Korean. J. Toxicol.
Vol. 6, No. 1, pp. 61-73, 1990

1990 Spring Seminar
Korean Soc. Toxicol.

BIOLOGICAL HUMAN MONITORING OF CARCINOGEN EXPOSURE: A NEW STRATEGY IN CANCER PREVENTION

Byung Mu Lee

College of Pharmacy, Sung Kyun Kwon University,
Chunchun-Dong 300, Kyunggi-Do, Suwon City 440-300, Korea

(Received May 20, 1990)
(Accepted June 15, 1990)

ABSTRACT: *Human exposure to environmental carcinogens can be detected by a number of methods including immunoassay, ^{32}P-postlabeling assay, and fluorescence technique. These assays have been applied to measure biological markers of carcinogen-adducts formed with macromolecules such as DNA, RNA and protein. In an attempt to investigate causal relationships between carcinogen exposure and tumor formation, specific carcinogen-adducts have been quantitated from human tissues and body fluids of cancer patients, occupational workers heavily exposed to certain carcinogens, smokers and controls. Carcinogens studied for biological human monitoring include benzo(a)pyrene, aflatoxin B1, UV light, ethylene oxide, 8-methoxypsoralen, 4-aminobiphenyl, vinyl chloride, N-nitrosamine, cisplatin and other chemotherapeutic agents. Relevance of human monitoring for cancer research, progress in this field, methods to detect carcinogen-adducts are reviewed here. It is hoped that these approaches will be used for the risk assessment of carcinogen exposure, cancer etiology study and cancer prevention in humans.*
Keywords: *Carcinogen-adducts, Biological markers, Biological human monitoring, Risk assessment, Cancer*

INTRODUCTION

Humans are exposed to various carcinogens in the environment, air, water, food, and soil. They can be classified into biological, physical, and chemical carcinogens. Biological agents associated with human cancer are a few cases of viruses: hepatitis B virus (HBV) to liver cancer, Epstein Barr virus to lymphoma and nasopharyngeal cancer, and papilloma virus to cervical cancer (Mark, 1989).

Ionizing (*e.g.* x-ray, gamma-ray) and nonionizing radations (*e.g.* UV light) are physical agents which are involved in leukemia, lung cancer, and skin cancer in humans. The next category is chemical carcinogens which are ubiquitous in the en-

vironment and humans are easily exposed to them. Particularly, workers will be directly exposed to high levels of carcinogens in the industry when they handle hazardous carcinogens improperly.

Much research has been carried out to identify human carcinogens based on short-term test, long-term animal bioassay, and epidemiology study. Few years ago, the International Agency for Research on Cancer (IARC) advised by experts in chemical carcinogenesis was able to classify carcinogens into five groups, depending on the degree of evidence for carcinogenicity (IARC, 1987). Group 1 includes 50 human carcinogens such as aflatoxin, vinyl chloride, and tobacco smoke. Next category is Group 2A which has limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animal study. Benzo(a)pyrene, ethylene oxide and benz(a)anthracene are few examples of probable 37 human carcinogens listed in this group. In order to evaluate the carcinogenicity of chemicals, we often rely on *in vitro* and *in vivo* tests. Although animal experiments can provide dose-response data and are considered useful predictors for human response, it should be emphasized that regulatory agencies value epidemiologic studies as the most conclusive evidence for human carcinogenicity. Epidemiology is more relevant to human study than animal bioassay, but time and cost are the limiting factors. In addition, epidemiology data can only suggest evidence for an association rather than identification of specific causative factors. However, epidemiology has been greatly supported by the introduction of molecular cancer epidemiology or molecular epidemiology which combines toxicology and analytical epidemiology. The principle of molecular epidemiology is based on human monitoring of carcinogenic dose and preclinical response in multi-stages of chemical carcinogenesis. A number of biomarkers and analytical tools have been developed for biological human monitoring in cancer research.

