DOSE-RESPONSE RELATIONSHIPS IN CHEMICAL CARCINOGENESIS: FROM DNA ADDUCTS TO TUMOR INCIDENCE

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SUMMARY

Mechanistic possibilities responsible for nonlinear shapes of the dose-response relationship in chemical carcinogenesis are discussed. (i) Induction and saturation of enzymatic activation and detoxification processes and of DNA repair affect the relationship between dose and steady-state DNA adduct level; (ii) The fixation of DNA adducts in the form of mutations is accelerated by stimulation of the cell division, for instance due to regenerative hyperplasia at cytotoxic dose levels; (iii) The rate of tumor formation results from a superposition of the rates of the individual steps. It can become exponential with dose if more than one step is accelerated by the DNA damage exerted by the genotoxic carcinogen. The strongly sigmoidal shapes often observed for dose-tumor incidence relationships in animal bioassays supports this analysis. A power of four for the dose in the sublinear part of the curve is the maximum observed (formaldehyde). In contrast to animal experiments, epidemiological data in humans rarely show a significant deviation from linearity. The discrepancy might be explained by the fact that a large number of genes contribute to the overall sensitivity of an individual and to the respective heterogeneity within the human population. Mechanistic nonlinearities are flattened out in the presence of genetic and life-style factors which affect the sensitivity for the development of cancer. For a risk assessment, linear extrapolation from the high-dose incidence to the spontaneous rate can therefore be appropriate in a heterogeneous population even if the mechanism of action would result in a nonlinear shape of the dose-response curve in a homogeneous population.

INTRODUCTION

The reaction of biological reactive intermediates with DNA can be investigated at low dose levels which would not give rise to a significant increase in tumor incidence in a standard animal bioassay on carcinogenicity. The shape of the dose-response curve for DNA adduct formation is therefore considered valuable information for a low-dose risk evaluation. In this presentation, general principles are derived to

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explain the available data. In addition, the value of these data for human risk evaluation is critically assessed.

From Carcinogen Dose TO DNA Adducts

Single-Dose Data: The processes and reactions leading to DNA adducts include diffusion as well as enzymatic and chemical reactions. At lowest dose levels, when the concentrations of the carcinogen and of its proximate and ultimate metabolite(s) are well below the Michaelis concentration of the enzymatic reactions, the formation of DNA adducts is expected to be proportional to dose. This hypothesis was first supported by data published by Neumann (1980) with trans-4-dimethylaminostilbene. Additional experiments which showed a linear dose-response relationship were reviewed by Lutz (1987). The only nonlinearity discussed in this review was the example of O6-methylguanine determined after administration of a methylating agent. It shows that the instantaneous repair by methyl transfer to an acceptor protein is exhausted above a certain level of DNA methylation.

Additional evidence of nonlinearities in an intermediate dose range of the dose-DNA adduct curve was reviewed by Swenberg et al. (1987). A sigmoidal shape of the dose-response curve was found with formaldehyde-induced DNA-protein crosslinks (Heck and Casanova, 1987), probably due to a saturation of metabolic inactivation. A superlinear shape was reported with the tobacco-specific nitrosamine 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and was explained on the basis of a low KM pathway for the metabolic activation (Belinsky et al., 1987).

A superlinear shape for DNA adduct formation is often seen at highest dose levels due to a saturation of the metabolic activation of the carcinogen.
In summary, while proportionality between dose and DNA adduct level is expected to hold at low doses, a number of saturation phenomena have been shown to generate nonlinear dose-response curves in an intermediate and high-dose range (Figure 1 top).

Multiple-Dose Data: The data referred to above are derived from single-dose experiments. For a correlation of DNA adduct formation with tumor induction, however, it is the level of dangerous adducts arising from chronic exposure which is the important variable. Since DNA repair is also an enzymatic reaction, the rate of DNA repair is expected to be proportional to the level of DNA adducts. Consequently, the DNA damage levels off to a steady-state when the number of newly-formed adducts equals the number of adducts repaired or lost during the same period of time. Proportionality between daily dose and steady-state adduct level has to be postulated under these conditions, unless DNA repair is induced. In this laboratory, DNA adduct levels were measured in rat liver after exposure for 4, 6, and 8 weeks to aflatoxin B1 and 2-acetylaminofluorene administered in the drinking water. Steady-state level of DNA adducts was reached during this period of treatment and was proportional to dose down to the limit of detection at one thousandth of the TD50 dose level (Buss and Lutz, 1988).

