Need for Convergence Technology Tools for Assessment of Toxicological Implications of Ionizing radiation

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Introduction

The tsunami damaged Fukushima nuclear power plant has released radioactive materials into the environment including nuclear fuel cycle fission products such as cesium-137 and activation products such as cobalt-60. These radioactive material releases increase the likely-hood of exposure to low-level ionizing radiation in the general population. The Japanese Ministry of Health, Labor and Welfare has announced radioactivity exceeding legal limits has been detected in milk certain vegetables produced in the Fukushima area, and measurements made in a number of locations have shown the presence of radioactive material on the ground[i]. Tokyo drinking water exceeded the safe level for infants[ii]. Seawater near the Fukushima plant has been found to have elevated levels of iodine-131, far beyond legal limits. Cesium-134 and 137 concentrations have also been discovered to be far beyond the legal limit near the damaged power plant. Many factors go into the determination of whether or not there is harm to people or other organisms that may be exposed to ionizing radiation. Factors include the type, intensity and duration of the radiation exposure, as well as the state of health, age, sex, diet and other variables related to the person or organism. The study of the mechanisms by which radiation exerts toxicological effects is an evolving field of toxicology. Research utilizing convergence technology can focus on identifying biomarkers and improving understanding of the specific mechanisms by which ionizing radiation generates toxicologically relevant end points. Published reviews of the biological effects of radiation and more in-depth discussion are available[iii] [iv] [v].

Scope

This report will discuss ionizing radiation and the biological effects of exposure to the three main types of ionizing radiation: alpha (α), beta (β), and gamma (γ) radiation. This discussion will not include non-ionizing radiation such as ultraviolet or microwave radiation. It will also include a summary of research in the development of toxicologically relevant tools for the study of this exposure.

Description of ionizing radiation

Ionizing radiation is not a substance that we can ingest, inhale, or absorb through our skin. Ionizing radiation is emitted from radioactive or radioactive contaminated materials, and these materials have the potential to be ingested, inhaled or absorbed

into the body. In worst case, radioactive materials become bound inside the body, emitting ionizing radiation into the surrounding tissue.

Mechanisms of radioisotope radiation release

Radioactive atoms, *or radioisotopes*, due to their unstable nuclear structure, will transform into another element by changing the number of protons in their nucleus. During this process of nuclear transformation the radioactive atom may emit alpha, beta and gamma radiation. Gamma radiation is also known as x-rays.

Atoms more massive than lead, which are all naturally radioactive, typically transform by emitting alpha particles. An alpha particle consists of a cluster of 2 electrons and 2 protons that is ejected from the nucleus at high velocity. The alpha particle is essentially a fast moving helium atom without the electrons. If the radioactive atom has a nucleus containing too many protons, it will be throw one of the protons out at high velocity in the form of a positively charged beta particle. Both alpha and beta particle generation processes are also accompanied by emissions of gamma rays. These radiation particles and rays carry enough energy that they can knock electrons away from atoms and molecules they collide with, thus creating ions. Inside organisms, ions can be created from molecules such as water, proteins or DNA.

Alpha particles

When an alpha particle passes near an atom, it can pull an electron away from the atom in a process called ionization. A single alpha particle can repeat this ionization process many times with many atoms. With each ionization event the fast moving alpha particle loses energy and it slows down. Because of their large mass and charge, alpha particles have the ability to ionize tissue very effectively. When the particle finally comes to rest it will take two electrons from surrounding atoms in a final, dual-ionization event and then it becomes a complete helium atom. The helium atom is inert and has no effect on the body. If the source of alpha particles is from a radioactive material that is outside of the body, the alpha particles will lose all their energy before penetrating through the human epidermis. For many organisms, the damage due to alpha radiation is a concern only if the radioactive material is ingested.

Beta Particles

Beta particles are high-energy electrons that are created with either a positive or negative charge, depending on the radioactive material that produces them. Most beta particles are negatively charged and much lighter and more penetrating than alpha particles. Some elements produce beta particles which have very little energy and can't pass through the epidermis, but most produce high energy particles that can blast through the epidermis and irradiate live tissue. Their penetrating power depends on their energy, which in turn depends on the radioactive element that it comes from. And

as with alpha particles, you can also be exposed to beta radiation from within if the beta emitting radioactive material is ingested, inhaled or otherwise finds a way inside the body. As with alpha particles, beta particles lose energy as they collide with and ionize atoms along their trajectory. When all of its energy is spent, a negatively charged beta particle becomes an ordinary electron that has no effect on the body. A positively charged beta particle will end with a collision with a negative electron, and this electron-positron pair turns into a pair of gamma rays, an event called annihilation radiation.