RATIONALE OF BIOLOGICAL HUMAN MONITORING IN CANCER RESEARCH

In 1775 Sir Pircivall Pott observed the high incidence of scrotal cancer among chimney sweepers who had been exposed to coal tar and soot (Pott, 1775). One hundred forty years later, this epidemiological observation was supported by Yamagiwa and Ichikawa who successfully produced malignant tumors on the ears of rabbits after repeated application of coal tar. Since this first example of experimental chemical carcinogenesis, much research has been carried out on understanding of the mechanism of chemical carcinogenesis. Now, it has been conceived that chemical carcinogenesis is a multiple stage (initiation, promotion, and progression) and a long latent period dependence. The first stage of initiation is a genotoxic event where electrophilic carcinogens covalently bind to nucleophilic center of macromolecules such as, DNA, RNA, and protein (Weinstein, 1982). The existence of carcinogen-DNA adduct, covalent binding of carcinogen to DNA, during DNA replication leads to mutation, the initial step for transformation and tumorigenesis at the late stage. General types of DNA damages and agents are listed in Table 1.

In order to minotor humans exposed to chemical carcinogens, carcinogen-DNA ad-

Table 1. Types of DNA damages and agents

DNA damage	Agent
1) Base modification	Chemical carcinogen
2) Cross-links	Chemical carcinogen
DNA-DNA	Radiation
DNA-protein	
3) Strand breaks	Chemical carcinogen
single strand	Radiation
double strand	
4) Pyrimidine dimer	UV light

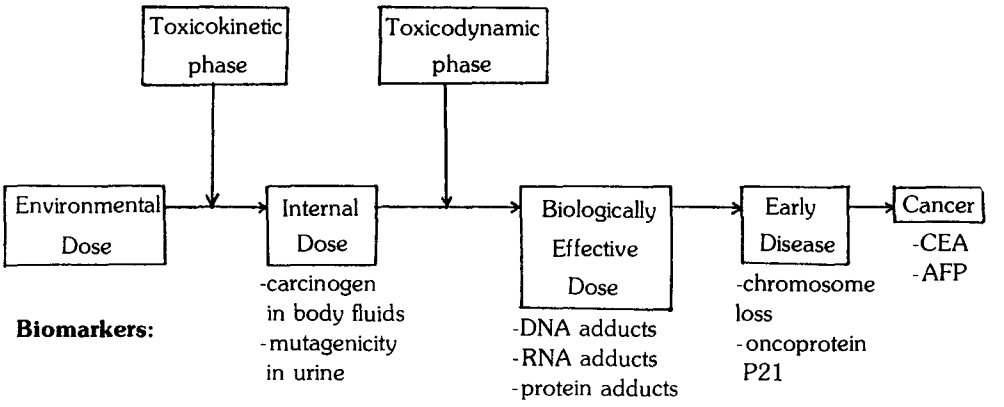

Fig. 1. Current approach to biological human monitoring in cancer research. CEA: carcinoembryonic antigen, AFP: alpha feto protein.

ducts have been studied since DNA is considered as the critical target macromolecule for chemical carcinogenesis. Carcinogen-DNA adducts can provide a marker of biologically effective dose, an alteration on normal biochemical or molecular process. Thus they have been measured in humans with various occupational or environmental exposure to chemical carcinogens (Perera *et al.*, 1982: Poirier *et al.*, 1986: Santella *et al.*, 1987). Studies for DNA adduct measurements have been successfully carried out because detection methods such as enzyme linked immunosorbent assay (ELISA), ^{32}P-postlabeling assay, fluorescence technique, and HPLC were well established. Among them ELISA has been widely used in human monitoring studies because of its advantage over other methods. Current approach to monitor humans exposed to carcinogens and biomarkers available for cancer research are in Fig. 1.

However, it is not always feasible to obtain sufficient quantities of human samples (at least, 1 gram of tissue, or 30-40 m*l* of blood) for DNA analysis by immunoassays. Thus measurements of chemical-protein adducts (hemoglobin (Hb), albumin) have been recommended as alternative markers of biologically effective dose since they are similar to DNA adducts in terms of the dose response of adduct formation.

