Annales de l'Association belge de Radioprotection, Vol.34, n°2, 2009
Annalen van de Belgische Vereniging voor Stralingsbescherming, Vol.34, n°2, 2009

THE BYSTANDER EFFECT.

A NEED TO CHANGE THE RADIATION RISK ESTIMATION ?

L. de Saint-Georges
Molecular and Cellular Biology
Institute of Environment, Health and Safety
Nuclear Research Centre (SCK•CEN)
Boeretang 200, B-2400 Mol, Belgium

April 2009

Abstract

Recent studies have provided evidence of bystander effect. The bystander phenomenon has been tentatively linked to an elevated risk of health effect at low dose in human (cancer, congenital abnormalities, neurological disease and hereditary effects) but none of those health effects has so far been scientifically shown to be associated with such radiation-induced effects. The possibility cannot be excluded but remains purely speculative. Further investigations are needed to clarify the nature and the importance of the bystander effect for the risk estimation in the low dose range.

INTRODUCTION

There is no doubt about radiation biological effect at high doses. Such effects for which a clear dose-effect relationship exists are called deterministic effects.
At low dose, whatever the low dose received, if a cancer should appear, the severity of the effect is not questionable and it is the probability of having the effect which becomes of concern. Such effects are called probabilistic or stochastic. The **risk characterization** is the estimation of the incidence and

severity of the adverse effects likely to occur. The **risk estimation** consists of the quantification of that likelihood. In this purpose any low dose effect should be carefully evaluated.

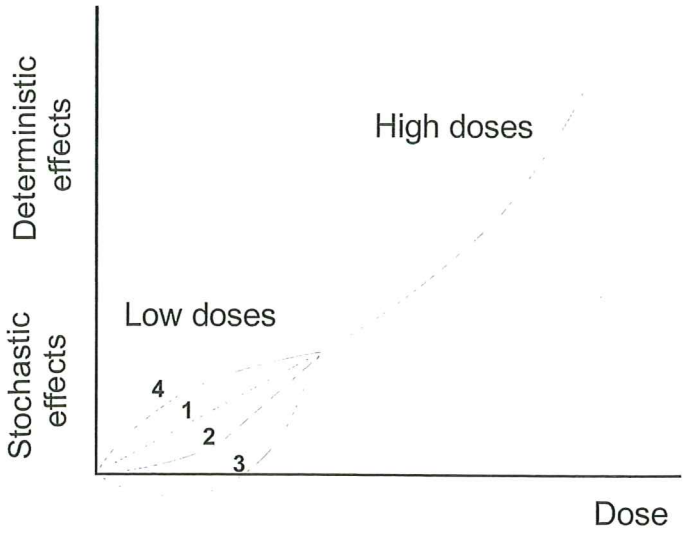
The problematic of studying the low dose effect resides in the statistical power of the studies. Indeed, the lower the doses, the lower the probability of a stochastic event such as chromosome aberration, mutation or cancer. The subsequent lack of evidence could indicate that either there is no harmful effect of radiation at such low levels of radiation or that the health effects, whatever they may be, are too few to be statistically significant.

To develop estimates of tumor frequencies at low radiation doses, it is necessary to extrapolate from responses at high dose. Different possibilities are to be considered: The choices generally are Linear Non Threshold, LNT (fig.«1»), non linear (fig.«2»), threshold (fig.«3») or greater than linear (fig.«4»). The Linear Non Threshold hypothesis estimates that the risk decreases when the dose decreases but the risk is never nil since a dose zero is impossible. (Natural radiation background). Non linear and threshold are respectively relevant for adaptive response and hormetic effect and would indicate that LNT is overestimating the risk. Greater than linear (fig.«4») indicates that LNT is underestimating the risk. The challenge in radiobiology is to establish which dose response curve shape best fits the tumor estimates at low doses.

Clearly any one of these three approaches has its own inherent sources of error and suppositions.

