Bystander effects induced by "Multiple Stressor" exposure to sublethal radiation and heavy metals in Atlantic Salmon

Seymour C.B[1] Mothersill C[1]., Denbeigh J[1]., Smith, R.W[1]., Heier L.S[2]., Teien H.C[2]., Rosseland B.O[2]., Oughton D.H[2],. Salbu B[2].,
[1.]McMaster University, Ontario, Canada, [2]Norwegian University of the Life Sciences, Aas, Norway.

Summary

These experiments were designed to identify cellular effects in 3 key organs in Atlantic Salmon (Salmo salar, L.) after exposure in vivo to very low doses of radiation, and subtoxic levels of aluminum (Al) and cadmium (Cd) alone or in combination. The data suggest that using bystander signal toxicity as an endpoint in a reporter cell line, sub-additive effects are most commonly seen following mixed exposures.. It is not clear if these result from saturation effects or represent toxic effects of one agent preventing the other from inducing apoptotic pathways.

Introduction

It is well known that pollutants are seldom present in the environment as single agents. Usually multiple agents are present, often at levels which all individually fall below action levels. Yet almost nothing is known about how these agents might interact. Increasingly it is becoming apparent, especially in radiation biology that low levels of radiation cause fundamentally different effects to high level (1). Most low dose effects are adaptive or induce protective proteomes. Other effects may be more sinister such as genomic instability and microsatellite mutation induction. Genetic background and epigenetic effects are known to be important (2-5). The study aims to examine multiple stressor effects directly using a fish model system. Direct effects on the tissue outgrowth and induction of non-targeted effects are both measured in the assay.

Materials and methods

The salmon (approx. 35g) were exposed to doses of 4, 18 and 75 mGy doses of gamma radiation, respectively, administered over 5 -48 hrs in untreated lake water or with Cd, Al or Cd+Al added to lake water. Six fish per group were sacrificed after exposure and the head kidney, fin and gill were dissected and sent for tissue culture. The Al Cu and Cd speciation in the water and the levels in fish gills were measured as well as physiological parameters associated with stress.

Small explants of each tissue were set up as tissue cultures using RPMI 1640 medium supplemented with serum, insulin, hydrocortisone and antibiotics. After 2 days, the culture medium was harvested and filtered then placed on a reporter cell line for determination of stress signal activity (bystander effects). The explant was grown on and stained for expression of pro and anti-apoptotic proteins using immunocytochemistry.

The scheme for the experiments is shown in Fig 1.

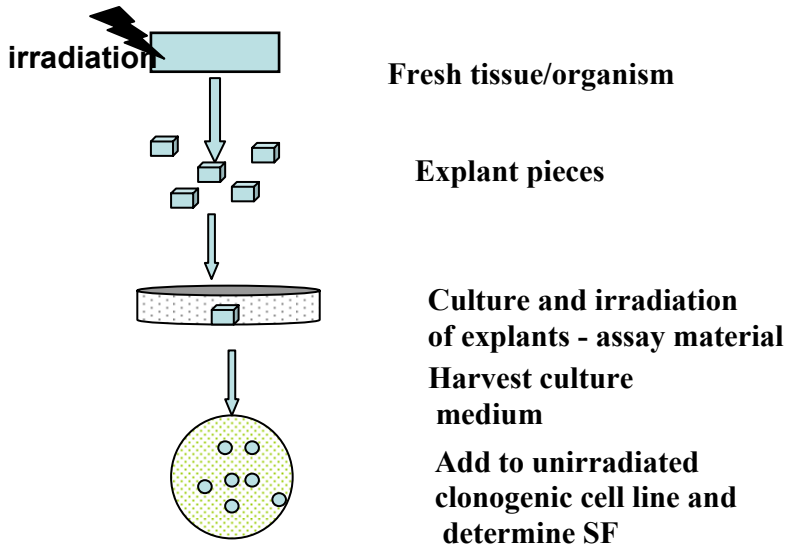


Figure 1. Experimental design for experiments with radiation and metals

Results and Discussion

Radiation doses as low as 4 mGy alone or in combination with Cd and / or Al, (Fig 2) caused bystander signals to be produced in tissues harvested from in vivo exposed salmon.

