CHAPTER 15

RADIATION RISKS IN THE CONTEXT OF MULTIPLE STRESSORS IN THE ENVIRONMENT – ISSUES FOR CONSIDERATION

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Abstract: The field of multiple stressors is highly complex. Agents can interact in an additive, antagonist or synergistic manner. The outcome following low dose multiple stressor exposure also is impacted by the context in which the stressors are received or perceived by the organism or tissue. Modern biology has given us very sensitive tools to access change following stressor interaction with biological systems at several levels of organization but the effect-harm-risk relationship remains difficult to resolve. This paper reviews some of the issues, using low dose ionizing radiation as a common stressor and chemicals known to act through similar mechanisms, as examples. Since multiple stressor exposure is the norm in the environment, it is essential to move away from single stressor based protection and to develop tools, including legal instruments, which will enable us to use response-based risk assessment. The problem of radiation protection in the context of multiple stressors includes consideration of humans and non-humans as separate groups requiring separate assessment frameworks. This is because for humans, individual survival and prevention of cancer are paramount but for animals, it is considered sufficient to protect populations and cancer is not of concern. The need to revisit this position is discussed not only from the environmental perspective but because the importance of pollution as a cause of non-cancer disease is increasingly being recognized. Finally a way forward involving experimental assessment of biomarker performance coupled with modeling is discussed.

Keywords: multiple stressors; radiation; low dose exposure; biomarkers

Introduction

Biological systems are highly complex. Modern biology has given us very elegant tools for investigating these systems and understanding mechanisms but where decisions have to be made about the safety of radiation or chemical

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pollutants in the environment, it becomes very difficult to determine the relationship between a detectable effect in a system, and the ultimate consequence for the organism or population, of that insult. This relationship is particularly obscure where the level of exposure to the agent is very low or when multiple agents occur in the system under examination. Much of the uncertainty surrounding the risk of exposure to low doses of single or multiple stressors is due to this inability to determine risk associated with molecular effects. Depending on the perspective of the "stakeholder" molecular effects can be interpreted as highly dangerous or just natural responses to environmental perturbations. Clearly environmental pollutants are not going to disappear so it becomes important to find objective methods for linking effects with risks and also to find regulatory and legal mechanisms for dealing with low doses of multiple pollutants. This paper will address some of the issues which complicate the field and lead to the uncertainty. It will then suggest possible approaches to solving the problems.

Mechanism Issues

It has been known for a long time that low doses of single agents can have fundamentally different biological effects to high doses. As early as 1500 Paracelsus famously said

> *"Alle Ding' sind Gift und nichts ohn' Gift; allein die Dosis macht, das ein Ding kein Gift ist."*

"All things are poison and nothing is without poison, only the dose permits something not to be poisonous."

In environmental protection, it is common to discuss four levels of "dose" of single toxic agents (see Figs. 1a and b), ranging from no effect doses through doses where organisms can accommodate the toxin, through to doses causing reversible damage and finally doses causing irreversible or lethal damage. The grey areas of concern in the multiple stressor field are the boundaries between the categories and how these might be changed if more than one stressor is present.

A less appreciated concern is that the actual mechanisms operating at low doses may not be in a continuum and that mechanistic switches may operate at specific dose thresholds. How these switches might be affected if multiple stressors are present is unknown. Similarly, many low dose non-targeted effects show saturable responses i.e. the dose response relationship is linear initially but then plateaus. The data for the direct dose v bystander effect for radiation is shown in Fig. 2, adapted from Seymour and Mothersill, (2000).

There is a clear saturation of the bystander effect at low doses while the direct effect is not obvious until a dose of 0.5Gy has been delivered.

