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CBRN Emergencies: Integrated Approach to Develop Innovative Technology and Training of Operators

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Introduction

A common feature of possible scenarios that could occur in case of CBRN (Chemical, Biological, Radiological, Nuclear) emergencies is the difficulty in forecasting. For this reason, it is necessary to establish interactions between professionals of different disciplines. The Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) encompasses a large part of the skills and instruments necessary for an integrated approach to CBRN emergency such as cyber-security, environmental contamination, bioterrorism (bacteria, toxins, viruses), early and retrospective dosimetry, measurement of environment radioactive contamination, electromagnetic characterization of materials and biological tissues, electromagnetic dosimetry and modelling, development of wireless technologies even in harsh environments, using robots for handling hazardous materials, carriers for transportation of sensors in territories unsafe, etc.

In particular, within the ENEA Division of Health Protection Technologies, several activities, including international projects (supported by NATO, IAEA, EU, EURATOM, MAECI and MATTM),[1] have been developed in the field of CBRN management during last decades. The running activities and achievements here introduced refer to defence against potential biothreat agents, retrospective dosimetry, early biomarkers to measure the received dose and applications in the field of electromagnetic radiation.

Facing deliberate or naturally occurring infectious disease outbreaks with plant-derived biotechnologies

Biological agents might be more lethal than chemical weapons, more difficult to detect than nuclear weapons and less expensive to be produced. Whether naturally occurring or man-made, biological threats pose a severe risk in an increasingly globalized world and it is necessary to rely on technical platforms able to cut down the time to tailor the eventual vaccine candidate to be effective to the epidemic. We present here two biotechnological approaches for biodefence purposes: i) plants as biofactories for the rapid production of biopharmaceuticals ('Plant Molecular Farming'), and ii) genetic vaccines with plant sequences with immune-enhancing activity. These platforms represent two promising and complementary approaches for the rapid and low-cost production of countermeasures (diagnostics and vaccine candidates) against emerging, re-emerging and bioterrorism-related infections.

i) The recent apparent success in fighting Ebola outbreak of 2014-2016 with plant-made antibodies (ZMapp™) brought renewed attention to plant-made biologics for human health.[5,8] Plants represent ideal platforms for quick and flexible production of high quality recombinant proteins due to lower manufacturing costs, reduced risks of contamination with human/animal pathogens and ability to perform eukaryotic post-translational protein modifications. Severe Acute Respiratory Syndrome (SARS) emerged in 2002 when its aetiological agent, the coronavirus SARS-CoV, crossed the species barrier to infect humans. The end of the SARS outbreak was declared by WHO in July 2003 but several local outbreaks were subsequently reported in China due to accidental laboratory contaminations or after contact with animals infected with strains different from those of 2002–2003 outbreak. SARS-CoV was defined as class C biological weapon and, currently, there are no approved antiviral treatments and novel diagnostics are needed. We demonstrated that plant (*Nicotiana benthamiana)* transient expression systems can be used to produce SARS-CoV antigens, in particular the structural N and M antigens, the most abundant proteins in the virus particle. The plant-derived N protein is able to reveal human N-specific antibodies in sera of SARS patients, thus providing an adequate instrument to develop novel immune-based (nano) diagnostic assays.[6]

ii) Several strategies are being developed to improve the efficacy of DNA vaccine efficacy (currently used in veterinary medicine). Some years ago we demonstrated that some plant proteins, involved in plant defence responses might have effects on tumours by modulating innate immune functions.[16] Recently, we developed a genetic vaccine where a plant protein signal sequence (ss-) was fused to the N-terminus of crucial viral antigens derived from the human papillomavirus type 16, HPV 16.[15] The immunological effects of the new DNA vaccines were studied in animal models for HPV, with a prime/boost schedule (implying the use of electroporation after intra-muscular immunization) demonstrating that the plant ss- enhances the humoral response to DNA-based vaccines.

To our best knowledge, this is the first demonstration that a plant protein signal sequence (-ss) is able to exert a biological activity in mammalian cells and enhance immunogenicity of an antigen of interest. This approach might work also for other antigens and for different pathogens, opening new perspectives in the design of DNA vaccines, especially to counteract infections where a fast and effective antibody response is needed, as in the case of emerging, re-emerging and bioterrorism-related infections.

