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Project Summary

Carcinogen-DNA Adducts: Introduction, Literature Summary, and Recommendations

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This report summarizes the literature concerning adducts formed by xenobiotics with DNA and protein in order to determine their feasibility as a monitoring tool for use in exposure and risk assessment and to propose compounds and methods that may be appropriate for preliminary field studies. This report is divided into three segments.

The first segment provides an introduction to DNA damage and its relation to carcinogenesis. This segment also discusses available methodology for the measurement of macromolecular (DNA, protein) adducts. The techniques were evaluated according to their sensitivity, selectivity, limitations, and future possibilities. The next segment provides a summary of the current literature on the individual chemicals found to form adducts in both man and in experimental animals. The information in this segment and additional information was tabulated and is presented in the appendix. Finally, the conclusion and recommendation section discusses the overall potential for the use of macromolecular adducts as a measure of dose, given the current technology. Recommendations on the analytical detection methodologies, applicable chemicals, and populations to be used for a human monitoring pilot study were offered.

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The Environmental Protection Agency (EPA) is charged to protect human health and the environment, and it has acted by placing restrictions and regulations on chemicals that have been shown to be detrimental to human health or to the environment. Accurate dose measurements are critical in the evaluation of health risks and in the development of regulations that may be needed for protection from chemicals released into the environment. Therefore, there is an intense interest in devising techniques that can be used as monitoring tools to quantitate exposure to xenobiotics and that can eventually be used in risk assessment. Consequently, the EPA has developed an initiative designed to develop, refine, and apply appropriate biomarkers that can be used in conjunction with other environmental monitoring data to provide a better estimate of risk to individuals and populations. By linking biological measurements to environmental monitoring measurements, it will be possible to determine relationships that exist between total exposure, dose, and disease.

The first stage of the EPA initiative is to evaluate the feasibility of using biomarkers as a monitoring tool for use in exposure and risk assessment. This will include a compilation of available biomonitoring methods for assessing environmental exposures and methods for predicting associated health risks.

One biomonitoring method is the measurement of the reaction products of possible carcinogens with DNA and protein Structural identification and quantitation of carcinogen-DNA and protein adducts that result from exposure to these xenobiotics could provide a more accurate measure of exposure

This report discusses the feasibility of the use of DNA and protein adducts as a valid biological dosimeter to monitor the integral dose of genotoxic environmental chemicals The report is divided into three segments The first segment provides an introduction to DNA damage and its relation to carcinogenesis This segment also discusses available methodology for the measurement of macromolecular (DNA, protein) adducts The analytical techniques were evaluated according to their sensitivity, selectivity, limitations, and future possibilities The next segment provides a summary of the current literature on the individual chemicals found to form adducts in both man and in experimental animals The information in this segment and additional information was tabulated and is presented in the Appendix Finally, the conclusion and recommendation section discusses the overall potential for the use of macromolecular adducts as a measure of dose, given the current technology Recommendations on the analytical detection methodologies, applicable chemicals, and populations to be used for a human monitoring pilot study were offered

Procedure

Two data bases were searched, Cancerline and Medline from 1981 to June 1986 The search terms used were environmental mutagens and carcinogens, pharmaceuticals and DNA or protein adducts In addition to the computer search, two separate manual searches were conducted The articles in volume 62 of Environmental Health Perspectives were searched for pertinent citations Also, Chemical Abstracts was searched back to 1976 for review articles on DNA adducts A total of 335 citations were located and 112 compounds were found to form DNA and/or protein adducts The 112 compounds were cross-referenced with the HEALS priority compound list and only one match was found, styrene The literature was summarized and the pertinent information was summarized in tabular form An introduction on carcinogenesis, DNA adduct formation, and the methods used to measure adduct formation was written and recommendations were made for compounds to study and methodology to use

Discussion

DNA or protein adduct formation occurs when electrophilic molecules enter or are created within the body These electron-poor molecules attack electron-rich sites within the body These sites are primarily found in DNA and proteins It is believed that adduct formation with DNA results in an alteration of the molecule that eventually results in the creation of a mutation within the genome Through poorly-understood steps, the DNA mutation transforms the normal cell into a cancerous one Although mutations cannot be easily measured, adduct formation with both DNA and protein can be measured