Gamma Radiation

Gamma radiation is not a particle but instead is pure energy in the form of very high frequency light. Radioactive atoms giving off an alpha or a beta particle during transformation may also give off gamma rays to release excess energy. These rays travel very long distances through air, body tissue and other materials, much farther than either alpha or beta radiation, and the source can be relatively far away and still create ionization exposure. Many gamma rays may pass through the body and not hit anything. But when a gamma ray does collide with an atom or molecule, the electromagnetic energy it contains can energize an electron in the atom or molecule such that the energized electron will bounce from atom to atom creating multiple ionization events within the surrounding tissue.

Radioactive material release

Radioactive material released into the atomsphere is carried by the wind and mixes with the air. If the radioactive material settles to the land or sea, it can be incorporated into the food chain. Rain and snow wash radioactive material out of the air, or dissolve it from contaminated soil that it passes over or through. Radioactive material may bind to suspended or settled solid particles and silt the bottoms of ponds, rivers or the ocean. Radioactive material may concentrate in animals and plants on land and in the sea. If this material finds its way inside the body, it can be mixed in the contents of the stomach and intestines, absorbed into the blood and deposited into living tissue. The emissions from this entrained radioactive material can cause damage to surrounding tissue during its entire journey inside the body.

Measuring affect on tissues and organs

While high levels of external beta contamination may lead to skin burns, resulting primarily from the gamma rays they generate, internally deposited radioactive material through inhalation, ingestion, or dermal pathways is generally more hazardous than external deposition. Internal exposures result in the deposition of energy to internal cells and organs. Depending on the radioactive material and exposure path, the radioactive material may be eliminated from the body within hours or it may remain there for years. Radioactive material is eliminated from the body by both the process of radioactive transformation as well as by the body's own biological removal systems.

The biological half-time (T_{biol}) is the time required for the sum of all of the available biological processes to eliminate one-half of the retained radioactivity and has the same

value for both stable and radioactive isotopes of an element. However, the biological half-time may be different from one organ to another. The time required for the mass of a radioactive element to be cut in half is the result of the combined action of both the radioactive transformation and biological elimination for the element. This is called the effective half-time (T_{eff}) described by the equation: $T_{eff} = (T_{biol} \times T_{phys})/(T_{biol} + T_{phys})$.

Dose units

In the context of radiation, "dose" refers to the fraction of the overall radioactive energy amount deposited in an organ or tissue. Absorbed dose is the energy absorbed per unit mass of the absorber. Units for measuring absorbed dose include the rad (1 rad = 100 ergs of energy deposited in 1 gram), or the gray (Gy), which is 100 rad (energy deposition of 1 J/kg). Another common unit is the sievert (Sv) where 1 rem = 0.01 Sv.

External radiation dose is obtained by multiplying the measured radiation dose rate by the exposure time. Internal radiation dose measurement is more complicated and obtained by calculating the mass of radioactive material ingested, the fraction of this material absorbed, the distribution and retention kinetics of the radioactive chemical species involved, the quality of the radiation(s) emitted by the material, the radioactive half-life of the material, as well as radiation energy transfer qualities for the tissues involved. For internal exposures, it is the effective half-life (T_{eff}) which will determine if the dose is considered acute or chronic in duration.

Gamma radiation is measured by a unit called the roentgen (R), which is the amount of air ionization the energy in the radiation is capable of producing. One roentgen produces 2.58×10^{-4} coulomb of ions per kg of air, and when exposed to tissue, is generally equal to 1 rad. In terms of toxicological study, gamma radiation would likely be measured in units or subunits of roentgens per hour.

