the cephalopods community react to the EU Directive 63/2010. Comparing papers published on 2010 and 2013, there was an implementation on data reported for source of water (natural or artificial seawater), water parameters (temperature, salinity, pH, dissolved oxygen, ammonia with nitrite and nitrate), water circulation system (flow-through system or recirculating aquaculture system -RAS), water purification system, tanks, light/dark cycle. Before the EU Directive 2010/63, researchers seemed not to consider and/or notify housing and water quality in their cephalopods facilities. On 2013, even if the EU Directive 2010/63 was not implemented in all EU countries: more data on housing and water quality have been controlled during

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experiments and more data have been shared in publications. There is still a huge gap to be filled regarding the understanding of best housing condition for cephalopods and the research community needs to be strongly encouraged to share more information about that, since standardization is fundamental to have comparable research data. Moreover, these data will have a great impact on the animal welfare and the 3Rs applied to cephalopods.

Noninvasive Ovarian Imaging in the Murine Models Using Ultrasound Biomicroscopy

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The laboratory mouse is a well-accepted model for reproductive biology studies and findings made in mice have been commonly translated to humans. C57BL/6J and BALB/c strains provide a robust experimental platform for polycystic ovary syndrome and ovarian cancer research in women. Serial assessment of ovarian morphometry and follicle dynamics may be performed non-invasively and reliably in mice by Ultrasound Biomicroscopy (UBM), with a near microscopic resolution. Our pilot study focused on the utility of UBM in murine models to measure size of ovaries and to count ovarian follicles over time. Three C57Bl/6J and two BALB/c adult mice (>8 weeks of age) were housed in standard conditions, with free access to food and water. Spontaneous estrous cycle was longitudinally evaluated by transcutaneous UBM (Vevo 770, VisualSonics, Toronto, Canada; center frequency of 40 MHz; focal depth of 6 mm, spatial resolution of 30 um) on a time period of 8-15 consecutive days. Mice were anesthetized with 1.5% isoflurane in oxygen and fixed in sternal recumbency on a heated stage. Trichotomy was performed over the thoracolumbar area, and acoustic gel was applied to the skin. Images of both ovaries were obtained in sagittal and axial scans, and processed offline using ImageJ software. Ovarian diameters, area, volume and follicles identification were obtained. To imaging results comparison, the phase of the estrous cycle was assessed in BALB/c mice by vaginal cytology. Ovaries were consistently localized in all mice, with a 10 minutes scan time. The changes of the follicles pattern and number overtime have wave-like fashion. In conclusion, UBM is a suitable method for measuring and for identifying the patterns in follicle development, ovulation and regression in mice in vivo, and to monitor the potential efficacy of novel therapies in murine models of ovarian pathologies.

Animal Care in a Mouse Model of Amyotrophic Lateral Sclerosis Atudied with [18F]DPA-714 Micro-PET/CT

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SOD1G93A mice are a transgenic model of Amyotrophic lateral sclerosis (ALS) to investigate in vivo neuroinflammation with innovative translocator protein radioligands and positron emission tomography (PET). The possibility to perform serial studies in the same animal, from the asymptomatic to the advanced stages, is highly desirable. Alterations of nutrition, hydration and of cardiovascular hemodynamics provide challenges for researchers and can adversely affect imaging studies. 10 hemyzigous SOD1G93A mice (aged 71-137 days) were evaluated at different clinical stages (CS, score range: 0-4) by [18F]DPA-714 PET/CT (GE Healthcare eXplore Vista, resolution: 1.8 mm FWHM/200 µm). 5 mice were longitudinally monitored. Weight, body condition (BCS, 0-5) and dehydration were daily monitored. From CS and BCS scored as 2, palatable source of hydration and energy were left on cage floor. Glucose 5% /Ringer lactate or NaCl 0.9% solutions (1-3 ml/day) with multivitaminic supplement were provided parenterally. Mice were housed in dry cage with soft bed, avoiding social isolation. Ocular discharges and ulcerative lesions were cleaned (NaCl 0.9%; iodopovidone). Imaging studies were performed under inhalant anesthesia (isoflurane 2% and oxygen 2 L/min). Radiotracer uptake (SUV) was measured on PET/CT fusion images in the cerebellum, brainstem, spinal cord normalized to those of the frontal cortex using Osirix software. In the single PET/CT group, 3 mice died after imaging study (CS: 1-2; BCS 3; weight gain +2.87g), while 2 mice were euthanized after 6 days (CS: 4; BCS 2; weight loss -3.04g). Mice performed from 2 to 5 imaging sessions, with a mean survival of 6 days after the last PET/CT (CS: 2-4; BCS 2-3; weight loss -1.68g). SUV showed an increased trend overtime in brainstem compared to age-matched controls. These findings underlie the relevant role played by the special care of transgenic murine models in order to improve longitudinal studies and minimize confounding variables.