From DNA Adducts To Mutations

DNA adducts alone are not sufficient to generate a heritable, initiated phenotype of a cell because adducts cannot be copied onto the progeny DNA strand. Only upon DNA replication can mutations arise in the new strand opposite an adduct. Since DNA adducts can be repaired, the probability of a fixation of the DNA adduct in the form of a mutation is dependent on the relative rates of DNA repair and cell division. All acceleration of cell division will therefore have a synergistic effect on the formation of mutations.

DNA adduct-forming carcinogens do not bind exclusively to DNA, but always also to RNA and protein. Above a certain level of macromolecular binding, the cell dies. This can induce regenerative hyperplasia in the surrounding tissue. Genotoxic agents at high dose, therefore, can stimulate cell division and accelerate the process of mutagenesis and carcinogenesis in the surviving cells. High-dose exposure to a genotoxic carcinogen (which is the usual situation in a bioassay; Hoel et al., 1988)

therefore results in a sublinear shape of the dose response curve for mutations (and tumors) in division-competent cells (Figure 1 middle).

In the low dose range which does not produce cytotoxicity, a linear relationship between level of DNA adducts and mutations can be expected. A nice example for such a linear -> sublinear shape can be given for the induction of liver tumors in female rats by nitrosodiethylamine (NDEA). In this study, NDEA was administered in the drinking water to groups of 60 rats at 15 dose levels (0.033 to 16.9 ppm; Peto et al., 1984). When the ratio observed/expected liver cancer incidence is plotted as a function of dose, a quadratic to cubic shape is seen. However, if only the low dose data are taken (up to 0.53 ppm), the increase in tumor response appears to be linear with dose and less rapid than the curve fitted to all data points (Zeise et al., 1987). This large experiment, using more than a thousand animals, afforded a unique opportunity to detect linearity at low dose levels and a power of the dose in the high-dose range. It is a good example to stress the idea that mode of action and potency of a carcinogen can change with the dose level.

From Mutations To Tumors

As a consequence of the multi-stage nature of carcinogenesis, the probability of cancer induction can be approximated by the product of the probabilities for the various steps (Armitage, 1985). Since all steps have a genetic basis to become heritable, DNA-damaging compounds could accelerate more than one step. The dose-response relationship then appears as a superposition of the curves for the individual steps. If, for instance, two events are affected, both proportionally with the level of DNA adducts, the product of the lines will be exponential (Figure 1 bottom).

Dose Response for Tumor Induction

The mechanistic analysis given above indicated a large number of possibilities for nonlinear dose-response relationships, especially at high exposure levels. In the high-dose range, data on tumor formation in animals and humans become available, and the following paragraphs will show whether the postulated steep slopes are indeed found.

Bioassays on Carcinogenicity

A comprehensive and recent review on this topic is available (Zeise et al., 1987). In the observable (high) dose range used in animal studies, several examples from well-documented studies suggest that nonlinearities are common. For instance, a sublinear curve is seen for bladder cancer caused by sodium saccharin and 2ãcetylaminofluorene, for liver cancer induced by diethylnitrosamine and dimethylnitrosamine, and for oesophageal cancer from diethylnitrosamine. In an extreme case, formaldehyde induced nasal tumors in rats with a frequency of 1 and 44 % after inhalation exposures of 5.6 and 14.3 ppm, respectively; Swenberg et al., 1983). Here, the tumor incidence increased with the fourth power of the dose. In all other examples, the respective exponent was smaller.

Epidemiological Evidence in Humans

In human studies, significant deviations from linearity are much more difficult to find. Only in two reports, namely lung cancer in smoking British physicians and bladder cancer from β-naphthylamine exposure is a sublinear fit clearly better than a linear one. The relationship in these two cases is approximately quadratic; higher exponents have not been observed (Zeise et al., 1987).

The fourth power of the dose seen in an animal experiment is clearly compatible with the multi-stage nature of carcinogenesis and with the mechanistic analysis discussed above. Astonishing at first glance is the fact that epidemiological investigations with humans in general showed a linear dose-response relationship, or, at most, a second order for the dose. The reasons for this difference between animals and humans might be found with the heterogeneity of the human population.