DETECTION METHODS OF CARCINOGEN-DNA ADDUCTS

Immunologic Methods for Specific DNA Adducts

Carcinogen-adducts can be detected by immunologic methods such as ELISA, immunoaffinity chromatography, immunoprecipitation, and immunohistochemical assays. A number of antibodies have been developed against specific carcinogen-adducts for these approaches. Competitive ELISAs are carried out to detect DNA adducts as described previously (Santella *et al.*, 1988). Briefly, polystyrene 96 microwell plates (Immulon 2, Dynatech Lab., Alexandria, VA) are coated with antigen. After washing with phosphate buffered saline (PBS) and incubation with fetal calf serum (FCS), a standard curve of antigen was obtained (mixed with antigen before addition to the plate). Antibody binding to antigen is conjugated with either alkaline phosphatase or peroxidase. Finally, corresponding substrates are added and color or fluorescent product is measured by microplate readers.

For monitoring human exposure to benzo(a)pyrene (BP), monoclonal and polyclonal antibodies have been developed for immunoassays (Poirier, 1981: Santella *et al.*, 1984). A number of studies have utilized these antibodies to monitor adducts on DNA isolated from various human tissues for quantitation of 7, 8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene-DNA (BPDE-I-DNA) adduct, the major DNA adduct in N2 position of guanine. The sensitivity of the ELISA is about one BPDE-I-adduct per 10^{10} nucleotides (Santella *et al.*, 1986), but multiple PAH adducts are measured by the immunoassy since antibodies developed against BPDE-I-DNA cross-react with DNA modified other diol-epoxides of PAH (chrysene, benz(a)anthracene, etc.) stereochemically similar to BPDE-I. In the first study utilizing these antibodies, four of eleven lung tumor tissues showed measurable BP-DNA antigenicity (0.14-0.18 fmol BPDE-I-dG equivalents/μg DNA) by ELISA and cigarette smoking did not appear to affect the assay results (Perera *et al.*, 1982). A well conducted study was also carried out with 35 Finnish foundry workers at higher risk of lung cancer. When exposure levels were classified as low, medium and high, BPDE-DNA adduct of WBC was clearly dose-dependent, but not significantly influenced by current cigarette smoking or by time since vacation (Perera *et al.*, 1988). Examples of carcinogens for DNA adducts in human monitoring are summarized (Table 2).

Aflatoxin B1 (AFB1)-DNA adducts have been measured from a Chinese population at high risk for liver cancer. AFB1-2,3-epoxide, an ultimate carcinogenic form of AFB1 after metabolism, forms a major adduct at the N7 position of guanine which either depurinates or ring opens. It has been known that hepatitis virus B is associated with human liver cancer, but aflatoxin exposure and alcohol consumption have been considered important cofactors. In order to investigate a causal relationships between aflatoxin exposure and liver cancer, AFB-albumin adduct study has been recently undertaken at large scale in cooperation with Taiwan at Columbia University, Cancer Center.

^{32}P-postlabeling Method for Unknown DNA Adducts

Another approach to quantitate PAH-DNA adducts is the ^{32}P-postlabeling assay

Table 2. Examples of carcinogens for human monitoring studies

Carcinogen	Cancer	Biomarker	Population	Reference
Benzo(a) pyrene	Lung	BPDE-I-DNA adduct	Lung cancer patients	Perera *et al.* (1982)
Cigarette smoking	Lung	BPDE-I-DNA dduct	Lung cancer patients, Smokers	Perera *et al.* (1987)
N-nitrosamine	Esophageal	06-alkyl-G adduct	Population at high risk	Wild *et al.* (1986)
Aflatoxin B1	Liver	Aflatoxin B1 -DNA adduct	Liver cancer patients	Wild *et al.* (1986)
Tobacco smoke	Cervical	PAH-DNA adduct	Smokers in pregnancy	Everson *et al.* (1986)
Cisplatin	Unknown	Cisplatin-DNA adduct	Chemotherapy patients	Poirier *et al.* (1986)
Betal	Oral	PAH-DNA adduct	Betel chewer	Dunn and Stich (1986)

*BPDE-I-DNA: benzo(a)pyrene diol epoxide-I-DNA, PHA: polycyclic aromatic hydrocarcon.

