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Commentary

Theoretical considerations for thresholds in chemical carcinogenesis

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ABSTRACT

There is increasing evidence for non-linear relationships for gene mutations, chromosomal aberrations and even tumor incidences in response to low doses of genotoxic carcinogens. To attain the biological relevance of such non-linear responses, there is a need to identify the underlying defense mechanisms that allow tolerance to low doses of genotoxicants. This communication discusses presumptive cancer prevention mechanisms that may contribute to thresholds, *i.e.* points of departure, for each endpoint, from initial DNA lesion to tumor formation. We discuss a sequential order of genome protection during carcinogenesis where genotoxicant scavenging, cellular efflux, DNA repair, elimination of damaged cells by apoptosis, autophagy, silencing by DNA damage-triggered replicative senescence, and finally, elimination of transformed (premalignant) cells by the immune system are thought to be responsible for a threshold in tumor formation. We highlight DNA repair, for which experimental evidence has been recently provided to dictate a role in PoDs. In conclusion, from a theoretical perspective it is reasonable to posit that tolerance to low dose levels exists for each requisite step of tumor formation and these tolerance mechanisms are critical in determining thresholds in chemical carcinogenesis.

1. Introduction

The linear dose–response for genotoxicant-induced gene mutations and chromosomal damage has been challenged. Sound experimental design and rigorous data analysis posit that low dose effects of genotoxicants are non-linear. Thus far, points of departures (PoDs) have been identified for mutagenic endpoints for DNA reactive genotoxicants (*i.e.* gene mutations and chromosomal damage). There is evidence of non-linear dose–responses for other endpoints such as pre-neoplastic lesions and subsequent hepatocellular tumors following treatment with DNA reactive methyl eugenol [1]. Incidentally, in a study of 40,800 trout, Bailey et al. [2] found that the incidence of liver and stomach neoplasms were comparable to control levels at low doses of dibenzo[*a*]pyrene (DBP), a genotoxic carcinogen in cigarette smoke. This and other data recently reviewed [3] suggest PoDs, here defined as thresholds, for DNA reactive carcinogens. The impetus of this work is to elucidate the respective cytoprotective mechanisms, which prevent mutations and cancer at low dose levels. The following work hypothesizes potential thresholds and protective mechanisms throughout the process from initial DNA damage induction to tumor formation. It is worth noting that such cytoprotective mechanisms will be dependent upon the mode of action of the genotoxicant. Recent evidence suggests that a PoD for mutation is influenced by the DNA adduct spectra and DNA repair proficiencies [4–6], as well as chemical clearance through detoxification for reactive oxygen species (ROS) [7]. Given that mutagenesis is an early event in the exposure-to-tumor scenario (the critical steps are summarized in Fig. 1), it is important to understand whether mutation prevention is the sole mechanism responsible to inhibit subsequent tumor formation at lower dose levels. Waddel and coworkers [8,9] predicted that sequential PoDs exist at increasing dose levels along the carcinogenic process in the order of adducts < gene mutation < pre-neoplastic lesion (foci) < cancer. It is possible that PoDs exist for each of these endpoints in a sequential manner during tumor development in chemical carcinogenesis as supported recently by expert members of the International Workshop on Genotoxicity Testing [10,11]. With growing acceptance of non-linear dose-tumor incidence relationships, this review will track the process of chemical administration to tumor development and comment upon putative mechanisms to account for a PoD at each step during chemical carcinogenesis (Fig. 1). Although this work contains a number of speculative inferences, it has been written with the intention of stimulating discussions of possible modes of action and opposing cytoprotective mechanisms that might be addressed experimentally in the future.

