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Review

Genotoxic thresholds, DNA repair, and susceptibility in human populations

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ABSTRACT

It has been long assumed that DNA damage is induced in a linear manner with respect to the dose of a direct acting genotoxin. Thus, it is implied that direct acting genotoxic agents induce DNA damage at even the lowest of concentrations and that no "safe" dose range exists. The linear (non-threshold) paradigm has led to the one-hit model being developed. This "one hit" scenario can be interpreted such that a single DNA damaging event in a cell has the capability to induce a single point mutation in that cell which could (if positioned in a key growth controlling gene) lead to increased proliferation, leading ultimately to the formation of a tumour.

There are many groups (including our own) who, for a decade or more, have argued, that low dose exposures to direct acting genotoxins may be tolerated by cells through homeostatic mechanisms such as DNA repair. This argument stems from the existence of evolutionary adaptive mechanisms that allow organisms to adapt to low levels of exogenous sources of genotoxins. We have been particularly interested in the genotoxic effects of known mutagens at low dose exposures in human cells and have identified for the first time, in vitro genotoxic thresholds for several mutagenic alkylating agents (Doak et al., 2007). Our working hypothesis is that DNA repair is primarily responsible for these thresholded effects at low doses by removing low levels of DNA damage but becoming saturated at higher doses. We are currently assessing the roles of base excision repair (BER) and methylguanine-DNA methyltransferase (MGMT) for roles in the identified thresholds (Doak et al., 2008). This research area is currently important as it assesses whether "safe" exposure levels to mutagenic chemicals can exist and allows risk assessment using appropriate safety factors to define such exposure levels. Given human variation, the mechanistic basis for genotoxic thresholds (e.g. DNA repair) has to be well defined in order that susceptible individuals are considered.

In terms of industrial exposures to known mutagens, knowing the dose relationships and protective mechanisms involved, offers the possibility of screening workers for susceptibility to mutation through examining DNA repair gene polymorphisms. Hence, thresholds may exist for certain mutagens, but there will undoubtedly be human subpopulations who are more at risk from low dose exposures than others and who should not be exposed, if possible. By studying polymorphisms in DNA repair genes, susceptible individuals may be identified, and additional safety factors appropriately targeted to these populations.

Contents

Abbreviations: MMS, methyl methanesulphonate; MNU, methyl nitrosourea; EMS, ethyl methanesulphonate; BER, base excision repair; MGMT, methyl guanine DNA methyl transferase.

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1. Genetic toxicology

Genetic toxicology, which evolved in the latter half of the twentieth century, is involved in the study of DNA damage and mutation and its impact on human health. Genotoxicity describes many different DNA endpoints including DNA adduct formation, point mutation, chromosome breakage and chromosome copy number changes. From its inception, genotoxicity has been used as a surrogate for cancer, as genotoxins are almost always carcinogens and cancer has traditionally been seen as a genetic disease characterised by acquired DNA mutations in growth controlling genes. Consequently, assessment of the genotoxicity of new chemicals is seen as a key regulatory requirement to minimise any deleterious effects that may be produced through genotoxic exposures within human populations. As part of the safety assessment of new chemicals (pharmaceuticals, consumer products, etc.), a tiered approach to genotoxicity testing is currently recommended. This tiered approach is well described by the Committee on Mutagenicity (COM) guidance (Guidance on a Strategy for testing of chemicals for Mutagenicity, 2000) and involves all chemicals entering so-called stage 1 tests where DNA damage induction is assessed in cells cultured in the laboratory. Negative results in these tests reassure the manufacturer that DNA damage is unlikely to be induced by that agent. Stage 2 tests are employed for positives from stage 1 tests and for compounds with medium to high exposure potentials and are carried out in animals. The stage 2 tests are designed to overcome problems with false positive results which can occur in stage 1 tests and also to more rigorously assess the risks to human health.

2. Linear dose–response relationships for genotoxins

In genetic toxicology a linear dose–response relationship has long been assumed to apply for direct acting genotoxic agents (Henderson et al., 2000). Fig. 1 displays the different theoretical dose–response relationships for genotoxins (linear and thresholded). In the linear model, DNA damage induction is believed to be directly proportional to dose; leading to the implication that there are no genotoxic doses, however low, devoid of a finite risk of genetic damage and hence cancer. This linear model has been implemented partly because of early experimental evidence and partly due to the precautionary principle. This linear concept has been controversial and has recently been challenged by ourselves and others, as it assumes a binary situation where chemicals are either genotoxic or not, but does not account for the effect of dose. As pointed out by Paracelsus in the 16th Century, "only the dose permits something not to be poisonous". In this context it is interesting to note that carcinogenesis has recently been shown to be induced in a non-linear manner with low doses of genotoxic agents failing to drive cancer formation in trout even when large numbers of animals were examined (>40,000) (Bailey et al., 2009).