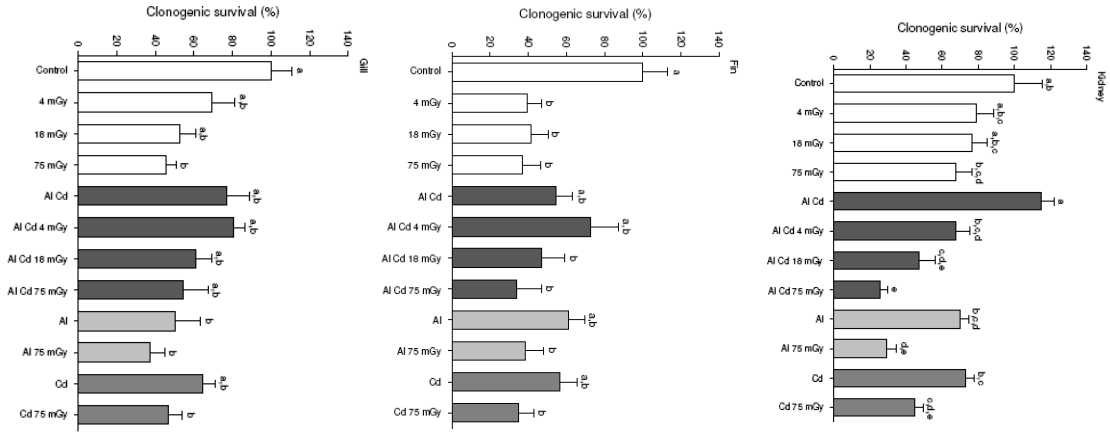


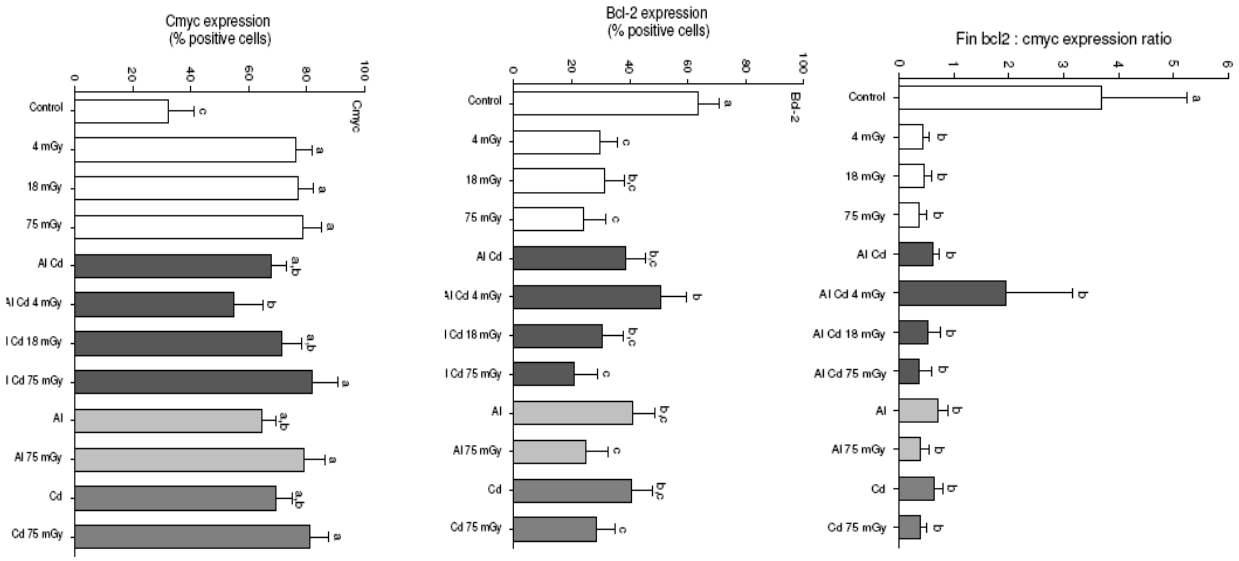
Figure 2. Toxicity of the bystander signal, measured as a reduction in the clonogenic survival of a reporter cell line exposed to culture medium harvested from explants of tissues removed from in vivo exposed salmon

The effects vary between different organs and are not consistently additive or synergistic for a given treatment. Individual results were recorded for each individual fish bu

t there was no consistent effect. Tissue type appears to be critical, with gill cells showing high degrees of synergism between radiation and metal exposure

Most data for Cd suggests that lower toxicity is found when the metal is used in combination with radiation exposure. This may be due to competition between calcium and Cd since calcium is a key component in the signal transduction pathway being followed. Bcl 2 which is an anti-apoptotic protein and cmyc which in this system is pro-apoptotic, correlated very well with tissue response (fig 3) suggesting they could be excellent bio-markers.

Figure 3. Expression (% positive cells) of Bcl 2(3a), cmyc (3b) and the ration of bcl 2/cmyc (3c), in fin tissue harvested from salmon exposed in vivo to low doses of radiation and metal combinations.



Whatever the mechanism for the subadditivity, the result raises an important point that additivity cannot be assumed, as is current practice if multiple stressors are considered atall. These data suggest antagonistic and synergistic effects may occur. They also point to the possibility that what appears to be an adverse effect of one agent may in fact be protective (eg apoptosis induced by low dose radiation) but that this effect may be negated by the presence of a second stressor such as a heavy metal which interferes with the signaling cascade.

Either way the results indicate that this stress signal response will be a useful indicator of combined environmental stress in species inhabiting aquatic ecosystems.

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