Editorial

Here comes the third part of our Special Issues dedicated to projects selected in CONCERT’s calls for tenders. LDLensRad was selected during the first call for projects, with a duration of 3 years. This project tackles a priority topic for MELODI and EURAMED: non-cancer effects and in particular the effects of irradiation on the eye and cataract induction. This Issue is not organized around the Work Packages of the project but rather around the outstanding results that have been obtained. It should also be mentioned that LDLensRad has designed a very ambitious Data Management Plan. The partners have worked together with the STORE database, not only at the ultimate stage of data storage, but from the very beginning of the project to design the data architecture, ensure the best storage and potentially the best re-use of the data.

Dr Laure Sabatier, CEA

The floor to...

Human studies, for instance of the Atomic Bomb survivors, have led to the conclusion that the lens of the eye is more sensitive to ionising radiation (IR) exposure than previously thought. Now, substantially reduced, dose limits came into force in Europe in early 2018. However, it is still very unclear how low dose IR might cause or be involved in development of cataracts. This is an important current public health issue, particularly for medical radiation workers, many of whom will need to amend their working practices despite a clear lack of understanding of the effects of low dose rate, low dose, IR exposure on the lens.

LDLensRad was a successful EJP CONCERT first call project, focused on the ‘Improvement of health risk assessment associated with low dose/dose rate radiation.’ The objective was to advance knowledge to solve the question of how radiation causes and/or promotes cataracts. The aims were to answer the questions:

1) How does low dose radiation cause cataract?
2) Is there a dose rate effect?
3) How does genetic background influence cataract development after radiation exposure?

In addition, the shape of the dose response (in time), the nature of radiation induced cataract (deterministic or stochastic, or both), bioindicators of global response and training of early career scientists were important considerations for the project.

So, to the results. Firstly, we have clearly demonstrated that both dose and dose rate of IR are important in terms of how the lens of the eye responds to IR. Importantly, doses as low as < 500 mGy, were found to cause quantifiable changes in the lens. Further, the long-term studies clearly demonstrate that genetic background, age and sex are also involved in the response and that factors influence each other.

However, unanswered questions concerning mechanism, latency and threshold remain. Our data were obtained using animal and cellular models and human studies need to be carried out to better understand the mutual influence of these and other factors and, further, the implication that the current radiation protection legislation and guidance might need to be reviewed in due course.

LDLensRad – Towards a full mechanistic understanding of low dose radiation cataracts

LDLensRad Coordinator:
Elizabeth (Liz) Ainsbury, Senior Scientific Group Leader
PHE Cytogenetics and Pathology Group

Partners:
PHE, HMGU, ENEA, OBU, DU, and advisory board members: Nobby Hamada (CRIEPI, Japan), Joe Dynlacht (Indiana University, US), Larry Dauer (MSKCC, US), Rick Tanner (PHE, UK), Tamara Azizova (SUBI, Russian Federation);

Informal collaborators:
Gabriele Babini (UNIPV, Italy), Paul Schofield and Michael Gruenburger (UCam, UK)

March 2020

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WP 6 News:

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Long term studies
Mouse models: Scheimpflug imaging and associated data on cataract over the mouse lifetime

WP 1 focused on collection of long and short term data on a variety of endpoints associated with cataract. Here are described the results of long term studies focused on cataract development in the mouse models over an 18 month (mth) period.

Mice were exposed to doses of 0 to 2 Gy at an acute, high dose rate (0.3 Gy/min) or at a more protracted, low dose rate (0.063 Gy/min). The impact of genetic background was assessed using 6 strains of mice currently or previously used as models for radiation cataractogenesis were included in this study, as detailed in the figure below.

Mice were irradiated at 10 weeks (wk) old (mature lenses) and at neonatal age (postnatal day 2), in order to investigate the ageing effect in this particularly age-sensitive strain. The mice were then followed for up to 18 mth post exposure, with Scheimpflug imaging (Dalke et al., 2018) every 1 mth, together with Optical Coherence Tomography (OCT) and histology to track cataract appearance and development.

Irradiations and long term Scheimpflug imaging: at HMGU with wildtype (B6C3F1) and heterozygous mutant Ercc2+/− mice, 19 mth Scheimpflug data demonstrate no significant radiation-induced lens opacifications in 10 wk irradiated mice given doses of 0, 0.5, 1 and 2 Gy at 0.3 Gy/min. Strain, and the interaction of sex and strain, strain and dose were, however, statistically significant.

