4 DNA Repair, Mutagenesis, and Risk Assessment

4.1 INTRODUCTION

The following analysis is based on the premise that improved methodology for risk assessment will issue from improved understanding of the fundamental processes underlying the formation of DNA adducts and their repair, and of mutagenesis, which may result should repair fail. Studies of the mutational specificity of DNA damaging agents can implicate specific DNA adducts as precursors to mutations and perhaps neoplastic changes. Molecular techniques are available by which such causal relationships can be tested. These studies are meant to identify specific DNA adducts that are expected to be the most reliable predictors of genotoxicity, and, hence, for monitoring human populations. Similarly, knowledge of the chemical structures of these adducts that result in genetic changes may provide insight into the exact nature of the metabolites that originally reacted with DNA. These data in turn may be used to identify specific adducts of a chemical and an amino acid within protein; such adducts could prove to be excellent dosimeters in blood proteins.

Several studies showing that differential kinetics of adduct removal in various organs provide a likely explanation for the organotropic effects of a DNA damaging agent. These results demonstrate the importance of studies of the removal of adducts from DNA. This argument is strengthened by the existence of human genetic disease (often associated with a high risk of cancer) associated with deficiencies in DNA repair. This association is especially prevalent for clinical syndromes such as xeroderma pigmentosum, where a defect in repair of UV light-induced damage is responsible for the disease. Speculatively, other sub-populations may exist in which DNA repair capability is diminished but not lacking totally. In principle, individuals in these groups are at elevated risk, and should be identified. However, no methodology exists to achieve this objective. The isolation of the proteins involved in repair and the genes that encode them should provide new tools by which an assessment of the risk of genetic disease can be made.

Finally, the relationship between repair of DNA adducts within specific gene sequences and changes of functional importance in tumor initiation or development is difficult to prove. Although mutational changes within oncogene or tumor suppressor gene sequences are known to occur with varying frequencies in cancers of different types, the molecular precursors to the mutational changes have yet to

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be identified. A possible modulating factor in carcinogenesis, which could explain inter- or intraspecies differences as well as organotropic effects, might be the selective repair of critical oncogenic loci. For example, a proto-oncogene sequence in a transcriptionally active gene might be subject to repair in a species refractory to carcinogenesis; whereas the same locus could be transcriptionally quiescent, and hence unrepaired, in a species sensitive to the carcinogenic regimen. Thus, differential DNA damage or repair as a consequence of differential gene transcription may possibly be contributing factors.

4.2 MONITORING OF HUMAN POPULATIONS

Several markers in humans are available to measure directly genotoxicant exposures and effects. These markers are being proposed to monitor exposures to mutagens and carcinogens. Implicit in proposals for human biomonitoring is that the process is of value to the individuals being studied. Benefits include: (a) identification of exposed individuals; (b) identification of the environmental mutagens/carcinogens; and (c) definition of range of susceptibility among humans to mutagens and carcinogens.

For an individual, the value of biomonitoring relies on the ability to differentiate between exposed persons likely to become ill from those unlikely to do so. This ability rests on the assumption that the markers being measured are valid surrogates of disease-producing events and that the endpoints measured are directly related to disease manifestation. This assumption can be tested directly in humans, providing a fourth advantage for human biomonitoring: Linking quantitatively genotoxicant exposure with disease incidence to estimate risk in other circumstances.

The genotoxicity markers addressed in this report include DNA adducts as markers of critical target tissue, chromosomal, and gene interactions. Damage to genes in somatic cells has also been analyzed at the molecular level to identify the spectrum of mutations that may indicate exposures to specific mutagens or carcinogens. At a less complex level, human population exposures may be described by either ambient monitoring or questionnaire. Finally, individuals can be characterized hypothetically, with respect to their ability to metabolize various classes of mutagens or carcinogens (pharmacogenetic characterization) and to their ability to repair DNA damage (genetic DNA repair deficiency states).

In a human population, the level of intervention justifiable for monitoring is that minimal amount needed to identify individuals with increased health risk due to genotoxicant exposures. However, intervention at this minimal level presupposes that markers of true disease relevance are known. Humans being exposed to genotoxicants are available to make this determination. Malignant diseases are being treated and cured in many individuals; unfortunately, curative therapeutic regimens often involve the use of mutagenic and carcinogenic agents. Exposure to such agents occasionally results in a second malignancy in treated individuals—usually haematological malignancy such as acute non-lymphatic

leukaemia (ANLL). Although relatively high in some patient groups (i.e., ≈ 5%), the risk of ANLL is quite acceptable, given the almost certain death from the original untreated malignancy. However, this situation provides a human population (knowingly exposed to relatively precise doses of specific mutagens and carcinogens) for study with currently available markers of genotoxicant exposure and effect. The findings of such studies would reveal relationships between markers and diseases and the degree of heterogeneity in human susceptibility.

Most current markers of genotoxicant exposures and injuries employ DNA from white blood cells or haemoglobin from red blood cells. ANLL is a disease of haematopoietic stem cells, whereby the tissue being monitored and that of the disease are identical. Although lymphocytes are usually the white blood cells being monitored, recent findings indicate a relationship between these cells and the cells involved in ANLL. ANLL is also a malignancy of short latency, with disease arising from 2 to 7 years after initial exposure to a carcinogen.

Retrospective case–control studies of only individuals who develop ANLL, and exposure-, sex-, and age-matched controls are the most efficient and relevant human population studies, as contrasted with studies using biomarker assays on large numbers of individuals. This conclusion is correct provided that blood samples are obtained for all treated cancer patients at defined times following treatment. Medical and other identifier information would be keyed to these cryopreserved samples, and updated periodically as part of patient follow-up. Virtually all cancer patients treated in medical centers in the USA, Europe, Japan, and Australia collected, stored, and updated this information; therefore, the systems exist for performing such an evaluation.

Blood samples could be cryopreserved periodically from small cohorts known not to be exposed to carcinogens or mutagens. Furthermore, this repository of samples and information could also be used to store samples from humans who have suffered large accidental exposures to mutagens and carcinogens. Some planning is required to rapidly obtain samples from accidentally exposed populations and store these samples, and later update relevant clinical information.

Once sufficient cases of secondary ANLL have been documented, retrospective case–control studies can be conducted, using "blind" study designs, relying on three groups of individuals: (a) treated patients who developed ANLL, (b) treated controls, i.e., those treated patients who did not develop ANLL, and (c) untreated controls. Only those experienced in performing biomarker assays should be relied upon to analyze the samples. Results can be correlated with disease rates to define reliable relative-risks or odds-ratios associated with the presence of a marker and the occurrence of the disease (ANLL).

Despite the limited intent of such study designs, some extensions may be possible. Individuals being studied can also be evaluated for pharmacogenetic or DNA repair characteristics. These latter measures may outweigh the value of predictors of genotoxic risk. No current marker may predict health risk with greater precision than does simple information concerning exposure, which may be useful to design in humans monitoring studies of exposures to mutagens and

carcinogens.

By extension, studies of biomarkers could also be used with rodent cancer bioassays. All markers used in human studies have counterparts in laboratory animals. Such a study would focus on the value of such biomarkers as predictors of the number and kinds of cancers induced in test animals. Such analyses can focus subsequent mechanistic studies in either cell culture or intact animals. In turn, these studies could assist in the design of additional biomarkers for human studies. As a result, delineation of mechanisms of action and the assessments of human risk can proceed as different facets of the same research undertakings.