THE BYSTANDER EFFECT

The major concern in the low dose exposure range is the increased risk with increased radiation dose. The conventional approach is to consider that at low dose only some cells in our body are hit by the radiations, that their total number is dose dependent and that the probability or the risk to get



a cell transformed (a cancer cell) depends on the number of the cells being hit.
Recently several works (1, 3, 4, 6, 7, 8, 9, 11) have shown that at low doses, if effects are observed in radiation hit cells, effects also can appear in cells not directly hit by radiation but damaged by signal sent by neighboring cells. Such effects are referred to as untargeted effect or bystander effect.

Bystander effect would suggest that the target for radiation is larger than an individual hit cell and that a linear extrapolation of risks from high to low doses could underestimate the risk at low dose (fig.«4»).
Bystander effect has been demonstrated, especially after high-LET exposure, with various biological end points, chromatid exchange (5, 11), clonogenic survival (8, 10), micronucleus induction (13, 15), chromatin damage (14), chromosome aberrations (11) and apoptosis (4). A signal can be transferred by cell-to-cell communication or via the culture medium. The factors involved in the transmission of the effect have only partly been characterized. They may involve the diffusion of cytokines or long lived reactive oxygen species (ROS), the diffusion of paracrine proapoptotic or antiapoptotic factors induced by up-regulation of p.21. Bystander effect was reported to be suppressed by adaptive response induction.
Bystander effect is independent of dose. There is therefore no threshold. The lowest dose used to evidence a bystander effect *(single alpha particle track to one cell or low dose to a cell population)* caused the same amount of bystander end points as doses that were orders of magnitude higher. Bystander effects reported for γ-ray are with dose of 500mGy and above. For α-particles and other high-LET radiation used in bystander studies, the dose to the nucleus was calculated to be 130-500 mGy per particle traversal. The most critical question remains therefore whether the bystander effect exists for low-LET radiation dose <100 mGy.
Data from the literature show pronounced bystander effect in a variety of cell lines. Recently T. Groesser et al. (2) pointed a lack of bystander effect from high–LET radiation for early cytogenetic end points. These results were in contradiction with those of several published reports (7, 10, 14, 15, 16) but were confirmed by Mothersill who tested in her laboratory the same cells. However when changing the culture medium, a bystander effect appeared.

To reconcile such conflicting data it is suggested that the epigenetic status of the specific cell line used or the precise culture conditions and medium supplements such as serum could be critical for inducing bystander effect (2).
It has been proposed that the bystander response could be the initiating event in radiation-induced genomic instability (4). The instability induced by bystander effect is frequent and *nonclonal* but tumors do have *a clonal* origin. Bystander effect therefore does not appear to be directly involved in cellular transformation but, by the induced genomic instability, would favor its occurrence and increase the cancer incidence above the estimation provided by LNT hypothesis.

It is also important to note that the experimental results supporting the bystander effect involve only *in vitro* model systems. To evidence bystander effect in *in vivo* systems appears clearly not possible. Mancuso et al. (6) working on shielded cerebellum reported the first proof-of-principle that bystander effects are factual *in vivo* events with carcinogenic potential, and implicate the need for re-evaluation of approaches currently used to estimate radiation-associated health risks. We might however consider that such long distance bystander effects described by these author's are more likely related to the abscopal effect, a well known systemic effect for which the mechanisms might be totally different.

CONCLUSION

If bystander effect is important, we should consider that it has already operated in the population over many thousands of generations and is included in any low dose effect studies. Epidemiology should then clearly indicate that LNT underestimates the risk.
Epidemiological evidence however supports the LNT hypothesis. International epidemiological research on health effects of low doses of ionizing radiation has progressed in a classical way through dose estimations of exposed populations.
The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the ICRP (International Commission Radiological Protection) and the US National Academy of Sciences (BEIR VII) reviewed the scientific progress worldwide and recently came to conclusions still supporting a linear non threshold hypothesis as best fit to assess and manage low level

exposure to ionizing radiation in the current context of uncertainty.
New investigations are needed to understand the mechanisms of bystander induction, the factors involved in the signal transmission, the role the bystander effect can play in vivo and verify if bystander effect is linked exclusively to ionizing radiation exposure or is a cell reaction to any stress. Only clear answer to those questions can allow to estimate the impact, if any, of bystander effect in the low dose radiation risk.

