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STRATEGIES FOR USE OF BIOLOGICAL MARKERS OF EXPOSURE

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SUMMARY

A major public health concern is the degree to which environmental or occupational exposures to exogenous chemicals result in adverse health effects. Biological markers have the potential for helping to answer this important question by providing links between markers of exposures and markers of early stages of the development of disease. However, that potential requires in-depth, mechanistic research to be fully realized. Biological markers of exposure have been extensively investigated, and mathematical models of the toxicokinetics of agents have been developed to relate exposures to internal doses. The field of clinical medicine has long used clinical signs and symptoms to detect disease. However, the critical area of research needed to improve the application of biomarkers to environmental health research is mechanistic research to link dose to critical tissues to the development of early, pre-clinical signs of developing disease. Only if the mechanism of disease induction is known can one determine the "biologically effective" dose and the earliest biological changes leading to disease.

BIOLOGICAL MARKERS OF EXPOSURE

Biological markers of exposure are exogenous substances or their metabolites, or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism [1]. Because biomarkers of exposure are all measures of internal substances, they are biological markers of dosimetry that are the result of exposures (Fig. 1). As shown in Figure 1, one could potentially have biomarkers for each of the indicated steps that link an exposure to a clinical disease. Even the markers of steps that are not directly in the line leading to the disease process can be useful, if one can link them

quantitatively to steps leading to disease. For example, if one knows the quantitative relationship between levels of hemoglobin adducts for a specific chemical (example of noneffective macromolecular adducts) and biologically effective liver DNA adducts (example of a biologically effective dose) for a liver carcinogen, one could theoretically use the more available blood adducts as a predictor of the biologically effective dose.

The emphasis on quantitation is to meet the requirements for setting regulations for allowable exposures and for predicting the likelihood of adverse health effects. If one only needs to know if an exposure has occurred, the presence of a biological marker specific for the chemical of concern may be all that is needed. However, for the purposes of risk assessment, that is, determining the potential for a given exposure to an exogenous substance to cause adverse health effects, one needs a biomarker that is 1) quantitatively relatable to prior exposures to a specific chemical and 2) quantitatively relatable to, or predictive of, later developing disease. Strategies to meet these needs are described below.

STRATEGIES FOR USE OF BIOLOGICAL MARKERS OF EXPOSURE TO ASSESS PRIOR EXPOSURES

Many commonly measured pharmacokinetic values, such as parent compound or metabolites in exhaled breath, blood, or urine, macromolecular adducts or degradation products of such adducts that appear in urine, can be used as biomarkers of exposure. To make quantitative assessments of the relationship of such markers to prior exposures, one must determine the rate of formation and removal (clearance) of the marker. From this information, one can predict the steady-state concentrations of the marker following various exposure scenarios. Also, with information on the rate of formation and removal of a marker,

and the factors that influence those rates (such as gender, dose, repeated exposures, route of exposure, rate of exposure), one can develop a mathematical model that will describe the concentration of the marker under different exposure conditions. While the concentration of the marker cannot be used to indicate a unique exposure scenario, the marker can indicate the types of exposure regimens that would produce the indicated level of biomarker.

From a practical viewpoint, one cannot use human populations to determine the rate of formation and clearance of markers and the influence of various factors on those rates. Therefore, most toxicokinetic studies are conducted in animal models. From detailed studies in animals, mathematical models are derived based on the animal toxicokinetic data, animal physiological data, and the physical/chemical properties of the compounds of interest (such as partition coefficients). The models, which are often referred to as physiologically based pharmacokinetic models, can then be modified for humans based on human physiological data and metabolic studies conducted with human tissues *in vitro*. Such models must then be validated with limited studies in humans. This strategy has been discussed by Henderson and Belinsky [2].

A second strategy for the use of biomarkers to help describe prior exposures is to use a battery of biomarkers with differing half-lives [3]. Some biomarkers of exposure have half-lives of minutes or hours (volatile parent compound in exhaled breath, some blood or urinary metabolites); other biomarkers may be present for days or weeks (some DNA adducts, blood albumin adducts); while others may accumulate over longer periods of time due to longer half-lives (blood hemoglobin adducts, some DNA adducts, products of DNA repair in the urine). There are also differences in the fraction of the internal dose of a chemical that is converted to each type of biomarker. In general, some of the markers with shorter half-lives,

such as urinary metabolites, represent large fractions of the internal dose, while macromolecular adducts, many of which have longer half-lives, represent only a small fraction of the dose. By combining knowledge of the half-lives of markers and the amount of marker formed relative to the total dose, one can obtain more information about a prior exposure using a battery of biomarkers than a single biomarker. For example, if multiple markers of a single chemical are determined in an individual, one should be able to distinguish between someone who has had a recent exposure, someone who is receiving an ongoing exposure and someone who was exposed repeatedly in the past but has had no recent exposures. If someone has had only a recent single exposure to a chemical, the shorter half-lived, more abundant biomarkers in the form of urinary metabolites should be readily detectable, but there should be very little of the longer half-lived, less abundant DNA adducts present. If the person has had an ongoing exposure for many years to the same chemical, there should be relatively high amounts of both the urinary metabolites and the DNA adducts. If the person was exposed some time ago, but not recently, then only the longer-lived DNA adducts or hemoglobin adducts may be detectable.

