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GUEST EDITORIAL

The ups and downs of low dose ionising radiobiology research

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I entered the world of low dose ionising radiobiology with a genetics and molecular/cancer biology background and without pre-conceptions. Almost all of the literature at the time was on high doses of radiation. High doses of radiation were proven to increase cancer risk. Radiation regulators had jobs to do and the linear no-threshold (LNT) model for regulation was workable. The problem was that LNT had only been validated at high doses. What was happening at radiation doses that were actually relevant to population exposure? Was there a threshold radiation dose below which there were no net damaging effects? There was even some evidence that a little bit of radiation stimulated cell defence mechanisms leading to a net health benefit. I discovered the emotive world of radiation and fear (regardless of the dose). There were pro-LNT and anti-LNT factions. Were CTs saving lives but giving people cancer? Was it more dangerous to evacuate after nuclear accidents than to get an increased, but still low, radiation dose? To some people, if you did not agree that LNT was a valid model at low doses, then you were clearly a proponent of nuclear power and/or part of a conspiracy theory (Say what!?). I just wanted to study the biological effects of low dose radiation exposure. I had never viewed low doses of radiation as being particularly more damaging than any other agent at low doses in the environment, nor that you could identify any one agent at low doses as being the sole cause of a cancer.

Everyone wants a simple yes or no answer to the question "Are low doses of radiation safe?" However the question requires context. Safe from what? At low doses, deterministic health effects are not detected and so only longer term outcomes are relevant, and usually cancer is the fear. How safe? Completely safe? No such thing–living is not a safe occupation. What type of radiation? Ionising or non-ionising? What dose? What dose-rate? Whole-body/partial body? Internal or external emitters? What dose is low? and so on. Each radiation scenario needs to be assessed before attempts at an answer can be given.

Before even mentioning actual doses of ionising radiation, we need to clarify how a cancer forms. The formation of cancer is a *normal* process as a part of life. We are aerobic organisms so we make high levels of reactive oxygen species (ROS) during normal metabolism in all cells. ROS are highly damaging to cellular molecules including our DNA, so we live in a catch 22 situation. We need ROS to live but at the same time they are highly damaging. The redox status of cells is very important in cancer formation and is affected by the sum of everything the cell/organism is exposed to at any one moment in time. From the time you are conceived you are on the stochastic road to cancer formation. The longer you live the more

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chance you will win the cancer lottery. The carcinogenic process is multi-step and different for every cancer in every individual unlucky enough to win that lottery because something else has not killed them before-hand. Also, the process of cancer formation is not just about DNA damage, it involves repair and surveillance mechanisms, including the immune system. So what affects this stochastic process and hence the risk of getting cancer? The chance of getting a cancer is dependent on the genes that you inherited from your parents and then what happened stochastically to all the molecules in every cell in your body, for every replication of your cells, the microbiota in your body, everything you have ever drunk, eaten, been exposed to (including infections), or done in your entire life. All of this combined can be called biological "stress". Each day our body responds to variations on this "stress" theme. The majority of us do not get cancer at an early age largely because we did not inherit strong cancer causing genes from our parents and we are well designed to cope with/counteract most of these stochastic interactions. The environment is full of low doses of potentially cancer forming agents and almost all (radiation included) have been around throughout evolution. We have evolved on a planet where we are exposed to radiation from the solar system and the earth, which is made up of a mixture of different chemicals, some of which are radioactive. They are a part of our normal environment. No agent in this world is specifically carcinogenic. *It is the dose of the agent that defines its carcinogenicity*. At high doses almost everything can make a major contribution to cancer formation if it doesn't kill you in some other way first. Even at high doses, not everyone will get cancer. If you could add up all the stochastic effects of all the stress interactions resulting from low doses of the vast mixture of everything that your body is exposed to in life, you could never determine that any particular agent started off the cancer in amongst the normal metabolic stress. It is only when the dose gets to an amount that overwhelms homeostatic responses that the rate of damage from any one specific agent can speed up the chance of getting cancer. High doses of ionising radiation, like any high dose of a potentially toxic agent (e.g. DNA damaging chemicals) will potentially overpower our defensive mechanisms. It should be noted that not having enough of some agents in the environment can also result in being outside homeostatic boundaries. Too little of most agents in the cell environment can put things out of balance in much the same way as too much.