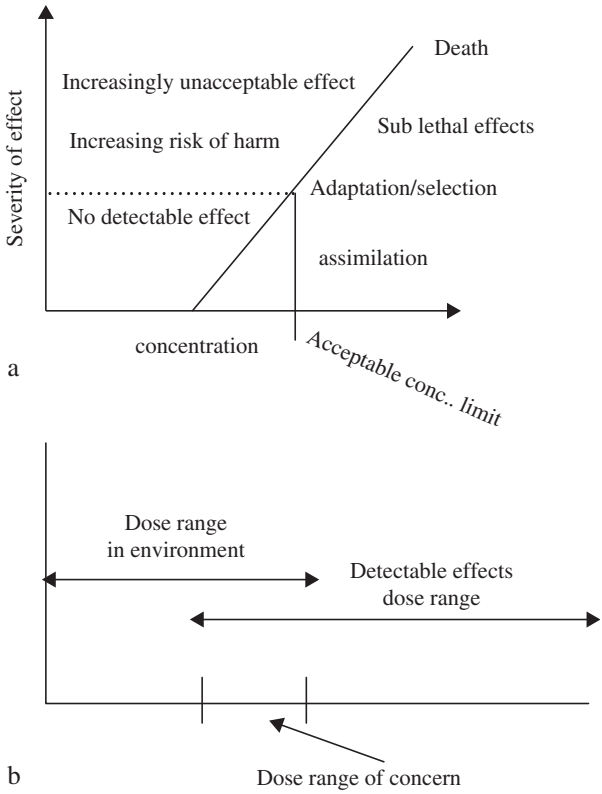

Fig. 1. (a) Levels of dose response categories for mono-stressor regula*tion.* (b) Simple model of mono-stressor protection

Increasing direct radiation then shows a linear dose response relationship. A key question is whether this saturable low dose bystander effect could lead to apparent "protective" effects if two stressors operating by similar mechanisms are present? Stressor 1 might fully use all the receptors or induce the maximum response which the system can produce, meaning that stressor 2 is not seen as inducing any additional effect. This is where the link between effect and harm or risk is critical to understand because "no additional effect" does not mean "no additional harm". The harm could be as a result of the system switching to a new level of response which may not be the one being measured.

Another issue is that of the "critical compound" in the mix. Since some stressors are much more toxic at a given dose than others, it will be necessary to devise "iso-effect" curves linking stressors in terms of some indicator of effect/harm/risk. This was done for single stressors by our group (Mothersill et al., 1998) using the induction of delayed cell death, which is associated

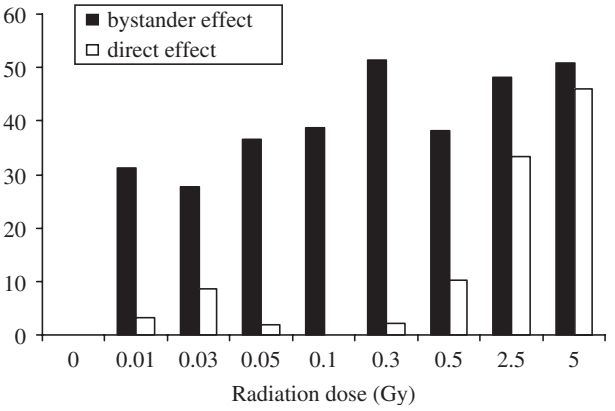

Fig. 2. Direct v bystander effect

with genomic instability (Morgan, 2003), as an endpoint. A useful approach may be to adopt the concept of "relative biological effectiveness" used in radiation biology. This allows doses of different radiation qualities to be added by applying weighting factors to the more toxic forms of radiation. The approach is controversial since it assumes a single mode of damage for all the radiations and that the same weighting factor can be applied for a variety of different endpoints of effect, which is probably not true.

Another mechanistic issue is the role of "enabling mechanisms" such as genomic instability or bystander effects (Morgan, 2003; Lorimore and Wright, 2003; Mothersill and Seymour, 2006). These mechanisms can be induced by one agent and make mutations or apoptosis etc much more likely if another agent is experienced by the system. There are concrete examples of this (Lord, 1999; Hoyes et al., 2000, 2001; Lord and Woolford, 2002; Barber et al., 2006; Barber and Dubrova, 2006) although the stressors were not applied at the same time or even to the same generation, again highlighting the complexity of this area.

Hormesis is a very controversial area but one which must be considered in the multiple stressor field. Hormesis is defined as beneficial effects occurring after low dose exposure to agents which are toxic at high doses. Calabrese has reviewed this field extensively in over 230 papers (e.g. Calabrese and Baldwin, 2001; Calabrese, 2005), and has concluded that beneficial effects at low doses are the norm not the exception. What will happen when multiple stressors are present is unknown. Hormetic mechanisms are thought to include adaptive responses, such as DNA repair induction, which condition cells or organisms making them more able to respond to stress. However, immune system stimulation or metabolic stimulation are also likely mechanisms (Sato et al., 1984; Boonstra et al., 2005; Sakai, 2006). A critical element in the

context of multiple stressors must be whether the stressors are all present at the same time or whether one stressor can make the system less or more vulnerable to other agents. There is considerable evidence for reduced effects in the adaptive response field (Broome et al., 1999; Mitchel et al., 1999; Hall et al., 2000) where for example, heat stress can adapt cells to subsequent radiation stress and vive versa. The best evidence for adverse effects occurring is the transgenerational evidence already alluded to where exposure to of parents to ionizing radiation made the progeny more likely to develop cancers in response to a chemical carcinogen.