STRATEGIES FOR USE OF BIOLOGICAL MARKERS OF EXPOSURE TO PREDICT DISEASE OUTCOME

To be able to relate markers of exposure to health outcome, one needs to know which markers can be associated with the disease outcome and the degree of that association. That is, given a certain level of a biomarker of exposure, what is the probability of getting a disease? This query is certain to be made by participants in any study in which biomarkers of exposure are assayed in workers or in the general public. Currently, we have very little

information on which to base an answer. The inability to use biological markers of exposure to predict health outcome represents a major gap in our knowledge and decreases the potential usefulness of the markers. What we need are valid markers of risk.

How can we improve our knowledge is this area? Perhaps the most fruitful area of research for discovering biomarkers of exposure that can be linked to disease outcome is the study of mechanisms of disease induction. One cannot define a marker of a "biologically effective dose" unless one knows the mechanism by which the biological effect is induced. Likewise, one cannot know the earliest biological events that lead to a disease, unless one understands the mechanism of disease induction. Such mechanistic studies should provide the markers for steps that link the biological markers represented by traditional toxicokinetic measurements (left side of Fig. 1) and the biological markers represented by traditional clinical markers of disease (right side of Fig. 1).

In addition to knowledge of the mechanism of disease induction, one must define the quantitative relationship between the level of the marker and the probability of progression to an adverse health effect. Pharmaco- or toxicodynamic modeling describing the kinetics of disease development is required in a manner similar to the toxicokinetic modeling used to describe the kinetics of internal dosimetry. For example, if one wants to use chemical-specific DNA adducts to predict cancer induction, the following pieces of information are required. First one must identify the various DNA adducts formed by the chemical. Then one must determine the biological half-lives of each adduct (How long will they be present before they are repaired?) and the mutagenic potential of each adduct (How much harm will the adducts cause if they are present?). If adducts are formed that have relatively long half-lives and high mutagenic potential, one can determine if the mutations induced by the adduct

in *in vitro* studies are present in tumors induced by the chemical. Once enough is known about the disease induction to form an hypothesis for the process, one can use intervention studies, in which the proposed path to disease is blocked, to validate the path as the active disease-generating process. Finally, toxicodynamic models can be generated that describe the quantitative relationship between adduct levels and cancer induction. Such models will require knowledge of cellular dynamics involved in tumor formation.

The initial part of this approach can be illustrated by the studies of the mechanism of induction of liver tumors by vinyl chloride (VC) [4]. The four major DNA adducts formed by VC are the 7-(2-oxoethyl)-deoxyguanosine (OEdG), 3, N^4-ethenodeoxycytidine (EdC), 1,N^6-ethenodeoxyadenosine (EdA), and N^2,3-ethenodeoxyguanosine (EdG) [5]. The most abundant adduct formed is the OEdG, but this adduct has the shortest half-live of all the adducts. Also, the mutagenic potential of the OEdG adduct, as estimated by fidelity of DNA replication assays, is low [6], while the other adducts do induce mutations, especially the EdG adduct [7]. The relative amounts of the different adducts in the livers of VC-exposed adult rats were compared to the amounts in livers of similarly exposed newborn rats, which are more sensitive than adult rats to VC-induced hepatic tumors. The molar concentration of EdG was almost 4-fold higher in the newborn livers than in the adult livers, while the other etheno adducts were similar in both age groups [8]. This information indicated that the EdG adduct was a better measure of the biologically effective dose (and of risk) than were the other adducts.

As in the case of relating biological markers to prior exposure, the relationship of the levels of markers to expected health outcomes can be studied first in animal models, as discussed above for studies on VC-induced tumors. But the models developed in animals

must be validated by studies in humans (*in vitro* studies using human tissues, epidemiological studies). Studies reported by Qian et al. [9] are examples of how epidemiological studies can determine the most valid biological markers of risk for a disease. In a study of Chinese men exposed to aflatoxin in their diet, the investigators found that levels of urinary aflatoxin-N^7-guanine were a good predictor of the risk for hepatic cancer rather than other markers studied, including total AFLATOXIN-metabolites in urine and dietary intake of aflatoxin as assessed by questionnaires. Intervention studies, either in animals or in epidemiology studies, are also valuable in determining if the selected biological markers of risk are valid.