Dosimetry models

Biokinetic dosimetry models are used to estimate internal doses of radioactive material and consider the mass of material entering the body, the factors affecting their movement or transport within the body, the material's distribution and retention in the body and the energy the material emits into surrounding organs and tissues. The dose may also be influenced by the route of entry of the material into the body. Ingestion of radioactive materials from contaminated food, water or air may result in toxicity from intestinal absorption of the material, irradiation of the gastrointestinal tract during the passage of the materials through it, or a combination of both. Absorption into the bloodstream from the gastrointestinal tract will depend on the specific chemical and physical form of the radioactive material as well as the affected organism metabolic and physiological factors, including diet and age[vi]. Inhalation is perhaps the most common route of exposure with the size of the particles being inhaled determining where they are deposited in the lungs. Retention of the material in the lungs will depend on the site of deposition, the physiological condition of the lung, as well as the specific physico-chemical properties of the inhaled material. Biological mechanisms for eliminating material from the lungs include ciliary clearance in the upper respiratory tract where large sized inhaled particles are deposited and then either coughed up or swallowed, and

phagocytosis and systemic absorption of smaller particles that find their way deeper into the respiratory tract[vii].

Biological effects

Radiation interactions within the body produce sub-cellular effects that may result in cellular damage which may ultimately produce observable effects in organs or tissues such as the skin or thyroid. Biological factors that can influence the effects of radiation exposure include the affected organism species, age, sex, location of exposure and the repair mechanisms that are naturally available to that organism. Cells are particularly sensitive to radiation damage if they exhibit a high rate of mitosis, have a long mitotic cycle or are undifferentiated[viii]. All of these cellular features describe the workings of DNA in the cell. Particularly radiation sensitive cells include stem cells, blood precursor cells, hair follicles and gut epithelium cells. Thus, DNA is a primary molecule of concern for radiation toxicity, and undifferentiated and actively dividing cells are particularly sensitive to ionizing radiation.

Molecular damage, which includes damage to the DNA, can occur in one of two ways from an exposure to radiation. Ionizing radiation can interact directly with DNA by breaking the DNA strands or un-bonding base pairs[ix]. Radiation can also ionize surrounding molecules, such as water, to produce free radicals and active oxygen species which interact with DNA and/or cell membranes, organelles, lipids and other macromolecules to produce a wide range of damages. The success or failure of the organism's ability to repair itself depends on factors such as the dose rate received and the affected tissue. If the organism cannot repair itself completely, mutations can accumulate and the result can lead to cell apoptosis, altered cellular function or the development of neoplasticity. Even if the damaged cells recover and relatively normal function is restored, DNA alterations may be expressed later as mutations and/or tumors. Damage in susceptible cell types may result in cell death, and extensive cell death may produce permanent damage to a tissue or organ and may result in the death of the organism. Because the cells of younger people are dividing and growing more actively than adults, they are much more vulnerable to radiation exposure. Rubin and Casarett[x] devised a classification system categorizing cells from the most sensitive type, such as stem cells of the bone marrow and gastrointestinal tract, to the least sensitive cell types found in muscles and fully developed nerves.

Only a few incidents of general population exposures to radioactive materials have been of sufficient size to produce quantifiable effects. These include a rise in thyroid cancer rates associated with the Chernobyl accident and a surge of childhood leukemia cases and other cancers in the general population after the Hiroshima and Nagasaki bombings[xi].

Tools for toxicological assessment

Cytogenetic analysis of peripheral blood lymphocytes has long been the standard biomarker of radiation exposure but it requires time consuming and sophisticated processes such as mammalian cell culture, chromosomal aberration detection or micronucleus assays. Minimally invasive methods would allow greater access to assessment.

The ability to distinguish cancers by profiling the constituents of serum protein was first demonstrated more than 30 years ago[xii] but only recently have technological advances led to high-throughput and comprehensive analysis of serum proteins[xiii]. While use of serum proteins is providing impressive results, blood sampling is not always feasible. If samples could be taken from urine instead of blood, they would be easier to obtain. Besides being less invasive, urine analysis has the added advantage of delivering a metabolic picture over time. Metabolites accumulate in the bladder and are collected there over recognized periods of time as opposed to the snapshot in time that a single blood sample offers. Prostaglandins[xiv], neurotransmitters and their metabolites that identify stress, including radiation stress, have been used to measure radiation damage.

These approaches to biomarker identification have been predicated on known or suspected biological effects of ionization radiation such as DNA damage or inflammation. These approaches use the methods of the field of study called *metabolomics*, as a means of measuring small-molecule metabolite profiles and fluxes in biological matrices after an event such as genetic modification or an exogenous challenge such as ionizing radiation exposure. Metabolomics is an important component of systems biology used alongside other disciplines including genomics, transcriptomics and proteomics. These multi-disciplinary areas of study have high potential for technology convergence.