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High Frequency Ultrasound Guided Microinjection in Mice Models of Human Mammary Carcinoma: A Feasibility Study

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The mouse is an important model for exploring metabolic and biochemical pathways for human carcinoma in vivo. Injection in specific sites is currently based upon surgical visualization of organs or estimated position, with many possible complications. High frequency ultrasound (HFUS) allows real-time non-invasive visualization of mouse organs. We evaluate the feasibility of ultrasound guided needle microinjection of carcinoma cells and mammary cell carcinoma growth after ultrasound guided injection with conventional xenografts orthotopic model. Eight, six-week-old, female, balb/C nude mice were anesthetized with isoflurane 2% and Oxygen 0,8 L/min. HFUS equipment, Vevo 2100 (VisualSonics Inc., Toronto, Ontario, Canada) mounting a multifrequency (22-55 MHz) probe was used in all procedures. Each mouse (n=4) was positioned in dorsal recumbency on the handling table of the imaging station and a thick pad of warm gel was used over mammary gland. Once the mammary fat pad

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was identified, a syringe mounted on the Injection Mount of the Rail System was made advancing until the needle tip entered the field of view. The syringe was prefilled with 2 x 106 MB -MDA-231cells with in ~20 µL. When the needle was in contact with the skin, it was further advanced using the micro-manipulation controls until the tip was within the mammary fat pad; the cells were gently injected. Mice were allowed to recover from anesthesia. The xenografts were injected of the same number of cells subcutaneously on the right flank on manually restrained awake mice (n=4). Tumor size was assessed at regular intervals up to XX days using US. HFUS-guided microinjection was fast and all mice recovered well from anesthesia. Growth rate was faster and final tumor volumes were larger in HFUS-guided compared with standard xenograft models ($p \leq 0.05$). Furthermore, the HFUS-guided procedure resulted less stressful for the animals.

Behavioral Characterization of 5-HT1A/1B Agonist, Eltoprazine, in Experimental Parkinsonism

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Long-term treatment of Parkinson's disease (PD) patients with L-3,4-dihydroxyphenylalanine (L-DOPA) is, at present, the most effective therapy for motor symptoms relief. Major side effects of chronic administration of L-DOPA are abnormal involuntary movements (AIMs) known as L-DOPA induced dyskinesias (LIDs). Many studies support the involvement of raphe-striatal serotonin neurons as crucial for the onset of LIDs, as they are able to convert exogenous L-DOPA to dopamine (DA) but lack the cellular components for the feedback machinery to regulate synaptic DA levels and, therefore, may contribute to the pulsatile stimulation of DA receptors in PD treatment. Here we focus on the therapeutic effects of the mixed 5-HT1A/1B receptor agonist eltoprazine in unilaterally 6-OHDA-lesioned rats modelling PD. In lesioned rats, which developed LIDs after chronic treatment with L-DOPA, one week coadministration of L-DOPA and eltoprazine reduced AIMs score by more than 50%. However, such regimen was not able to restore motor coordination neither to saline controls nor to eltoprazine controls levels, as revealed by RotaRod test performances. Moreover, sham-operated rats administered with chronic eltoprazine showed increased traveled distance in the Open-field test and increased conditioned responses in the Active Avoidance paradigm. Deficits in reward function, which underlie anhedonia, were assessed by Sucrose Preference Test: sham-operated animals administered with the 5-HT1A/1B receptor agonist failed to show 0,8% sucrose solution preference. Taken together, these results may account for 5-HT1A/1B agonist therapeutic effects on LIDs but, on the other side, possible cognitive side effect need to be further investigated.

A Cyclic Peptide Derived of Urokinase Receptor Generates a Potent Inhibitor of the Growth and Invasion in a Mouse Model of Chondrosarcoma