So what is the lowest dose of radiation that overwhelms the homeostatic system and if it does, does it increase cancer risk? Research in the low dose radiobiology area has been trying to answer this question but studying low doses of anything has inherent difficulties and requires very sensitive experimental systems. According to the US and European low dose radiation research programs a low dose of radiation has been defined as <100 mGy of low linear energy transfer (LET) radiation. Therefore, much of the research has been based around trying to look at, or just below, 100 mGy low LET radiation. This cut-off was chosen because there is no evidence for increased cancer formation below 100 mGy exposure in the atomic bomb survivors. Much of the low dose radiation research has been conducted in highly artificial in vitro experimental systems which are much cheaper and easier to perform than studies in animals. These studies can inform on aspects of mechanism but they are far too often extrapolated to the much more complex situation of a health endpoint in whole animals. For example, DNA mutations can be studied in vitro and in vivo, but the two are not identical in terms of health outcome, as in vitro systems only provide some of the damaging/defence mechanisms existing in vivo. At high doses, in vitro and in vivo comparisons correspond more closely because the response is so high that any biological system will be in SOS damage mode. The effect is so large that stochastic detrimental effects are now so frequent that the outcome is clearly going to be damaging.

If studying a more directly relevant end-point such as cancer, we need to use animal studies or human epidemiology. When studying low doses of anything, epidemiology studies are plagued by confounding factors. Given the enormous genetic variation in the population, the high background cancer incidence, the inability to determine the exact dose of radiation and also other agents that individuals have been exposed to—the number of people required to be studied is limiting (millions). It is unlikely that epidemiology studies will be able to answer the low dose question below 100 mSv or to chronic low dose-rate exposures at somewhat higher doses, i.e. the doses most relevant to public exposure. There are some long-term mouse studies that are particularly interesting where single low doses and/or chronic low doses of radiation have been shown to increase life span, reduce diabetic and atherosclerosis symptoms and protect from cancer caused by higher doses of radiation [1–5]. Most of these types of studies are performed in animals that are prone to a disease type or have also been exposed to a high dose of radiation as well, and so the effects are particularly striking. The doses and dose rates used vary between studies, with doses around or below 100 mGy broadly seeming to define doses that can be protective. These types of experiments are very expensive and time-consuming and are only performed in a very few, and unfortunately, ever-decreasing number of laboratories around the world. This is an important area of research which needs expanding as it is the most likely to be able to help answer the low dose questions from a health end-point. Animal studies have the advantage of reducing

variation in end-point data as everything else including genetic composition, diet, temperature etc., can be controlled, so that the effect of a specific agent such as radiation can be studied in isolation. Although it is agreed that "mice are not men", whole mammals do eat, drink, reproduce, have similar organs with similar functions and the all-important immune system. The health end-point in experimental whole animal models incorporates these essential requirements for potential extrapolation to humans.

The new genomics techniques are revolutionising biology and contributing to our knowledge on low dose radiation effects. We can now examine each DNA molecule, each RNA transcript, and each protein in cells. The amount of information from these experiments is enormous and we do not, as yet, have the knowledge to interpret the vast majority of the down-stream interactions, nor the long-term effects of these changes, into a specific health end-point. We have learnt that the sets of genes responding to low doses of radiation are largely different to the sets of genes responding to higher doses of radiation [6]. In other words, low doses are not just less of a high dose response. They are different responses. The initial responses may be similar or dissimilar but the downstream responses are not similar. This is interesting, but it does not tell us whether these doses cause cancer or not. Certainly many of the genes responding at low doses are involved in many of the pathways that we know are important in maintaining homeostasis. Once homeostasis is no longer maintained then disease can result. Again these experiments are usually confined to in vitro experimental protocols.