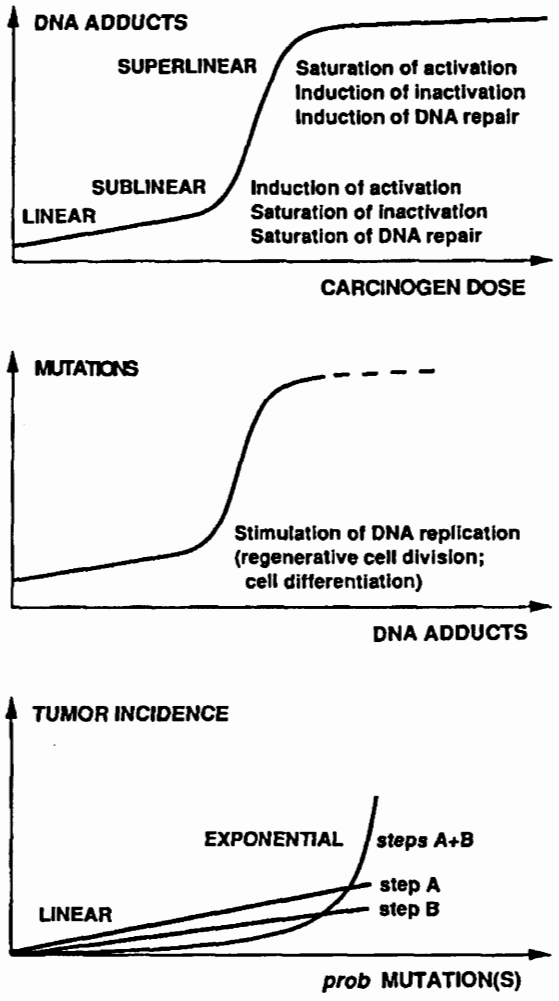

Figure 1. Schematic representation of the dose-effect relationship for three aspects of tumor induction by genotoxic agents.

Homogeneous vs. Heterogeneous Population

So far, carcinogenesis has been discussed mechanistically, as a process in one individual or in an animal population which is relatively homogeneous both with respect to the genetic predisposition and the life style (Fig. 2 top). The epidemiological evidence with humans, on the other hand, is based on a highly heterogeneous population. For instance, interindividual differences in the metabolism of chemical carcinogens and repair rates of DNA adducts are large and reflect acquired and inherited host factors that may influence an individual's risk for development of cancer (Harris, 1989). For instance, genetically controlled metabolic capacities contribute to a predisposition of humans to bladder cancer (Kaisary et al., 1987).

If one single gene was to govern the sensitivity to a carcinogen, the human population would be separated into a population A where a given dose leads to a tumor in almost all exposed individuals whereas the remaining people (population B) develop a tumor only at higher dose levels (Fig. 2 middle). The mechanistic aspects discussed above would then only become important for an evaluation of the dose-response curve within each subpopulation.

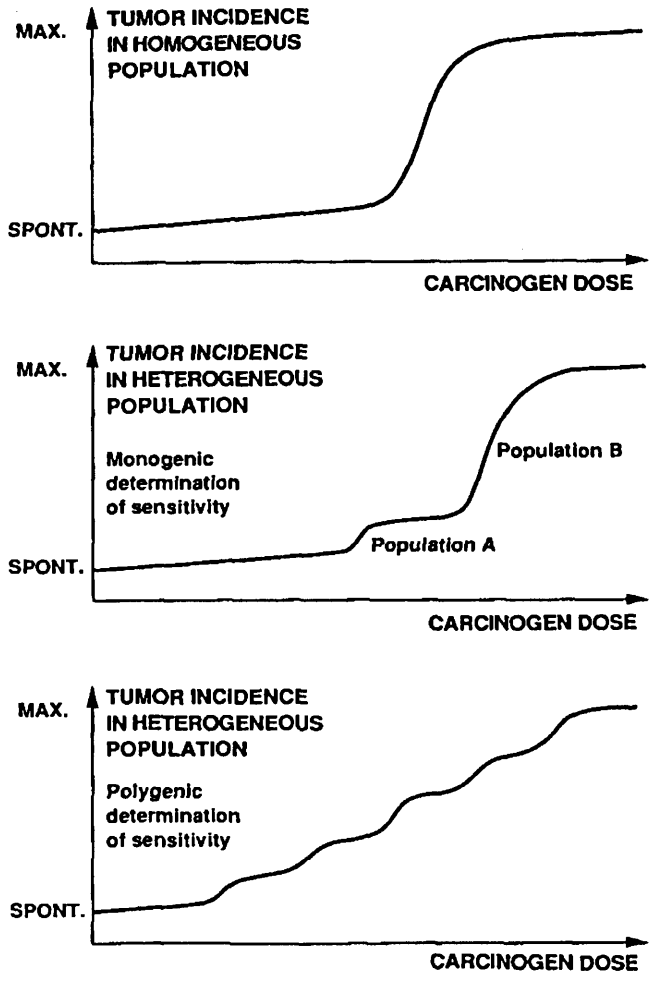

Figure 2. Schematic representation of the effect of population heterogeneity on the dose-effect relationship in chemical carcinogenesis. Top, homogeneous population; middle, heterogeneous populations with monogenic determination of the sensitivity; bottom, polygenic determination of the sensitivity.