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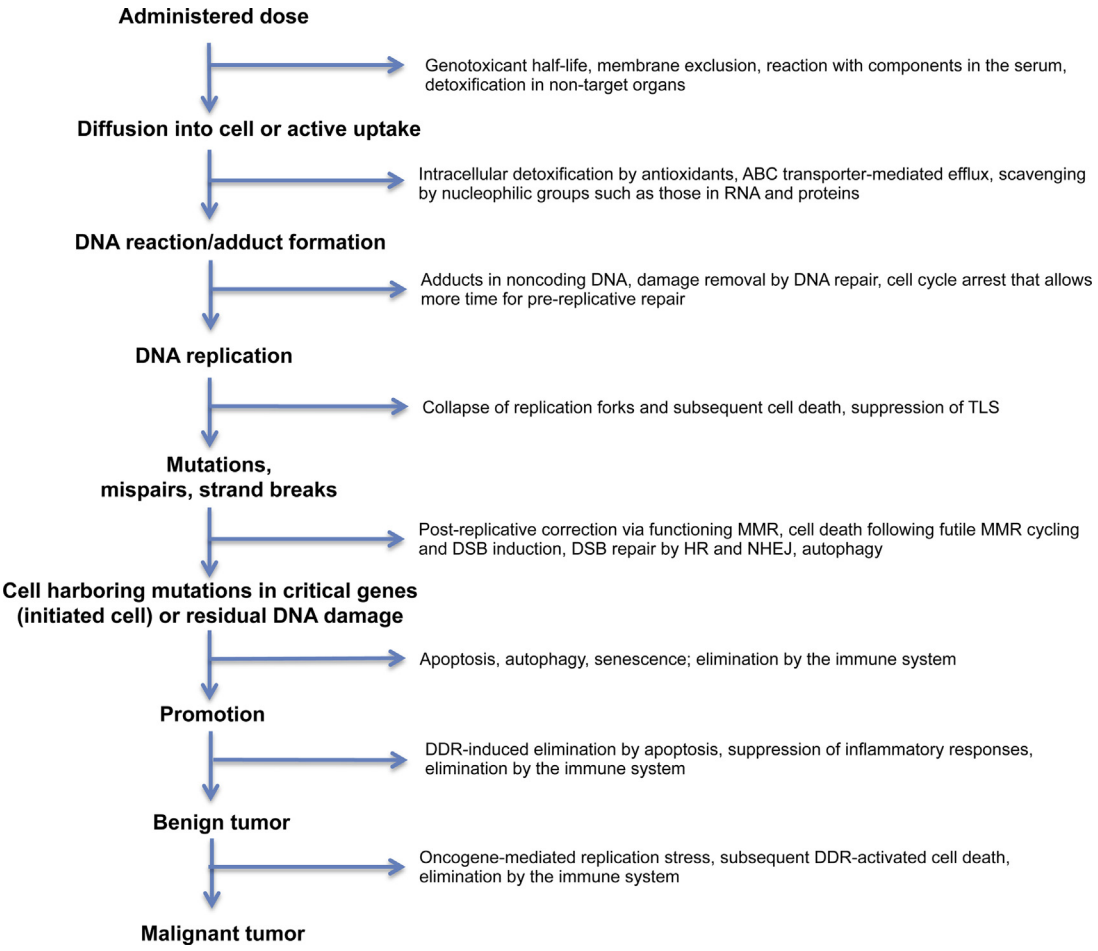

Fig. 1. The process of chemical carcinogenesis (vertical arrows) and potential protective mechanisms involved in points of departures (PoDs; thresholds) at each stage (horizontal arrows). ABC, ATP binding cassette; TLS, translesion synthesis; MMR, mismatch repair; HR, homologous recombination; NHEJ, non-homologous end-joining; DDR, DNA damage response.

2. Prevention of DNA damage

Not unlike the *in vivo* situation, in cell culture administered chemicals have a number of barriers to traverse before a molecule reaches the DNA target. Potential reaction with culture media and cellular components (proteins and RNA) would reduce the effective dose of administered chemical to reach and react with DNA. Under the linear hypothesis, it is predicted that, upon DNA reaction, every molecule causes one adduct. Although current data supports this [12], it is prudent to assume that all administered molecules reach the DNA and cause adducts. As an example, mass spectrometry data quantified *N*7-methylguanine (*N*7MeG) adducts as biomarkers of exposure. A dose of 0.025 μg/ml *N*-methyl-*N*-nitrosourea (MNU) caused 9.1 *N*7MeG adducts/10^8 nucleotides and a significant increase in mutant frequency in human lymphoblastoid cells, which can be considered as greater than the PoD for MNU-induced point mutations [5]. The linear hypothesis would predict a maximum of 3.19×10^5 *N*7MeG adducts/10^8 nucleotides following treatment with 0.025 μg/ml MNU if all administered molecules reacted with the DNA. Given that only 9.1 *N*7MeG adducts/10^8 have been detected with this dose of the mutagen, the calculation shows that the theoretical ratio is far too high (35,054-fold higher) for the "one molecule administered—one adduct" interpretation of linear hypothesis. It is clear that not all molecules administered will reach the DNA. Thus, cellular macromolecules and serum proteins are substrates for carbamoylation by alkylating agents (used in this example), Moreover, a very low half-life of the compound in solution at the given pH (for MNU at pH 7.5, $t_{1/2} = 0.3$ h [13]), cytoplasmic exclusion or reaction with other cellular nucleophilic components (*e.g.* RNA, which is ubiquitous within the cytoplasm) would reduce the number of molecules and propensity for DNA reaction and may produce a true null effect at very low doses.

The kinetics of influx into the cell may also be non-linear. Some drugs are internalized by passive diffusion and, others, by active transmembrane carriers [14]. In this regard, cisplatin is subject to uptake by copper transporters and also by passive diffusion [15]. The concentration may play the deciding role as to the mechanism of uptake, where at low doses, transporter proteins mediate cellular influx, whereas at higher concentrations, following saturation of active mechanisms, non-charged small molecule-drugs passively diffuse into the cells until the concentration equilibrates over the plasma membrane [16]. A mathematical model has illustrated this principle for doxorubicin [17]. Interestingly, the kinetics of cellular uptake reflected the cell killing potential of doxorubicin and suggests that uptake may be a crucial factor in dose–response relationships for some drugs, notably large lipophilic compounds.

Once intracellular, the remaining molecules of exogenous genotoxicant may be subject to detoxification and clearance to further limit DNA reaction. Detoxification has been shown as a cytoprotective mechanism preventing reactive oxygen species (ROS)-induced mutations. Thus it was shown that upon enhancement of antioxidant defenses (glutathione) by *N*-acetylcysteine