Biological dosimetry for individual retrospective dose assessment in subjects accidentally exposed to ionizing radiation

In the period 1960 - 2000, the ENEA Biological Dosimetry laboratory was the Reference Centre at the national level for retrospective dose assessment for populations accidentally exposed to ionizing radiation. The team participated from 1992 to 1998 to International Cooperation Projects for retrospective biodosimetry studies on subjects coming from contaminated areas after Chernobyl and Southern Urals accidents.[18,20] From 1995 to 1997 ENEA participated to the International Project INTAS (L. n. 449/1997) "Assessment of the Radiological Consequences for Man and Environment from Nuclear Tests in Kazakhstan" performing retrospective dosimetry studies by cytogenetic methods on Kazakhstan population heavily overexposed to ionizing radiations.[9,21] The ENEA Cytogenetic Dosimetry staff is currently part of the Division of Health Protection Technologies and is involved in several activities within International Biodosimetry Networks and Associations. From 2012 to 2015 it participated in the coordinated action "*RENEB*" (Realizing the European Network in Biodosimetry) (http://reneb.eu) within the EU FP-7 Fission Project 2012, a project aiming to create a sustainable network in Biological Dosimetry that involves a large number of experienced laboratories throughout the EU acting in collaboration in case of a large-scale radiological emergency.[12,13] Within this project, ENEA laboratory was involved in inter-comparison exercises on triage-dose reconstruction by using both dicentrics and micronuclei assays.[17] In the same period it took part in the EU-EMRP Joint Research Project "*BioQuaRT*" (Biologically weighted Quantities in RadioTherapy) (http://www.ptb.de/emrp/bioquart.html) aiming to develop measurement techniques for characterizing charged particle track structure on different length scales, and to correlate at the cellular level the track structure properties with the biological effects of radiation. Within the Work Package on Radiobiology, ENEA laboratory performed the analysis of chromosome aberrations induced in cells exposed to charged particles delivered by a microbeam facility.[19]

The same ENEA Division is also involved in the activities of the WHO Global Biodosimetry laboratories network for radiation emergencies, *BioDoseNeT* (http://www.biodosenet.net), an international network whose role is to support management and decision-making in cases of large radiation emergency events. ENEA is a member of the European Radiation Dosimetry Group "*EURADOS*" (http://www.eurados.org), a non-profit association that promotes research and development and European cooperation in the field of the Dosimetry of ionizing radiation. ENEA is involved in EURADOS activities including "Retrospective Dosimetry" (Working Group 10) that aims to establish a network of contacts and collaborations throughout European laboratories with expertise in the area of physical and biological retrospective dosimetry.

Since 2014, members of the ENEA Division of Health Protection Technologies are included in the expert panel of the European Parliament on Safety and Security technologies (Tender Lot 9) and, since 2016, participated in the activities of the International Commission on Radiation Units and Measurements (ICRU) Report Committee on "Retrospective Assessment of Individual Doses for Acute Exposure to Ionizing Radiation".

Early management of CRN emergencies

Due to the urgency of triage decisions, new dose-based tools for sorting of subjects exposed to radiation within the first hours after exposure are strongly needed. The validation of such sorting methods is the aim of the ongoing NATO SPS Italy-Egypt funded project: "A Panel of Biomarkers as Novel Tool for Early Detection of Radiation Exposure" (http://www.nato-biorad.org/en/). Biomarkers were chosen because they provide results in a few hours, they measure the radiation exposure with a dose-effect relationship, they do not need very experienced staff and complex and expensive equipment. Thus, they could be used in structures or in countries that do not have the possibility to invest considerable resources for R/N emergencies. Biomarkers measure: blood cells genetic damage (Comet assay, pH2AX foci) oxidative stress and radio-sensitive proteins alteration (FlT3, citrulline, amylase, IL 6 etc.). Results are compared with the established micronuclei count test.

This study is conducted on patients treated with irradiation of therapeutic dose (2, 3, and ≥ 5 Gy) in Italy (Regina Elena National Cancer Institute IRE-IFO, Rome) and in Egypt (HIPH Alexandria).

A main implementation of the proposed project is a concrete collaboration between Italy and Egypt for building a platform for early assessing the radiation absorbed dose, to be integrated into the R/N emergencies management by the indicated End-users and possibly to other NATO countries. Egyptian young scientists are trained in the Italian and Egyptian laboratories. As soon as possible, inter-laboratory comparison will be performed. Recently, an extension of this project has been funded by the Italian Ministry of Foreign Affairs and International Cooperation (MAECI) in order to study the inflammation early response to radiation and the presence of circulating exosomes.[3]