Much more realistic is the situation where the sensitivity is governed by a large number of genes. As a result, the dose-response curve will become flatter with each modulatory factor (Fig. 2 bottom). If, in addition, the sensitivity is modulated by the lifestyle, near-linearity can be produced between the 'spontaneous' tumor incidence (Lutz, 1990) and the high-dose data. This polygenic-multifactorial situation might explain the finding that dose-response curves in human epidemiological studies are linear in most cases, and never show the steep slopes observed in animal studies.

Risk Extrapolation

This analysis has revealed a large number of mechanistic possibilities to generate nonlinear dose-response relationships in chemical carcinogenesis. On the other hand, the dose-response curve is linearized in a heterogeneous population, if the sensitivity of an individual is determined by its specific set of genetic factors and is further modulated by the lifestyle. The larger the number of these factors, the more will the

dose-response curve become linear. Extrapolation of tumor incidence from the high dose range to low dose levels can therefore be linear in a heterogeneous population even if the mechanism of carcinogenesis in an individual produces a nonlinearity.

REFERENCES

Armitage, P. (1985). Multistage models of carcinogenesis. *Environ. Health Perspect.* **63**, 195-201.

Belinsky, S.A., White, C.M., Devereux, T.R., Swenberg, J.A., Anderson, M.W. (1987). Cell selective alkylation of DNA in rat lung following low dose exposure to the tobacco specific carcinogen 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res.* **47**, 1143-1148.

Buss, P., Lutz, W.K, (1988). Steady-state DNA adduct level in rat liver after chronic exposure to low doses of aflatoxin B1 and 2-acetylaminofluorene. *Proc. Amer. Assoc. Cancer Res.* **29**, 96.

Harris, C.C. (1989). Interindividual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair. *Carcinogenesis* **10**, 1563-1566.

Heck, H.A., Casanova, M. (1987). Isotope effects and their implications for the covalent binding of inhaled (3H) and (14C) formaldehyde in the rat nasal mucosa. *Toxicol. Appl. Pharmacol.* **89**, 122-134.

Hoel, D.G., Haseman, J.K., Hogan, M.D., Huff, J., McConnell, E.E. (1988). The impact of toxicity on carcinogenicity studies: implications for risk assessment. *Carcinogenesis* **9**, 2045-2052.

Kaisary, A., Smith, P., Jaczq, E., McAllister, C.B., Wilkinson, G.R., Ray, W.A., Branch, R.A. (1987). Genetic predisposition to bladder cancer: ability to hydroxylate debrisoquine and mephenytoin as risk factors. *Cancer Res.* **47**, 5488-5493.

Lutz, W.K. (1987). Quantitative Evaluation of DNA-Binding Data In Vivo for Low-Dose Extrapolations. *Arch. Toxicol.* (Suppl) **11**, 66-74.

Lutz, W.K. (1990). Endogenous genotoxic agents and processes as a basis of spontaneous carcinogenesis. *Mutat. Res.*, in press.

Neumann, H.G. (1980). Dose-response relationship in the primary lesion of strong electrophilic carcinogens. *Arch. Toxicol.* (Suppl) **3**, 69-77.

Peto, R., Gray, R., Brantom, P., Grasso, P. (1984). Nitrosamine carcinogenesis in 5120 rodents: chronic administration of sixteen different concentrations of NDEA, NDMA, NPYR and NPIP in the water of 4440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting and of species. *IARC Sci. Publ.* **57**, 627-665.

Swenberg, J.A., Barrow, C.S., Boreiko, C.J., Heck, H.A., Levine, R.J., Morgan, K.T., Starr, T.B. (1983). Non-linear biological responses to formaldehyde and their implications for carcinogenic risk assessment. *Carcinogenesis* **4**, 945-952.

Swenberg, J.A., Richardson, F.C., Boucheron, J.A., Deal, F.H., Belinsky, S.A., Charbonneau, M., Short, B.G. (1987). High to low dose extrapolation: critical determinants involved in the dose response of carcinogenic substances. *Environ. Health Perspect.* **76**, 57-63.

Zeise, L., Wilson, R., Crouch, E.A.C. (1987). Dose-response relationships for carcinogens: a review. *Environ. Health Perspect.* **73**, 259-308.