In conclusion, biomarkers can be valuable for reducing uncertainties in assessing risk for disease from chemical exposures. However, to make the markers more useful, more information is needed on the mechanisms of disease induction by chemicals; such studies will suggest the most appropriate biomarkers of risk for the disease. Much research effort will be required to establish quantitative relationships between the level of markers present and both the degree of prior exposure and the predictability of health outcome.

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FIGURE LEGEND

Figure 1. Biomarkers for risk assessment. Toward the left are biomarkers of dosimetry resulting from exposures; most of these markers represent values obtained from toxicokinetic studies. Toward the right are biological markers of effect; many of these markers are standard signs and symptoms familiar to clinicians. One of the greatest needs in biomarker research is to obtain more information on the link between biologically effective doses and the early, initial biological changes that can lead to disease; such values will come from studies on mechanism of disease induction.

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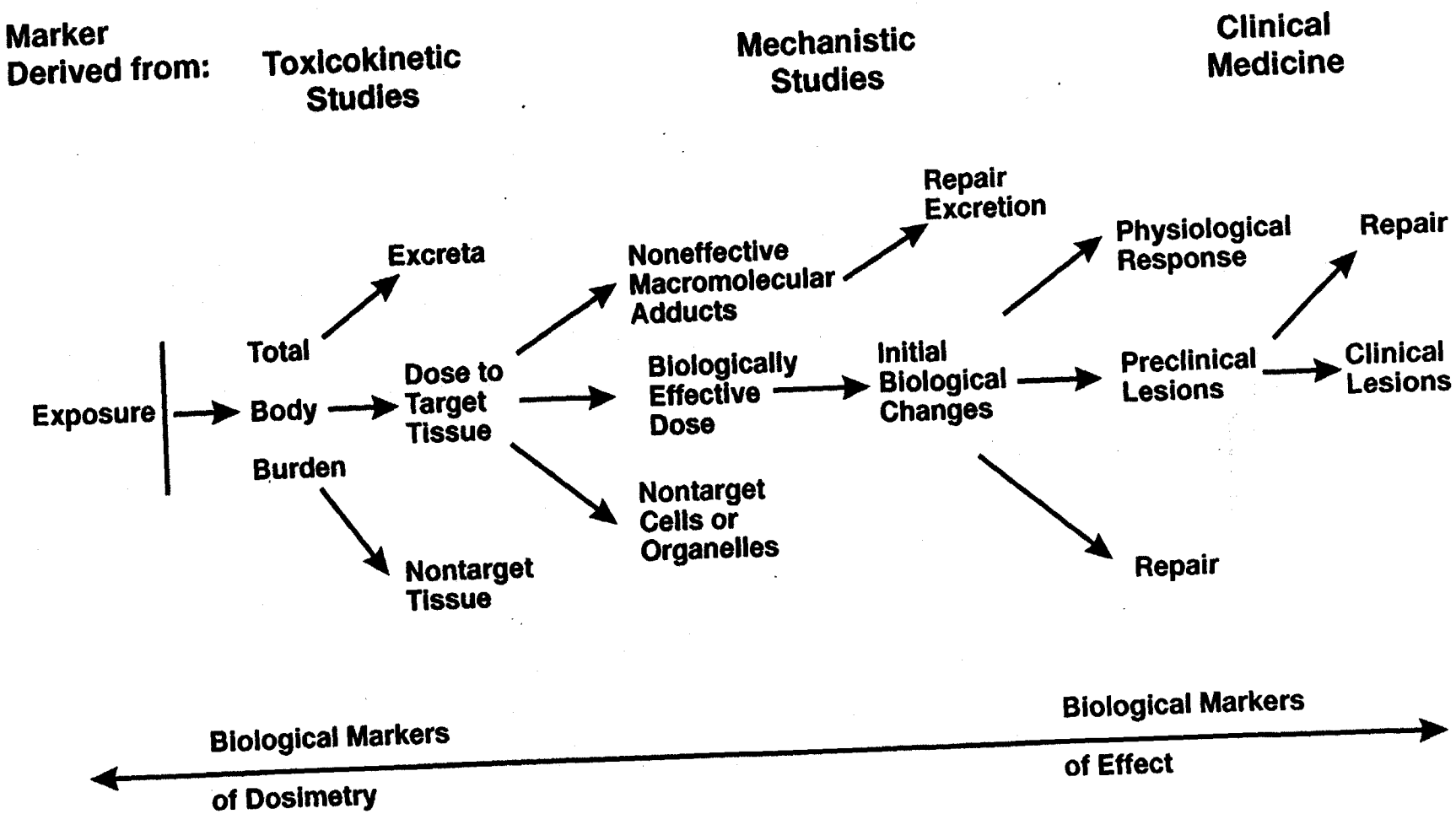
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