A number of low dose radiobiology experiments both in animals and in vitro identified responses that are termed as low dose radiation effects. Terms like radio-adaptive response (where cells respond to low doses of radiation in a manner that prepares the cell to be able to defend itself against subsequent doses of radiation) and bystander effects (where cells which have not directly received radiation respond) have become mainstream buzz-words in radiation research. These terms do not necessarily describe separate processes, nor are they necessarily radiation specific, and the term bystander has often been used in rather a confused manner in the literature [7, 8]. Effectively they are alternative words for outcomes from intra- and inter-cellular signalling. This is not a new concept but because it involves radiation it seems to be erroneously considered unique from other biological processes, just as low dose radiation is considered to be unique in its effects compared with other potential carcinogens. As doses of radiation get lower and lower, low dose radiation research likely becomes part of mainstream redox biology research. Cell signalling is mainstream to all processes that go on in our bodies in response to everything that we come into contact with throughout life. It is important to note that low doses of radiation can not only change the subsequent response to high doses of radiation but also to other forms of stress such as chemical exposure. Therefore, the low dose radiation-induced response can change the response to other agents in the environment just as any change in response to any agent will affect a multitude of biological pathways, and interact with responses due to everything else added into a biological system. In vitro end-points possibly point towards some adaptive and bystander responses being potentially protective or detrimental. However, again these end-points cannot be directly extrapolated to health effects. The one area where this has been achieved is in the adaptive response animal experiments where a health endpoint, including cancer formation, has been studied.

The adaptive responses observed with low doses of radiation forces one to think of dose responses in more than one direction. Hormesis describes dose responses where the effect of an agent differs for an end-point at different doses, not just in magnitude but in direction. Hormesis is not the exception, it is the norm [9]. It is only active for a limited period of time in response to each stimulus but can affect stochastic processes during that time. Radiation, like almost everything you can test that is in the environment exhibits hormesis, where low doses relevant to normal population exposure show different responses to high doses of the same agent for many endpoints. Hormesis is almost always identified if the experimental design of studies covers a large range of doses and the endpoint studied can be detected both above and below endogenous levels. Many experiments that do not show hormesis, would not be able to detect hormesis under the experimental designs used. However, again, hormesis should not be interpreted as being beneficial or detrimental unless a health end-point can be clearly demonstrated. A health outcome is exemplified where a low dose of radiation can increase the time to formation of cancers in mice induced by a high dose of radiation. The animals given the low dose of radiation still got cancer but it took longer for the cancers to form, therefore there was presumably protection from an earlier stochastic event. There is some evidence that low dose chronic radiation stimulation is more effective in sustaining the adapting radiation effects over time, possibly by protecting from a greater number of stochastic events over time. The major outcome of interaction of low or high doses of radiation with biological material is ionisation of water to form ROS. There is substantial evidence that low doses of radiation can induce anti-oxidant responses which can decrease stochastic ROS effects and could explain the low dose induced protection from cancer. Levels of ROS are important in the immune system for fighting infection and also for recognising and removing pre-tumour cells as part of our natural anti-cancer defence mechanisms. Low

dose radiation has been shown to upregulate this process in vitro with potential protective effects [10]. One of the very exciting areas of low dose radiobiology research is the study of living organisms when they are placed in areas with extremely low background radiation. Studies are being performed in underground laboratories where the level of radiation is well below the average background radiation levels. This allows study outside of the normal homeostatic boundaries due to lower (vs higher) radiation exposure than a biological system is used to. Changes in the ability of cells to respond to DNA damage have been identified in vitro in these types of experiments. Cells at very low background radiation exposure are less able to respond to stress in the environment; their homeostatic boundaries have been reduced and anti-oxidant pathways are involved [11]. It will be important to perform these types of experiments in in vivo models to determine what effect lower than average background levels of radiation has on cancer and other health end-points.