An automated micronucleus assay using flow cytometry has been developed to improve monitoring methods.[xv] The ability to efficiently survey many dose levels and many more cells per specimen relative to the traditional method, which relies on microscopic examination, is particularly a benefit to studies designed to identify no observable effect levels or lowest observable effect levels that are relevant to low dose exposures. Other advanced tools such as Surface Enhanced Laser Desorption and Ionization (SELDI) Time of Flight Mass Spectrometry (TOF-MS) have been used to examine the profiles of binding that exist between large, highly abundant proteins such as albumin, to the low-molecular-weight targets[xvi] that typically bind to them. This method could also enable very useful and accessible biomarkers in blood or urine. Another innovative biomarker method uses blood plasma and investigates radiation–induced apoptosis rates using flow cytometric identification of cells displaying apoptosis-associated DNA condensation[xvii].

Conclusion

Of the major mechanisms by which low-level ionizing radiation exerts it toxic effects, macromolecules - in particular DNA - are the critical molecules that may be damaged. Damage occurs by direct ionization of DNA itself or indirectly through the formation of toxic chemicals in the body, such as free radicals. Because DNA contains critical

information for cellular function, when it is disrupted, a wide range of biological responses may be encountered including both carcinogenic and non-carcinogenic end points. Other bio-molecules, such as proteins, amino acids, lipids and carbohydrates can also be damaged. By determining the specific mechanisms by which ionizing radiation generates these end points, research utilizing convergence technology can focus on identifying and developing methods for measuring biomarkers to assess the effects of low-level radiation exposure that the Fukushima reactor release has created.

i IAEA Briefing on Fukushima Nuclear Emergency,19 March 2011, 14:00 UTC

ii Anxiety in Tokyo over radiation in tap water, Business Week, 23 March 2011

iii Kondo S. 1993. Health effects of low-level radiation. Osaka, Japan: Kinki University Press and Madison, WI: Medical Physics Publishing.

iv ICRP. 1984. International Commission for Radiation Protection. A compilation of the major concepts and quantities in use by ICRP. ICRP Publication 42. Oxford: Pergamon Press.

v BEIR V. 1990. Health effects of exposure to low levels of ionizing radiation. Committee on the Biological Effects of Ionizing Radiations, National Research Council. Washington, DC: National Academy Press.

vi NCRP. 1987d. Use of bioassay proceedures for assessent of internal radionuclide deposition. National Council on Radiation Protection and Measurements. Report No. 87. Washington, DC

vii James A, Roy M. 1987. Dosimetric lung models. In: Gerber G, et al., eds. Age-related factors in radionuclide metabolism and dosimetry. Boston, MA: Martinus Nijhoff Publishers, 95-108.

viii Casarett and Alison 1968, Radiation biology, Prentice-Hall, Englewood Cliffs, N.J

ix Mettler F, Moseley R. 1985. Medical effects of ionizing radiation. New York, NY: Grune and Stratton.

X Rubin and Casarett 1968, Clinical radiation pathology, Saunders, Philadelphia

xi Pierce BA, Yukyko S, Preston BL, et al. 1996. Studies of the mortality of atomic bomb survivors. Rep Rad Res 146:1-27.

xii Phillips RK, Dewhirst MW, Gillette EL, Clow DJ. Electrophoretic evaluation of sera from dogs with cancer. Am J Vet Res 1978;39:1482–6.

xiii Conrads TP, Zhou M, Petricoin EF III, Liotta L, Veenstra TD. Cancer diagnosis using proteomic patterns. Expert Rev Mol Diagn 2003;3:411–20.

xiv R. M. Wolfram, A. C. Budinsky, B. Palumbo, R. Palumbo and H. Sinzinger, Radioiodine therapy induces dose-dependent in vivo oxidation injury: evidence by increased isoprostane 8-epi-PGF(2 alpha). J. Nucl. Med. 43, 1254–1258 (2002)

xv Dertinger SD, Chen Y, Miller RK, et al. Micronucleated CD71-positive reticulocytes: a blood-based endpoint of cytogenetic damage in humans. Mutat Res 2003;542:77–87.

xvi Lowenthal MS, Mehta AI, Frogale K, et al. Analysis of albumin-associated peptides and proteins from ovarian cancer patients. *Clin Chem* 2005; **51**: 1933–45.

xvii Menz R, Andres R, Larsson B, Ozsahin M, Trott K, Crompton NE. Biological dosimetry: the potential use of radiation-induced apoptosis in human T-lymphocytes. Radiat Environ Biophys 1997;36:175–81.