Previous studies have been carried out on radiation effects such as genomic instability on irradiated mice;[10] different sensitivity of genes to radio-induced fragmentation;[1] the role of membrane rafts' component in the transmission of cell signalling; the spatial distribution of antioxidant enzymes acting as protective shield around the cell nucleus;[7] the radio-protective properties on antioxidant compounds or nanoceria treatment;[2] environment reference organisms for studying uranium and depleted uranoium toxicities;[11] a Bilateral Italy-Hungary program: "Exposure to Ionizing Radiation: Risk Assessment, Early Biomarkers, Recognition of Countermeasures in Treated Patients and in Animal Models" (HU11MO6, 2012-4). Before 2000, ENEA researches have also been involved in studies about the effects induced by inhalation of airborne substances (such as organic solvents, nanoparticles, ozone) on the respiratory tract.[22]

Dissemination and training activities in the emergency field are being carried out, such as: teaching at the IAEA Radioactive Waste Management Summer School, 2009-2013, ISPRA JRC, Varese (Italy); invited professor course on "Ionizing Radiation Effects", 2009-2012, Medicine Faculty, University Tor Vergata (Rome).

Participation to the ConRad-NATO Symposia from 2011; organization of the practical courses: i) "Radiological and Nu-

clear Emergencies' Management: Biological Aspects" Casaccia, ENEA Center (Rome), 5-7/11/2012; ii) "From Dosimetry to Biological Effect: Radiobiology as Guide to Clinical Practice in Nuclear Medicine, 5-8 November 2011, Sorrento (NA), Italy.

Edited Handbooks: i) Practical course on biological dosimetry [ISBN: 978-88-8286-264-0 2012]; ii) "How to Communicate the Radiation Risk" – "Comunicare i rischi delle radiazioni" [ISBN 978-88-88648-36-1]; iii) "CBRN Risks in Maritime and Land Containers Transport" [ISBN 978-88-8286-347-0].

Electromagnetic technologies for nuclear safety

Wireless technology has matured to a level where it has seen extensive deployment in many industries for measurement, diagnostics and condition monitoring.[4,14] These applications have demonstrated benefits in terms of reduced wire installation time and costs, and increased flexibility of process instrumentation and control. The nuclear industry could benefit in a similar manner, but issues including, but not limited to standardization, unique environmental conditions, cybersecurity concerns and a more conservative view of adopting new technologies have limited the widespread deployment to date.

The Coordinated Research Project (CRP) 1000145 "Application of Wireless Technologies in Nuclear Power Plant Instrumentation and Control Systems" (2015–2018) of the International Atomic Energy Agency (IAEA) (https://www.iaea.org/NuclearPower/Engineering/CRP/AWT/index.html) aims to study the feasibility of application of electromagnetic wireless technologies for control safety measures at nuclear power plant facilities, as well as to provide indications for deploying potential benefits of wireless technologies by offering an alternative to wired solutions for transferring process and diagnostic information. The CRP covers both novel wireless research and support the adoption of existing wireless technologies. Research topics include: a review of existing codes and standards associated with wireless network technologies and their applicability to the nuclear industry, the effects of the nuclear specific environments on the operation of wireless systems, a range of technological challenges including optimal wireless system design and selection to meet the needs of nuclear applications, the role of energy harvesting and quality of service issues. The IAEA acts as a coordinator for this research to bring together diverse organizations worldwide to address key scientific and technical challenges in an emerging domain relevant to the international nuclear I&C industry. The outcomes of the CRP will facilitate the more widespread adoption of wireless communication in existing and future plants by providing guidance on the deployment of the technology. Results are planned to be published in a Nuclear Energy Series report when the work of the CRP is completed, and will serve as a source of information to support the application of wireless technologies in nuclear power plants in the IAEA Member States.

Under this framework, ENEA (Physics of Fusion Division (FSN-FUSPHY), in collaboration with ENEA Division of Health Protection Technologies coordinates the task "Evaluation of Electromagnetic Fields from Wireless Technologies in Nuclear Plant" whose aim is to perform a feasibility study on the application of wireless technologies at nuclear power plant facilities, in order to assess whether the required level of reliability can be achieved for transmitted data. At first, a survey on currently proposed wireless technologies for nuclear facilities and plants has been carried out. Afterwards, for selected scenarios, the electromagnetic field propagation has been investigated by way of electromagnetic simulation tools and the nuclear plant environment has been simulated by modelling the presence of engineered barriers. Finally, the feasibility of application of wireless technologies at nuclear power plant facilities will be assessed on the basis of the results achieved from the simulated scenarios.

Conclusions

ENEA (www.enea.it), with its 9 Research Centres distributed all over the Italian territory, represents a multidisciplinary entity already committed at the international level in the field of CBRN, as also documented by the work performed in the Division of Health Protection Technologies.

These peculiar characteristics make ENEA a suitable national reference point for the development of innovative technologies and for practical and theoretical training related to the 'Culture of Security' and CBRN emergencies.

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