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The receptor for the urokinase-type plasminogen activator (uPAR) is a widely recognized master regulator of cell migration and uPAR88-92 is the minimal sequence required to induce cell motility. We and others have previously documented that the uPAR88-92 sequence, even in the form of synthetic linear peptide (SRSRY), interacts with the formyl peptide receptor type 1 (FPR1), henceforth inducing cell migration and invasion of chondrosarcoma cells. In this study, we present evidence that the cyclization of the SRSRY sequence generates a new potent inhibitor of chondrosarcoma cell invasion. We show that motility of human chondrosarcoma cellsexpressing a considerable amount of uPAR and FPR1 on cell surface, may be prevented by the cyclized peptide [SRSRY] which we have previously found to block uPAR/FPR1 interaction (*Yousif AM, et al., PLoS One. 2015 May 4;10(5):e0126172*). To analyse the effect of peptide [SRSRY] on the growth and invasion of chondrosarcoma cells *in vivo*, ten six-eight week old, CD1 female nude mice received an injection of human chondrosarcoma cells into the right flank as a single-cell suspension (1x106 cells in 100µl PBS, 96% viability). When [SRSRY] was i.p-administrated at 6 mg/Kg every day for 10 days, animals survived to the treatment schedule without clear changes in body weight. Measurement of tumor volumes revealed that [SRSRY] reduced the growth of tumors by 69% as compared to untreated mice. To quantify circulating tumor cells, retro-orbital blood samples collected from each mouse just before the sacrifice, were subjected to DNA extraction. Real-time quantitative PCR using primers capable of amplifying ALU sequences revealed the presence of human DNA in blood samples from 4/5 untreated mice and from 2/5 mice treated with the peptide [SRSRY]. Our findings indicate that the peptide [SRSRY] may be considered a valid compound for counteracting chondrosarcoma cell invasion.

MiR-199 Enforced Expression Inhibits Tumor Growth in a HCC Transgenic Mouse Model

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer and one of the most deadly tumors worldwide. With the exception of the multi-kinase inhibitor Sorafenib, there are no effective systemic treatments available for this disease. New perspectives in HCC treatment emerged in the last decade with microRNAs (miRNAs), a large class of short RNAs frequently altered in human cancer and affecting crucial cancer-associated pathways. miR-199 has been reported to be consistently down-regulated in HCC and is involved in HCC pathogenesis through the regulation of several target genes as mTOR and c-Met. The expression of miR-199 in HCC cell lines and in mice tissues was analyzed by quantitative RT-PCR. The apoptotic effect of miR-199 on HCC cell lines were assessed by MuseTM Annexin V. Mimic-199 oligonucleotides were introduced in vivo by intraperitoneal injection and Sorafenib was administered orally. Livers of transgenic mice were histologically examined to assess number and extent of lesions. In this study, we firstly demonstrated how the enforced expression of miR-199 through miRNA-mimic oligonucleotides or Adeno Associated Viruses (AAV) leads to inhibition of cell viability and increase of apoptosis in HCC cell lines. According to these data, we subsequently proved that a long-term administration of miR-199 mimics in tumors arising in a HCC transgenic mouse model leads to a reduction in number and size of liver nodules in comparison with the untreated control animals. Interestingly, results obtained with miR-199 administration were comparable to results obtained in mice treated with Sorafenib. This work suggests that miR-199 replacement might have a significant therapeutic value and could provide new therapeutic opportunities for HCC treatments.

Exposure to Airborne Biological Agents in a Conventional Laboratory Animal facility

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Working with laboratory animals (LA) may expose personnel to a complex mixtures containing, besides the well-known allergens, microorganisms and their components that can lead to adverse health effects. This paper reports the results of a microbiological environmental monitoring carried out in a conventional laboratory facility housing about 7,000 animals including mice and rats. The primary objective was to measure and characterize airborne fungi, bacteria, endotoxins, and (1/3)-b-D-glucans through air sampling with the aim to determine which factors or working tasks were associated with the greatest level of exposure to biological agents. Bioaerosols were collected from seven animal rooms, a storage area, two washing rooms and some offices. In animal rooms air samples were taken over three consecutive days: the day in which the cages are changed, the day before and the day after changing cages. Mesophilic bacteria levels ranged from 2.5 to 885 CFU/m3, with the detection, during the change of cages, of Gram-negative bacteria belonging to risk group 2 (*Escherichia coli*, *Enterobacter cloacae*), while moulds and yeasts levels ranged from 0 to 335 CFU/m3. Mean concentrations of endotoxins and (1/3)-b-D-glucans were below the exposure limits in all workplaces except the storage area, where values respectively of 210.7 CFU/m3 and 5,145 pg/m3 were recorded during the preparation of litter and distribution of feed. The one-way ANOVA test showed a statistically significant increase (p-value <0.05) of all microbiological parameters during the change of cages. Our results identified the working phases carried out in animal rooms and in the storage area as the more "critical" with regard to the potential exposure to biological agents. The data suggest that rodent bedding could be a source of accumulation and release of endotoxins and fungal components. Further investigation is needed to estimate the personal exposure in order to implement proper control and prevention measures.