So, back to the main question of whether low doses of radiation that the public are exposed to are safe? At the end of the day there is no consistent significant detrimental change in a health end-point for doses in the 1–100 mGy range. Very little research has been performed at lower doses than 1 mGy. The extraordinary aspect of these data is that the dose-rate used in studies for these doses varied enormously. So is a 10 mGy single exposure CT scan having the same overall biological effect as 10 mGy spread over a year of the same or a different type of ionising radiation? It depends on whether the dose/dose-rates fall within the homeostatic boundaries of the cell/organism. This is again an area requiring systematic research [12]. In other words, no statistically significant health effects at these low doses that are relevant to public or occupational exposure have been categorically or consistently observed. This is not to say that stochastic effects are not happening (because they are), nor that there is not an increase in cancer risk. If there is an increase in cancer risk then it is too small to see statistically. It is interesting that the upper limit of 100 mGy for low dose radiation research is at the upper end of yearly background radiation doses across the planet. Perhaps biological homeostasis can handle these types of doses.

Background radiation levels, involving many different forms of radiation as well as other agents in the environment, vary enormously due to geography. When we travel, we do not ask what the background radiation level is at our holiday destination, nor if moving house to another area or country. So presumably we are happy to double or triple our radiation exposure for short to long periods of time, because it is "natural". Also, unlike most low dose radiation experiments, these changes in environment result in a change in low doses of a variety of different types of radiation based on differences in geography. Every day we eat a multitude of foods. All foods have the potential to affect metabolism (and hence ROS) and contain a myriad amount of very low amounts of potentially carcinogenic agents, some of which are radioactive. We are restricted in how much food we can physically eat at any one time and so we are, to some extent, limited in the dose-rate at which we take up chemicals via food. The food ingested across the planet by individuals varies enormously both in type and amount over their life-time. There is no logical rationale for people to be worried about highly regulated negligible radiation from industry while eating unregulated amounts of food and/or chemicals/supplements from the "health-food" shop (containing doses that are potentially much higher than background doses of these chemicals in food). At really high and really low doses these agents (radioactive or not) are potentially deleterious in some way or other. Vitamins and minerals are the classic example—too much or too little can cause disease. We need to find a chemical/molecular (radioactive or not) composition that comprises the "happy medium" for a healthy life.

We live in a world where radiation plays a significant role in the quality of our daily life including via medical procedures and in many aspects of industry. It is time to step back, clear the mind of dogmas and put low doses of radiation into perspective in terms of safety. Hopefully research on low dose radiobiology will expand our knowledge in this area but what of the attitude of the media and the public? The fear of low dose radiation appears to far outweigh the fear of low doses of other potential carcinogens in the environment. This is largely historical. In this context medical practitioners and allied health workers can often be included in this group. Communication of risk is a major challenge in this area. Cancer (C) arises in an insidious manner and the C word is as scary to most people as the radiation (R) word. Put them together and you have a powerful dread. Several key messages need to be conveyed: cancer formation is the product of every molecule encountered throughout life and that no one agent at low doses is solely responsible for causing cancer, or preventing cancer, for that matter; radiation is present naturally in the environment and is handled within normal biological homeostatic mechanisms, as are non-radioactive substances in the environment at low doses; medical radiation doses to the population far exceed doses from industry, and there are benefits to the use of both. As scientists we need to deal with the facts and not be driven by dogmas. At present we cannot pick low levels of radiation that are completely safe, because there is no such thing as completely safe, but from the evidence so far the risk is low. Meanwhile, low dose radiation research has identified some fascinating biological responses where low doses of radiation may act like an immune system, sparking off mechanisms where either short-term or long-term protective effects are observed in health end-points including cancer, diabetes and heart disease in animals. These pathways should be further explored and potentially harnessed.

Perhaps we should be asking the question: is protecting from low unknown risks of low doses of radiation any more important than protecting from low doses of anything else in our environment, or protecting from larger known health risks in our community?

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