Apart from the implications of multiple stressor mechanisms for risk assessment, it is important to continue efforts to understand how multiple or single stressor non-targeted low dose effects happen and whether it is possible to modulate them. In the radiation field progress in this area has been reviewed extensively and oxidative stress is now known to be a key cellular effect, which perpetuates non-targeted effects (Morgan, 2003; Little and Morgan, 2003; Prise et al., 2006; Mothersill and Seymour, 2006). Antioxidants work at the cellular level and in tissue models to reduce non-targeted effects of ionizing radiations (Dahle et al., 2005; Prasad, 2005; Seymour et al., 2005; Konopacka and Rzeszowska-Wolny, 2006; Lyng et al., 2006) although there are no data available about their effectiveness in vivo. With chemicals there are problems conclusively demonstrating non-targeted effects because the persistence of the chemical cannot be excluded and therefore it is difficult to distinguish between true delayed or non-targeted effects and effects due to residual chemicals. However, there are convincing data from Glaviano et al. (2006) who looked at Chromium and Vanadium induced delayed chromosomal damage over 30 days in the progeny of cells originally exposed for only 24 h to sub-toxic doses. Other evidence comes from work by our group in collaboration with Salbu's group in Norway (Mothersill et al JNER submitted). Here rainbow trout were exposed to sub-toxic levels of aluminum and cadmium the exposed to low doses of Cobalt 60 gamma rays. About four tissues were examined for production of bystander signals using a reporter system. Results (ms in preparation) show that the metals interact with radiation but the manner of the interaction (synergistic, additive or antagonistic) varies with the tissue and no overall universal pattern is seen. Again this is not surprising from the biological standpoint but it complicates regulatory issues!

Possible Biomarkers

By implication, a move away from dose driven mono stressor regulation, means that biomarkers of exposure or response must be selected and validated. Following multiple stressor exposures, response biomarkers are

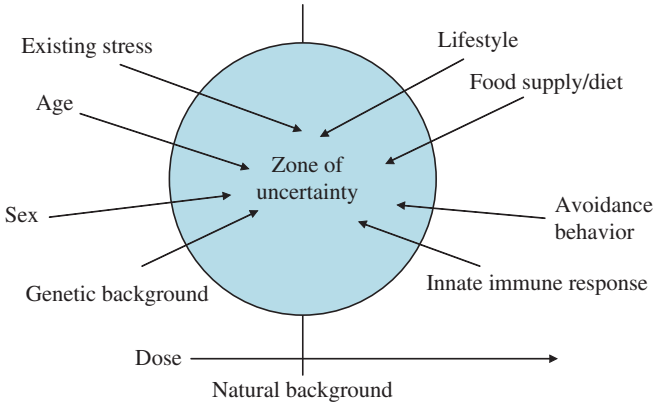

Fig. 3. Factors influencing outcome and uncertainty in multiple stressor scenarios

likely to be more useful and easier to validate because otherwise, causality becomes an issue while when monitoring response, it is possible to define a cell/tissue/organism as, for example, "stressed" without having to identify which of the stressors contributed most to the stress. An important caveat when looking for multiple stressor biomarkers is to accept that a biomarker is probably only good as an indicator of one aspect of the response and results cannot be extrapolated to generalize from for example a biomarker such as elevated ROS to carcinogenic activity or mortality. Validating biomarkers as being relevant and meaningful requires their use in situations where the link between the stressor and the response is already known. Alternatively it is useful just to know a system has been stressed. This could provide first line screening for adverse multiple stressor effects. Generic stress biomarkers at the cellular level could include elevated ROS, P450 up-regulation, calcium influx, mitochondrial membrane depolarization effects and elevated apoptosis. These effects all occur after low doses of many stressors (Mothersill and Mullenders, 2006). They do not however indicate risk, merely that the system has induced a cellular stress response. They are highly dependent on genetic background and on "context" i.e. other environmental factors such as lifestyle, diet and existing health factors (Fig. 3). At the organism level, generic stress responses include elevated cortisol and immune system effects (e.g. Roberts, 2000; Bilbo et al., 2002; Yang et al., 2002). Stress responses at population/species levels are generally behavioral or related to fecundity but discussion of these is outside the scope of this review.