Acknowledgements
These studies were supported by the NOTE IP 036465 (FI6R), Euratom specific programme for research and training on nuclear energy, 6th FP of the EC.

Bibliography

1- O.V. Belyakov, A.M. Malcolmson, M. Folkard, K.M. Prise and B.D. Michael, Direct evidence for a bystander effect of ionizing radiation in primary human fibroblasts. *Br J. Cancer* **84**, 674-679 (2001).

2- T. Groesser, B. Cooper, and B. Rydberg, Lack of Bystander Effects from High-LET Radiation for early Cytogenetic End Points, *Rad. Res.* **170**, 794-802 (2008)

3- E.J. Hall and T.K. Hei, Genomic instability and bystander effects induced by high-LET radiation. *Oncogene* **212**, 7034-7042 (2003)

4- F.M. Lyng, C.B. Seymour and C. Mothersill, Initiation of apoptosis in cells exposed to medium from the progeny of irradiated cells: a possible mechanism for bystander-induced genomic instability? *Rad. Res.* **157**, 365-370 (2002)

5- J.B. Little, E.I. Azzam, S.M. de Toledo and H. Nagasawa, Bystander effects: intercellular transmission of radiation damage signals. *Radiat. Prot. Dosimetry,* **99**, 159-162 (2002)

6- M. Mancuso, E. Pasquali, S. Leonardi, M. Tanori, S. Rebessi, V. Di Majo, S. Pazzaglia, M. P. Toni, M. Pimpinella, V. Covelli, and A. Saran.,

Oncogenic bystander radiation effects in Patched heterozygous mouse cerebellum.
PNAS **105**, 34, 12445–12450 (2008)

7- C. Mothersill and C. Seymour, Medium from irradiated human epithelial cells but not human fibroblast reduces the clonogenic survival of unirradiated cells. *Int J. Radiat. Biol.* **71**, 421-427 (1997)

8- C. Mothersill and C. Seymour, Cell-cell contact during gamma irradiation is not required to induce a bystander effect in normal human keratinocytes: evidence for release during irradiation of a signal controlling survival into the medium. *Radiat. Res.* **149**, 256-262 (1998)

9- C. Mothersill and C. Seymour, Radiation-induced bystander effects: past history and future directions. *Radiat. Res.* **155**, 759-767 (2001)

10- C. Mothersill, R.J. Seymour and C.B. Seymour, Bystander effects in repair-deficient cell lines. *Rad res.* **161**, 256-263 (2004)

11- H. Nagasawa and J.B. Little, induction of sister chromatid exchanges by extremely low dose of alpha particles. *Cancer Res.* **52**, 6394-6396 (1992)

12- M.V. Sokolov, L.B. Smilenov, E.J. Hall, I.G. Panyutin, W.M. Bonner and O.A. Sedelnikova, Ionizing radiation induces DNA double strand breaks in bystander primary human fibroblasts. *Oncogene* **24**, 7257-7265 (2005)

13- C. Shao, Y. Furusawa, M. Aoki and K. Ando, Role of gap junctional intercellular communication in radiation-induced bystander effects in human fibroblasts. *Radiat. Res.* **160**, 318-323 (2003)

14- M. Suzuki, H.Zhou, C.R.Geard and Y.K. Hei, Effect of medium on chromatin damage in bystander mammalian cells. *Rad. Res.* **162**, 264-269 (2004)

15- H.Yang, N. Asaad and K.D. Held, Medium-mediated intercellular communication is involved in bystander responses of X-ray-irradiated normal human fibroblasts. *Oncogene* **24**, 2096-2103 (2005)

16- H. Yang, V. Anzenberg and K.D. Held, The time dependence of bystander responses induced by iron-ion radiation in normal human skin fibroblasts. *Radiat. Res.* **168**, 292-298 (2007)