Data from the wildtype (WT) and Pch1−/− mice on C57 (C57Bl6) and C57 backgrounds irradiated at 10 wk and 2 days can be summarised as follows: Age related changes are clearly strain dependent. CD1 mice had more opacities than the C57 mice and, in contrast to the PHE C57 data, the C57 mice showed no increase in opacification with age over 18 mth of observation. There was a strain dependent dose rate effect, but in 10 wk mice no ‘clinically significant’ opacification was seen. In the P2 mice, there were significant strain dependent dose and dose rate effects resulting in opacification of the lens up to ~40% (likely vision impairing), which also markedly impacted survival.

OCT and histology data clearly show that OCT is the preferred modality for detection of PSC, compared with Scheimpflug imaging. New measurement techniques and new phenotypes have been defined and there is a clear effect of IR for posterior and anterior changes. In the 10 wk mice, at 12 mth post irradiation, there appears to be a threshold of approximately 2 Gy for alterations in lens structure, however, by 20 mth the dose response is linear.

Visual acuity was impacted by radiation with sex and genotype influence. Only for female mutants was there a linear correlation between visual acuity and corneal clouding (opacification). This leads to further questions regarding the impact of radiation on vision in general – and, possibly, as the data can be interpreted to support a stochastic model for cataractogenesis, regarding the ICRP definition of detriment. Effects in female mice were greater than those in male mice throughout. C57 mice showed a similar dose response but no dose rate effect.

The results of long term studies focused on cataract over an 18 month (mth) period.

Keywords:
Mouse models, cataract, ionising radiation, Scheimpflug imaging, OCT

Partners involved in WP1:
• PHE, United Kingdom
• HMGU, Germany
• ENEA, Italy
• DU, United Kingdom
• OBU, United Kingdom

Duration:
36 months

Total project budget:
~ 2.5M€

Infrastructures:
PHE, HMGU and ENEA Co-60 gamma irradiation facilities

Open Access of produced data:
Yes: https://www.storedb.org/store_v3//study.jsp?studyid=1111

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Related to:
MELODI EURAMED
Further to the long term results described in the previous WP description, for each strain, dose and dose rate, lenses extracted from groups of mice were assessed for: initial DNA damage at 0, 4 and 24 hours (h) following exposure; intracellular communication, cell cycle effects, biochemical analyses and genetic pathway analyses at 0, 4 and 24 h, 4 and 12 mth; proliferative and morphological effects at 24 h, 4 and 12 mth; miRNA content using Next Generation Sequencing (NGS) at 4 h and qRT-PCR at 24 h, 4 and 12 mth.

In terms of initial DNA damage responses in the lens epithelial cells, a key output of the project is that an inverse dose rate effect for DNA damage (53BP1 foci) was observed. The different mouse strains showed differential repair in terms of detection of 53BP1 foci at 4 and 24 h post-irradiation, indicating Ercc2+/− mice are either more efficient in DNA damage repair or there is less damage induced in the lens. The influence of single strand breaks was discussed together with a hypothesis around the potential role of growth factors as discussed in Barnard et al., 2019 and transcription factors for the XPD DNA damage repair protein, in which the Ercc2+/− mice are deficient. In lymphocytes, in vivo, repair was slower, perhaps suggesting differential repair mechanisms in the lens as compared to other tissues. Further investigation into this interesting phenomenon is still to be carried out.

To date inverse dose rate effects, whereby lower dose rates lead to larger effects, have only been reported at low doses in very few studies, these references should be reported with these data to prompt further investigation into the potential implications.

The proliferation analyses completed to date in the PHE and HMGU mice indicate that IR reduces proliferation and also cell density, with region of the lens epithelium (i.e., the central or peripheral areas), dose and dose rate all significantly contributing to the response.

The morphology data indicate slow, but dynamic, development of opacities associated with radiation exposures of 0.5 - 2 Gy, 12 mth post exposure, with clear evidence of lens repair too, and gender and strain effects, similar to those seen in other tissues.

Regarding the NGS carried out at ENEA, initial analysis of miRNome identified miRNA indicative of a variety of well known radiation responses.

