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Commentary

Application of Biomarkers to Risk Assessment

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Due to difficulties in conducting epidemiological studies, most estimates of cancer risk are based on data from animal bioassays. Extrapolation of cancer risk estimates in animals to humans requires an assumption of equal potency across species based on the average daily dose. The purpose of this paper is to examine the ability to predict tumor incidence across species from DNA adduct concentrations resulting from exposure to carcinogens. A 100-fold range of structurally diverse adduct concentrations corresponding to the same tumor incidence raises questions about quantitative predictability across chemical classes and across species. Differences in adduct structure, mutagenic efficiency, adduct repair rates, and cellular proliferation could account for some of the differences. For specific carcinogen–DNA adducts, the steady-state levels associated with a 50% tumor incidence appear to vary over a narrower range. An equal incidence of liver tumors was obtained at equal concentrations of aflatoxin B_1–DNA adducts for rats and trout. A 2- to 3-fold range of 4-aminobiphenyl–DNA adduct concentrations between mice and dogs appears to be associated with nearly equal bladder tumor incidence, on the basis of limited data. In humans, a 5-fold higher concentration of a 4-aminobiphenyl–DNA adduct in bladders of smokers than of nonsmokers is compatible with the relative risk of bladder cancer due to smoking. DNA adduct concentrations certainly can be used to improve quantification of chemical exposures for epidemiological studies. Although promising, more data are needed to judge the usefulness of DNA adduct concentrations to predict cancer incidence across species.

Introduction

The estimation of disease risk generally involves use of a dose–response model. The goal is to estimate risk (proportion of adversely affected individuals) as a function of the dose of the active chemical that reaches the target tissue. Biologically based dose–response models are generally based on the dose of the active chemical at the target tissue. For example, the Moolgavkar–Venzon–Knudson (*1,2*) model of carcinogenesis assumes a mutagenic change of a normal cell to an initiated premalignant state, followed by proliferation of initiated cells, and finally a mutagenic change from an initiated state to a malignant state. If either one of the mutagenic rates is proportional to the dose at the target tissue, in the absence of a change in the cell proliferation rate, the incidence of tumors at low doses is approximately proportional to the dose at the target tissue. Proportional relationships have been observed between tumor incidence and DNA adduct concentration (*3*), which indicate that DNA adduct concentration can serve as a biomarker of dose at the target tissue in the experimental dose range. A nonlinear dose–response relationship indicates that a chemical affects more than one mutagenic rate and/or affects the cell proliferation rate. At the least, use of the dose at the target tissue, as measured by DNA adduct concentration, removes nonlinearities introduced by conversion of the whole-body administered dose to the effective dose at the target tissue. In the absence of pharmacokinetic information, it is generally

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assumed that the dose at the target tissue is directly proportional to the dose administered to the whole animal. No doubt, many nonlinear dose–response curves are the result of the pharmacokinetics involved, even though the basic mechanism of carcinogenesis may be a linear relationship between cancer incidence and dose at the target tissue. Measurement of DNA adduct concentrations may assist in the elucidation of carcinogenic mechanisms and in the prediction of cancer risk.

Although it is recognized that it is preferable to base cancer risk estimates on human data, epidemiological studies are often severely hampered by lack of data on exposure. Populations under investigation are generally divided into exposed and unexposed groups on the basis of occupation, residence, or social habits. Misclassification of individuals into exposed and unexposed groups weakens the capacity of an epidemiological study to detect effects. Quantification of exposure is often not attempted and is generally limited to years of exposure. Individuals exposed to high levels for short periods may receive higher effective doses than individuals exposed to low levels for longer periods. This bias also weakens the capacity of a study to relate risk to dose. Even if current environmental concentrations of chemicals can be measured, they may not reflect earlier levels, which may be more pertinent for cancer risk estimation. The identification and measurement of molecular biomarkers in individuals will certainly increase the number and type of epidemiological studies that can be conducted by better identifying exposed individuals and by providing information on dose for quantitative risk estimation. Furthermore, molecular biomarkers that are related to disease end points can be used to identify individuals who may be at higher risk because of inherent genetic factors or lifestyles.

Tumor Incidence Prediction from DNA Adduct Concentration

Beland and Poirier (*4*) provided a list of DNA adduct concentrations corresponding to 50% tumor incidence induced in animals by several carcinogens (Table 1). The 100-fold range of DNA adduct concentrations associated with the same tumor incidence raises questions about predicting tumor induction potential on the basis of DNA adduct levels across species. Some of the differences may be due to nongenotoxic promotional effects (e.g., cell proliferation rate) of carcinogens. For example, there is a nearly linear relationship between liver tumor incidence in mice and DNA adduct concentration with 2-acetylaminofluorene (*3*); however, the relationship between bladder tumor incidence and DNA adduct concentration with 2-acetylaminofluorene is highly nonlinear. Cohen and Ellwein (*7*) attribute this nonlinearity to the proliferative cellular effects of 2-acetylaminofluorene on the mouse bladder. Other differences in DNA adduct concentrations associated with identical tumor incidences may be due to differences in repair rates. The observation that identical levels of aflatoxin B_1–DNA adducts in rats and rainbow trout correspond to the same incidence of liver tumors (*8*) indicates that it may be possible in some cases to make comparisons across species.