Methodological Approach for the Study of Laboratory Animal Allergy: A Proposal to Harmonize the Procedures of Risk Evaluation

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Animal facilities are environments in which workers are potentially exposed to biological, chemical, and physical agents, therefore they can develop allergic diseases of different severity. The allergy risk arises from exposure to allergens of animal origin (such as mice and rats) whose main sources are hair, dander, urine and saliva. In 1998, the LAA (Laboratory Animal Allergy) has been officially recognized as occupational risk in the USA and has been studied for years in other countries, where institutional recommendations have been issued in this regard. In Italy, this allergic condition has received little attention, especially in terms of legislation and regulations. About a third of those working with laboratory animals may experience allergic symptoms within the first three working years and of these, a percentage higher than 10% can develop occupational asthma and rhinitis. The study proposes an analytical and descriptive flow chart that could be a reference tool for those dealing with such occupational hazard in order to harmonize the methodological approach. In this context, the first step is to find a commonly shared definition of LAA-positive workers – i.e. those serological IgE-positives with clinical symptoms in order to harmonize the terminology and overcome the methodological discrepancy found in the literature. The following step is an innovative research approach based on both sequencing of new allergens and evaluating their presence in several environmental and biological matrices through molecular methodologies.

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Upgrading Gunn Rat Health and Welfare by Colony Rederivation: The Effect on the Animal Model

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Embryo transfer rederivation allows for pathogen-free animals; it ensues that animal welfare improves and experimental data are no longer biased by infection-dependent alterations. In this study we present the results of the rederivation of the hyperbilirubinaemic Gunn rats, an animal model for the human Crigler-Najjar syndrome. The SOPF, rederived colony (RGunn) was established in 2014 (with 1 heterozygous male and 4 heterozygous females) and was bred under microbiologically controlled conditions (barrier). The strain was analysed in terms of reproductive performance, total serum bilirubin, and cerebellum size, data were compared to those obtained from the original pathogen-positive colony (OGunn) that had been bred under standard conditions (conventional). Althougth RGunn heterozygous females showed a significantly ($P \leq 0.05$) lower fertility compared to the number of offsprings/mating observed in the OGunn strain, the percentage of deads/mating and the percentage of hyperbilirubinaemic pups/mating were comparable between groups. In hyperbilirubinaemic RGunn the peack of total serum bilirubin (TSB) was delayed and slightly increased (P17, about 17 mg/dL, vs P9 14.5 mg/dL) in respect to the hyperbilirubinaemic OGunn. Similarly, an increased TSB of 7.5 mg/dL was observed in older RGunn vs 4.8 mg/dl in the OGunn. Instead, the temporary (up to P9) mild (around 4 mg/dL) hyperbilirubinaemia present in heterozygous OGunn pups disappeared in RGunn. No differences in TBS were recorded in homozygous normobilirubinaemic RGunn after rederivation. Despite levels of TSB similar in the first 9 days after birth, and even higher afterwards, in RGunn the cerebellar hypoplasia was reduced vs age-matched OGunn (P9: 14.7%, $p<0.05$, vs 20% in the OGunn; P17: 28.9%, $p<0.0001$, vs 44% in the OGunn; P60: 39.9%, $p<0.0001$, vs 50% in the OGunn). Our findings strengthen the notion that strain rederivation leads to phenotypic plasticity and underlines the effect of the environmental/microbiological conditions on the variability in animal response/phenotype.

Electrical Membrane Properties of Oocytes Isolated from *Xenopus laevis* following Multiple Laparotomies

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Xenopus laevis oocytes are commonly used for studies in molecular biology, embryology, biochemistry and electrophysiology. Their large size facilitates direct microinjection of cRNA, cDNA and cell membranes to record the activities of exogenous receptors and ion channels functionally incorporated into the sarcolemma. To perform these kinds of experiments, oocytes are isolated from sexually mature females by laparotomy under general anesthesia. The number of surgeries performed on a single animal varies among different laboratories around the world and its effect on the quality of the collected oocytes have not been investigated. We used the resting membrane potential (RP), membrane input resistance (Rm) and the capability to incorporate functional exogenous nicotinic acetylcholine receptors (nAChRs) as parameters to evaluate the quality of oocytes isolated after multiple laparotomies (up to four; time interval between two surgeries: 6 months; on the same abdominal side: >1 year). Two-microelectrode voltage clamp recordings were performed in oocytes injected with membranes isolated from immature mouse skeletal muscle. We found the RP was significantly ($P<0.001$) more negative in oocytes isolated at the third and fourth laparotomy (-28.92±0.59 mV, n=160 and -42.39±1.01 mV, n=101, respectively), whereas Rm was significantly ($P<0.01$) increased already by the second surgery (1.11±0.03 MW, n=268). All cells were able to incorporate functional nAChRs, as confirmed by bath-applied ACh response, but interestingly ACh-current amplitudes were significantly ($P<0.05$) higher at the fourth surgery compared to those obtained after the first laparotomy (348.7±30.99 nA, n=54 and 254.11±20.85 nA, n=27, respectively). These results demonstrate that multiple surgeries may affect the passive electrical membrane proprieties of the oocytes, but not their efficiency to incorporate nAChRs.

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