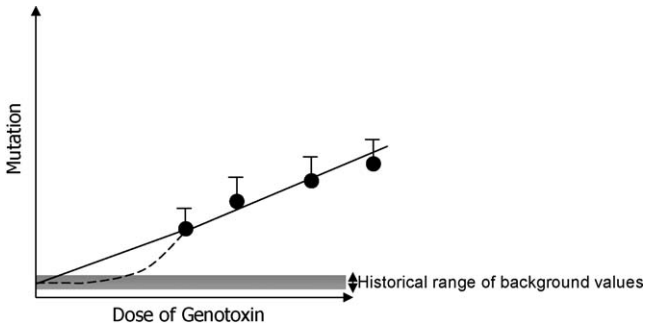

Fig. 1. Dose–responses induced by genotoxic agents. Theoretical mutagenic dose–responses for genotoxic agents are displayed. Linear response (solid line) implies no safe low dose. Note the line does not go through the origin, as background mutation levels are detectable in vitro and in vivo. The shaded area represents the background level (historial background ranges can define this region). Thresholded responses are depicted by the dotted line in the low dose range. At the low dose region no increase over background mutation level is seen, followed by a critical dose range where mutagenic responses are observed. The boundary between no effect and effect is represented by the no observable effect level (NOEL) and the lowest observable effect level (LOEL), and the threshold dose is statistically calculated where the slope of the graph first increases significantly.

In the case of indirect genotoxins which have non-DNA targets (aneuploidy inducing agents and agents interacting with DNA modifying enzymes), thresholds have now been accepted (Elhajouji et al., 1997; Lynch et al., 2003). Hence, this demonstrates the usefulness of solid experimental evidence in altering paradigms. However, for direct acting genotoxins, linear models are still assumed to apply. Recently, the role of dose in mutagenicity testing in general has been a major issue in the field and inappropriately high doses have been suggested to be responsible for many of the false positive results in stage 1 tests (Kirkland et al., 2007). High doses of chemicals have traditionally been used to ensure that DNA damaging effects are identified in the available tests (due to test sensitivity constraints) and because it has widely been assumed that the effects are induced in a linear manner, this is then extrapolated back to the low dose region. Therefore, if a high dose is positive for genotoxicity, then under this linear paradigm, a low dose will also be positive. Hence, the implications emanating from the linear model for genotoxins can be wide reaching and can impact scientifically and economically on the availability and use of certain chemicals. As the linear model is currently being challenged, this paradigm is subject to change which may affect future regulatory testing and allow some previously unavailable chemicals to be licensed for use in the future.

3. Theoretical arguments against a linear response for genotoxins

The main argument against a linear dose–response for genotoxins is the presence of natural defences which have evolved to cope with our daily exposure to genotoxins. Humans are constantly exposed to genotoxic substances like cytosolic oxidative agents, dietary amines, inhaled hydrocarbons and many others. Low level exposures to these genotoxins have occurred throughout evolutionary time and have led to the development of efficient homeostatic defences to protect organisms against the deleterious mutagenic consequences. DNA repair is one such homeostatic defence mechanism that may impact on the consequences of genotoxin exposure. Indeed, even simple bacteria have intricate defences (like DNA repair) against genotoxins. As multicellular organisms, humans have several tiers of protection against DNA damage including, but not restricted to:

1. Epithelial barriers to genotoxin entry.
2. Detoxification processes leading to excretion of water soluble genotoxins.
3. Compartmentalisation of tissues leading to reduced access for genotoxins.
4. Cellular and nuclear membranes reducing access of genotoxins to the nucleus.
5. DNA repair to remove damaged DNA sequences.
6. DNA redundancy (<1% gives are thought to code for proteins).
7. Apoptosis/autophagy/anoikis to remove damaged cells.

Hence, it is theoretically difficult for genotoxins to cause DNA damage in a manner proportional only to dose. This is due in part to the failure of the genotoxin to readily access the DNA of a target tissue. Even in a simple cell culture system, it is unlikely that true