A Possible Way Forward

As discussed earlier, what is needed in the multiple stressor field is a way to determine the risk of low doses of chemicals and radiation for human health and environmental health in its broadest context. The problem is that the new non-targeted effects dissociate dose from risk and there are no simple ways to determine the relationship; i.e.

- Dose is not proportional to effect even at the cellular level
- Effect is not proportional to harm even at the organ level and
- Harm is not proportional to risk at the organism level
- When moving to the environment, none of the above are simply related to survival at the ecosystem level.

In addition to this, the interactions are so complex and the species and stressors involved so diverse that really only a modelling approach can produce testable hypotheses. We propose a tight interaction between experimental biologists and modellers and suggest the approach outlined below as an example using the bystander effect as a test "biomarker of generic stress response at the cellular level.

Modeller–Experimentalist Interactions

To develop models which can predict multiple stressor risk is highly complex and needs to be broken down into a series of sub models. For the purposes of this review we have decided to use the bystander effect induced by radiation as an example and have attempted to define what data are needed in order to allow modelers to develop meaningful models.

Bystander effect example

In the specific case of what the bystander effect might do to the dose – risk equation

1. We need many more phenomenological experiments, repeated in different labs with different or similar systems
2. We need negative results presented and discussed. Much information is lost when negative results are not reported. For example, often enhanced survival following bystander protocols is dismissed as "no effect" or "system not responding or working". Significant effects of any sort are bystander effects.

The working model developed by our group for the bystander effect is shown later (Fig. 4).

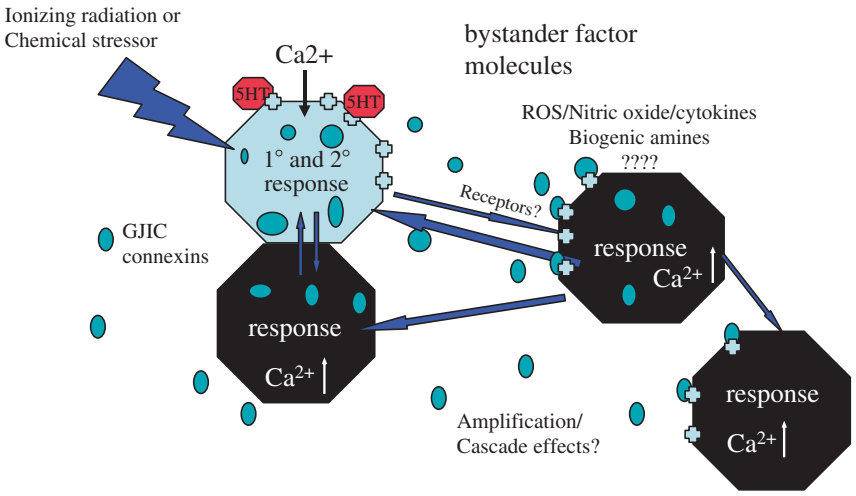

Fig. 4. Model of the bystander effect

This is an experimental model based on all we know from experiments done by our group using medium transfer techniques to test for the presence of the signal. Using this we can develop testable hypotheses and get pointers for the questions which need to be addressed. Some obvious ones are listed below as an example of how this approach might be useful.

Specific examples of data needed in order to model bystander effects:

1. We need quantitative data for several cell lines manifesting different types of bystander effects, specifically we need data about the signal strength in relation to cell number irradiated in defined media volumes. This appears only to have been done once in one cell line (Mothersill and Seymour, 1997). This is needed to determine irradiation volume – likely signal production relationship.
2. We need experimental data concerning dilution effects of signal in media – no data are published concerning this – our own unpublished data suggest even a 1:1 dilution completely loses the effect. This again relates to volume of exposed tissue/blood.
3. We need comparative and recent studies to establish if clastogenic factors and bystander effects are the same phenomenon. This would mean that the old studies of persistent clastogenic effects dating back to 1921 (reviewed in Marozik et al., 2007) could be included in modeling analysis.
4. We need to dissect out the signal production from the response to the signal in a quantitative way. This would allow models to be validated if inhibitors of production/response could be identified and effects quantified in stochiometric ways.