The lipidomic and proteomic work addressed the hypothesis that IR causes oxidative stress, leading to protein carbonylation and oxysterol formation from cholesterol respectively, ultimately causing cataracts. Oxysterol levels were impacted with a trend of dose dependent increase, with differential responses in the lens nucleus and cortex. Further analysis, including the in vitro work on the additional endpoints, is ongoing.
Lens as an indicator of global radiosensitivity: behaviour and pathological effects

The wider effects in the mouse brain together with behavioural testing were carried out concurrently and, at the end of the long term study, with the aim of comparing the data with the lens results, to test the hypothesis that lens effects can be used as an indicator of global radiation effects.

Studies on behaviour of the HMGU mice was focused on spontaneous and cognitive behaviour assessed by open field, prepulse inhibition, social discrimination and y-maze following IR of the HMGU (ERCC2+/− and wt) mice at 10 wk to 0 – 2 Gy (0.3 Gy/min) at 4, 12 and 18 mth post irradiation (PI). The strongest dose-dependent IR effects were observed at 4 mth PI, but overall the responses were clearly also dependent on age, sex, genotype, with various factor interactions identified for the different endpoints. At 4 mth PI, visual acuity was not yet affected, indicating that it is not the lens which is driving behavioural alterations at this timepoint. The relationship with neurogenesis is likely to be important.

Irradiation effects on the mouse brain at ENEA are still under investigation, but the role of the sonic hedgehog (shh) pathway for wide regulation of cell growth and differentiation (and which is deficient in Ptc1−/− mice) is now much better understood. Key outputs include that doses of IR > 0.5 Gy significantly reduced survival of the CD1 background mice due to development of medulloblastoma (MB); this was not observed in any unirradiated mice. There were no significant effects of dose rate on survival or MB induction in the CD1 mice. In the C57 mice, IR reduced survival less significantly, and there was no MB induction above background in the P2 irradiated C57 mice. Genetic background was thus the dominating factor in MB development in this study.

In terms of neurogenesis, the results show a significantly slower rate of basal neurogenesis in C57 (~50%) compared to CD1 mice pointing to important genetic background related differences between the two mouse strains. No difference in the long-term response (4 mth PI) of the neuronal population of the dentate gyrus to adult irradiation with 2 Gy were observed at any of the two dose-rates in WT CD1 and C57 mice, although the genetic background-dependent differences were maintained (Figure below).

At 6 wk post exposure, impairment of Sox2 and Dcx populations was observed in the C57-Ptc1−/− mice only. It is thus concluded that sensitivity of Ptc1−/− mice to irradiation was strongly exacerbated on a C57 background both after neonatal or adult irradiation. The link between these observations and the NGS data are still being considered. Efforts will continue to attempt to understand the molecular mechanisms accounting for the different response to IR in Ptc1−/−/CD1 and Ptc1−/−/C57 mice.

Genetic background-related changes in neurogenesis in CD1-Ptc1−/− and C57 (‘BL6’)-Ptc1−/− mice exposed to 2 Gy (0.3 Gy/min and 0.063 Gy/min) at 10 wk of age and examined 4 mth post-IR.
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**Future events:**

**CONCERT Short Courses**
15-29 March 2020
Monitoring strategies applied in NORM involving industries – evaluation of occupational exposure and environmental impact,
Central Mining Institute, Katowice, Poland
Contact: Boguslav Michalik

16-27 March 2020
Health effects induced by radiation and space conditions,
SCK•CEN Mol, Belgium
Contact: Sarah Baatout

30 March 2020
EU CONCERT Radiation Protection Research Projects and UK NIHR HPRU in Chemical and Radiation Threats and Hazards Medical Radiation Theme - Final Stakeholder Dissemination Meeting,
Newcastle, United Kingdom
Contact: Liz Ainsbury

20 April-1 May 2020
Assessment of long-term radiological risks from environmental releases,
Technical University of Denmark, Risø Campus, Denmark
Contact: Andrea Ottolenghi

18-29 May 2020
Modelling radiation effects from initial physical events,
University of Pavia, Italy
Contact: Andrea Ottolenghi

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Editorial Committee: Maria Panagiotopoulou, Jean-Michel Dolo, Laure Sabatier

Future events:

19-24 April 2020
ICRER: 5th International Conference on Radioecology & Environmental Radioactivity, Amsterdam, The Netherlands

5-8 May 2020
1st ISORED scientific and organisation meeting, Sitges, Spain

27-29 May 2020
6th NERIS workshop: Operational and research achievements and needs to further strengthen preparedness in emergency management, recovery and response, Barcelona, Spain

28 September-2 October 2020
ERPW2020: European Radiation Protection Week 2020, Estoril, Portugal
Deadline for abstract submission: 31st March 2020

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