After DNA adduct formation occurs, their levels can be modulated by the rate of cell division or by DNA repair Therefore, DNA adducts may be a good indicator of risk but may not be an accurate measure of exposure However, if animal studies indicate that a linear exposure-dose relationship exists, then DNA adducts would be a good indicator of exposure In general, protein adducts would give a better indication of exposure because little if any protein adduct repair occurs

Several methods exist for the detection of carcinogen-macromolecule adducts, but only a couple are suitable for human studies The use of radiolabelled carcinogens provides a very sensitive measure of adduct formation, but it is only of use in animal studies because human populations are normally not exposed to radiolabelled compounds

Spectrophotometric and fluorometric methods can be used to measure DNA to protein adducts that are formed with compounds that absorb strongly or that fluoresce The disadvantage of these methods are that they are either not sensitive enough or that they are useful only for certain compound classes Chemical derivatization methods are sensitive, but the methodology is relatively new and unproven It may also prove to be too expensive for use in studies involving many subjects

Conclusions and Recommendations

Two procedures appear to be ideally suited for human monitoring studies The first is the use of antibody techniques Antibodies are selective for a particular three dimensional structure and can be very sensitive Both polyclonal and monoclonal antibodies can be used, but monoclonal antibodies have the potential for being the most selective The antibodies are used in competitive assays where the sample adduct competes with a known amount of added adduct The sample adduct levels are calculated by using a standard curve

Another method has been developed that has the sensitivity advantage of radiolabelled compounds, and it does not require that the carcinogen be radiolabelled The method is referred to as ^{32}P-postlabeling, and the method is summarized as follows Adducted DNA is isolated from a tissue source and is digested to form 3'-mononucleotides ^{32}P is incorporated on the 5'-end of the neucleotides, and the adducts are separated by using multidimensional thin layer chromatography The separated adducts are quantified by using autoradiography Because of the high specific activity of the ^{32}P, this method can detect adducts at about one adduct per 10^{10} bases from a 1 μg sample of DNA This makes ^{32}P-postlabeling one of the most sensitive methods available This level of sensitivity may be required when one is looking for DNA adducts induced by environmental carcinogens in the general population because of the low exposure situations Both immunoassay and ^{32}P-postlabeling techniques should be adaptable to nearly any DNA adduct that is characterized It is suggested that a possible method to monitor DNA adducts would include a rapid screening with the immunoassay method followed by the ^{32}P-postlabeling method for those samples that show up negative on the immunoassay screening This procedure would be the most cost-effective as only those samples with very low levels of DNA adducts would be analyzed by the more expensive ^{32}P-postlabeling method Currently, protein adducts can only be monitored by using immunoassay techniques because the ^{32}P-postlabeling technique is specific for DNA adducts However, protein adducts should occur at higher concentrations than the DNA adducts because of the lack of protein adduct repair systems and because

greater amounts of sample are available. Therefore, sensitivity should not be an insurmountable problem.

Two main points were considered when the tissues or fluids to detect adduct formation were chosen. The first point was one of invasiveness. If the sample collection technique is too invasive, it will be impossible to obtain enough volunteers for the studies. The second point involved the usefulness of the sample. The further away the sample is from the target organ, the less representative the measured adduct levels will be of the adduct levels at the target site, unless animal studies indicate otherwise. Therefore, tissue and fluid selection was a compromise between these two points.

The best compromise fluid is blood. Sample collection is relatively noninvasive, and it contains several molecules that can contain covalent adducts. White blood cells contain DNA; therefore, DNA adduct formation can be monitored. However, one must remember that the DNA adduct level may not correlate with the DNA adduct level in the target organ(s) or with the dose received. In addition, protein adducts can be monitored. The two main proteins to be monitored would be hemoglobin and human serum albumin. Each protein would give different levels of integration data as the half-lives of the proteins are 120 and 20 days, respectively. Also, human serum albumin is extra-cellular and is not protected by a cellular membrane. Therefore, it might show a higher level of protein adduct formation. In summary, both integration and equilibration data can be obtained from blood.