Talaska et al. (*6*) observed that administration of 4-aminobiphenyl (4-ABP) at doses that produced urinary bladder tumors in 20 out of 24 dogs induced average steady-state levels of 100 fmole 4-ABP adduct/μg of DNA. In Table 1, a level of 150 fmole adduct/μg DNA corresponds to a 50% incidence of urinary bladder tumors in mice. The ultimate question is whether DNA adduct concentrations in humans could be used to predict cancer risk. Talaska et al. (*9*) observed an average of only 0.05 fmole of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl/μg DNA in the bladder of 13 smokers. Other aromatic amine adducts may also be associated with bladder tumors in humans. The total 4-ABP adduct concentration in the 13 smokers averaged 0.27 fmole/μg DNA. Smokers have a 2- to 10-fold increased risk over nonsmokers for developing bladder cancer (*10*). At a relative risk of 4.0, the incidence of bladder tumors in male smokers is estimated to be about 0.04. In the absence of data, if bladder cancer incidence is proportional to 4-ABP–DNA adduct concentration, the concentration corresponding to a 50% bladder tumor incidence in males can be estimated to be $0.27 \times (0.50/0.04) = 3.4$ fmole 4-ABP adduct/μg DNA. This is well below the concentration of 150 fmole 4-ABP adduct/μg DNA associated with a 50% incidence of

Table 1. DNA adduct levels in experimental animals associated with a 50% tumor incidence.[a]

Carcinogen	Species	Tissues	DNA adduct	Adduct level, pmole/mg
MNU	Mice	Bone, thymus	O^6-Me-dG	220
ENU	Mice	Bone, thymus	O^6-Et-dG	25
NDEA	Rats	Liver	O^4-Et-dT	2
AFB_1	Rats	Liver	$N7$-AFB_1-dG	3
	Trout	Liver	$N7$-AFB_1-dG	3
2-AAF	Mice	Liver	C8-AF-dG	110
	Mice	Urinary bladder	C8-AF-dG	250
4-ABP	Mice	Liver	C8-ABP-dG	250
	Mice	Urinary bladder	C8-ABP-dG	150
	Dogs[b]	Urinary bladder	C8-ABP-dG	100[c]

Abbreviations: MNU, *N*-methyl-*N*-nitrosourea; ENU, *N*-ethyl-*N*-nitrosourea; NDEA, *N*-nitrosodiethylamine; AFB_1, aflatoxin B_1; 2-AAF, 2-acetylaminofluorene; 4-ABP, 4-aminobiphenyl; O^6-Me-dG, O^6-methyldeoxyguanosine; O^6-Et-dG, O^6-ethyldeoxyguanosine; O^4-Et-dT, O^4-ethyldeoxythymidine; $N7$-AFB_1-dG, *trans*-8,9-dihydro-8-(deoxyguanosin-7-yl)-9-hydroxyaflatoxin B_1; C8-AF-dG, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; C8-ABP-dG, *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl.

[a]Adapted from Beland and Poirier (*4*).

[b]Bladder tumors in 20 out of 24 dogs (*5*).

[c]From Talaska et al. (*6*).

urinary bladder tumors in mice; however, other adducts may be associated with urinary bladder tumors in humans. Measurements in humans indicate that 4-ABP adducts account for 10.6% of total smoking-related adducts. If only 10.6% of human bladder cancer is associated with 4-ABP adducts, then the concentration in humans corresponding to a 50% incidence can be estimated to be $3.4 \times (100/10.6) = 32$ fmole 4-ABP adduct/μg DNA, which is nearer to the concentrations observed for dogs and mice. These calculations assume a linear relationship between bladder tumor incidence and 4-ABP adduct concentration in humans.

Talaska et al. (*9*) observed an average of 0.01 fmole of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl/μg DNA in the bladders of 29 nonsmokers. The relative adduct concentration of smokers to nonsmokers, $0.05/0.01 = 5$, provides a relative risk well within the range of that for bladder cancer among U.S. males. Even if adduct concentrations cannot be used to estimate absolute cancer risks, relative adduct concentrations may be useful for estimating relative cancer risks.

Conclusions

Too few data exist at this time to judge the capacity of DNA adduct concentrations to predict cancer incidence across species. Cross-species comparisons of aflatoxin B_1 liver adducts and 4-ABP bladder adducts are encouraging; however, differences in adduct repair rates and the effects of carcinogens on cellular proliferation complicate prediction of cancer incidence. The ratio of the average concentration of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl in the bladders of male smokers compared to nonsmokers is similar to the relative risk for bladder cancer among male smokers. Data on DNA adduct concentrations and tumor incidence in different species, involving more carcinogens, are required before it will be apparent if useful patterns are emerging for cancer risk estimation. Certainly, DNA adduct concentrations can be used to quantify exposure to chemical carcinogens.

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