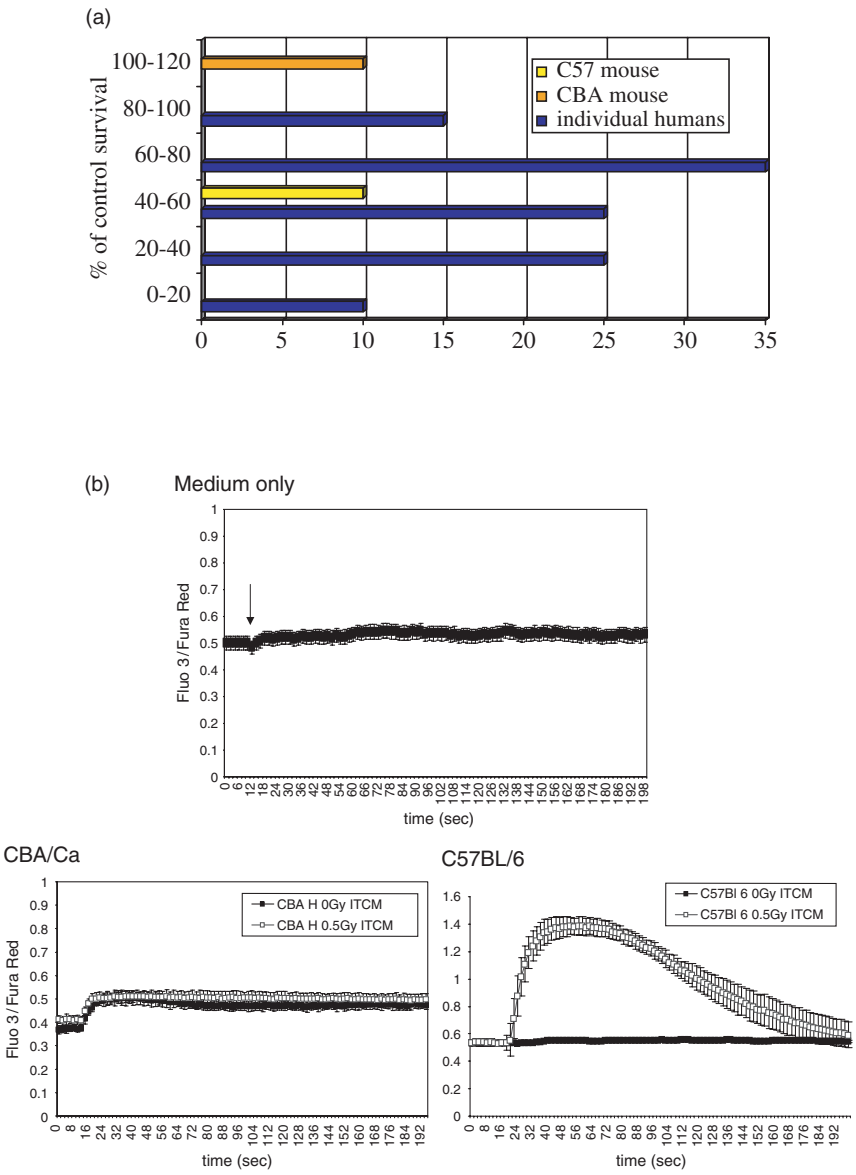

Fig. 5. (a) Individual/genetic variation in the cytotoxic properties of bystander medium (b) Calcium ratios in control and 0.5 Gy TBI CBA/CA and C57BL/6 mice

5. We need to look at bystander effects in animal strains know to be at risk of radiogenic cancer (or known to be resistant). These studies need to be quantitative and done at different doses including low and environmentally relevant doses. CBA strains and C57Bl 6 are obvious starters (see data adapted from Mothersill et al., 2005 Fig. 5a and b) but knockouts would also be good. Mix match experiments with these would also feed into point 4.
6. We need reporting of "positive bystander effects" these tend to get dismissed as negative results. They probably are real effects. Again mix match experiments would be valuable to allow "weight" to be attached to the relative importance of signal and response.
7. We need data about tissue specific bystander effects and whether signals produced by irradiation of one tissue can induce effects in other tissues
8. We need to confirm and extend studies suggesting that bystander effects "drive" genomic instability. This introduces temporal terms into the risk model
9. Signal production may be independent of dose but is not likely to be independent of target cell number or receiving cell number. It is likely that thresholds exist for molecules of signal needed to trigger target cell production of signal and recipient cell response. Quantitative studies needed.

Modelling multiple stressors could follow this type of approach and could help experimentalists define their experiments in a way which would actively support the development of testable models.

Conclusions

This paper discusses several issues relating to the management of risks associated with low doses of radiation. It suggests biomarkers which may prove useful as generic stress markers and proposes, using bystander effects as an example, a new approach to modeling multiple stressor risks using close interactions between experimentalists and modelers so that testable hypotheses can more easily be formulated.

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