Urine is also an excellent choice for monitoring DNA adducts. When DNA repair occurs, the removed adducts are excreted in the urine. However, not many carcinogen adducts have been monitored in the urine, so additional animal studies would have to be conducted. In one animal study, the levels of aflatoxin-DNA adducts found in the urine correlated with the administered dose of the carcinogen. Theoretically, any adduct that is repaired could be monitored by using this fluid.

Tissues obtained during a biopsy or autopsy would provide the most accurate information concerning the level of damage that occurs at the target site. The problem with biopsy samples is that they are very difficult to obtain, and it would be difficult to obtain volunteers for such a study. The question that needs to be asked is if target DNA adduct level information is really needed. If the main purpose of the planned studies is to obtain dose information, that is, levels of the carcinogen that have entered the body, then the information obtained from adducts measurable in blood may be sufficient.

In summary, the best approach to monitoring human subpopulations would entail collection of both blood and urine samples. This approach would allow the collection of the most data with a minimum of discomfort to the individuals.

Several compounds and compound classes were suggested. The primary consideration used was to select compounds that have the highest expectation of producing a usable exposure-dose relationship. Cisplatin, a cancer chemotherapeutic agent, was suggested as an ideal candidate compound for a study to prove feasibility because the exact amount of cisplatin administered is known. One could also determine if it is possible to correct for mediating factors such as drug metabolizing enzyme levels, glutathione levels, membrane transport, etc.

Other cancer chemotherapeutic agents might also be useful. For example, the Health Effects Research Laboratory (HERL), Research Triangle Park, NC, is conducting an exposure-dose relationship study with 2,5-diazeridinyl-3,6-bicarboethoxyamino-1,4-benzoquinone (AZQ). AZQ is used in the treatment of brain tumors. Unfortunately, long term studies are difficult because of the extremely poor prognosis for patients with brain tumors.

Psoralens are therapeutic agents used to treat various skin diseases. If adducts are found in the blood or urine after treatment, it may be that an exposure-dose correlation can be established. At present, EPA-HERL is conducting an exposure-dose feasibility study with the compound Psoraben in a collaborative study with Dr. Regina Santelli of Columbus University. They are looking at the dose-response of adducts formed with hemoglobin and white blood cell DNA.

Alkylating agents found in tobacco products, specifically 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN) would be good compounds to study. Again, the amount of carcinogen received can be accurately estimated. Snuff was suggested as the tobacco product to monitor because it contains high levels of NNN and NNK and does not contain polynuclear aromatic hydrocarbons that are derived from smoking. Other compounds that were suggested as possible candidates were aflatoxin B_1, o-toluidine, benzidine, 2-naphthylamine, vinyl chloride, acrylonitrile, styrene, and dimethylcarbamyl chloride.

In addition, several compounds have been identified that are on the EPA priority list and that may form adducts. Although little literature has been located on these compounds, it may be useful to carry out some preliminary experiments to determine if these compounds form adducts. The compounds are:

1. Dichloromethane
2. Carbon tetrachloride
3. Polychlorinated biphenyls
4. Chloroform
5. Toluene
6. Formaldehyde

An initial study with these compounds might include a simple feeding study to determine if the compounds do form adducts. If so, several log dose-response experiments should be conducted to check linearity with Hb, serum albumin, and DNA.

Although benzo(a)pyrene (BaP) does form DNA adducts, and although many animal studies have been conducted, it probably would not make a good test compound. Because BaP is ubiquitous, high background levels of BaP-DNA adducts are present and make the establishment of and exposure-dose relationship difficult.

This is not meant to be an all inclusive list as much as it is meant to comprise suggestions for compounds to be considered for environmental monitoring.

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The complete report, entitled "Carcinogen-DNA Adducts: Introduction, Literature Summary, and Recommendations," (Order No. PB 87-145 678/AS; Cost: $18.95, subject to change) will be available only from:
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