Table 3. Examples of carcinogen protein adducts in humans

Carcinogen	Cancer	Biomarker	Population	Reference
Benzo(a) pyrene	Lung	BPDE-I-albumin	Foundry workers Roofers	Lee *et al.* (in preparation)
EO	Leukemia	EO-valine -cysteine -histidine	Plant workers	Calleman (1986)
4-ABP	Bladder	4-ABP-Hb	Smokers	Bryant *et al.* (1987)
Aflatoxin B1	Liver	Aflatoxin B1-albumin	Population at high risk	Gan *et al.* (1988)

*EO: ethylene oxide, 4-ABP: 4-amino biphenyl.

which can detect one adduct/10^7-10^{10} nucleotides. The principle of the assay is that DNA is enzymatically digested (micrococcal endonuclease and spleen exonuclease) to 3′-phosphorylated adducts and normal mononucleotides. They are then ^{32}P-labeled at their 5′-hydroxyl groups by [γ-^{32}P]ATP and polynucleotide kinase to produce deoxyribonucleoside-3′,5′-biphosphates. Adducted nucleotides are separated from normal nucleotides by thin-layer chromatography or HPLC and quantitated. To enhance the sensitivity of this assay, nuclease P1 digestion is adopted to enrich adducts for labeling (Reddy and Randerath, 1986). This modified technique is useful when adducts are resistant to nuclease P1. With this assay, several DNA adducts were detected from

placental tissues of smokers but not nonsmokers during pregnancy (Everson *et al.*, 1986). Among smokers, one of the adducts was only weakly related to questionnaire and biochemical measures of the intensity of smoking exposures, which might suggest modulation by individual susceptibility factors. Although the adducts remain unidentified, the data indicate the assciation of cigarette smoking with covalent damage to human DNA. In addition, levels of smoking-related adducts from placental DNA were inversely associated with the birth weight of the offspring (Everson *et al.*, 1988). DNA from mononuclear and non-mononuclear cells of human bone marrow was analysed by the ^{32}P-postlabeling assay for the presence of aromatic adducts. With interindividual variations, all of 10 healthy adults showed detectable adduct levels of 1-9 adducts per 10^8 nucleotides. Similar adducts were also detected in the DNA of peripheral WBC of both smokers and nonsmokers, but at lower levels than in bone marrow (Phillips *et al.*, 1986). This result suggests that adduct formation in peripheral WBC may not be primarily related to smoking. DNA adducts were found in exfoliated cells from the oral cavity of nonsmokers and of three groups at high risk for oral cancer: betel nut chewers, tobacco chewers, and inverted smokers (Dunn and Stich, 1986). In foundry workers, 22-80% of WBC DNA samples were positive by the ^{32}P-postlabeling assay, depending on exposure level (Phillips *et al.*, 1988). Advantages of ^{32}P-postlabeling assay are high sensitivity, 1 adduct/10^{10} nucleotides and capability to detect many aromatic carcinogen adducts at once. The major disadvantages of ^{32}P-postlabeling assay that are unable to identify adducts, limitation of the use of radioactive ^{32}P, a time-consuming method. Nonetheless, this technique has become popular and has been widely used.

Fluorescent Method

An alternate assay takes advantages of the fluorescent properties of BP. For synchronous fluorescence spectrometry, DNA samples are acid treated and release BP-tetraols, more fluorescent than adducts, measured by scanning with a fixed difference between excitation and emissin monochromater (Rahn *et al.*, 1982). Using this assay, DNA adducts of WBC in humans were determined with a sensivity of 3 adducts/ 10^8 nucleotides (Haugen *et al.*, 1986). After 3 weeks of vacation, the level of adducts remained the same or decreased for 90% of the workers tested. In coke oven workers, about 68% of the samples (27 of 40) had detectable adduct levels in the range of 0.06-34.3 fmol BPDE-DNA/ug DNA. Antibodies to the DNA adducts were also found in the sera of a number of the workers (Harris *et al.*, 1985). In another study of coke oven workers, all 13 samples tested had detectable levels of adducts (mean $\pm$ 1.7 fmol BPDE-DNA/μg DNA: range from 0.1 to > 13.7). BP-like DNA adducts of WBC were also measured from aluminum smelter workers at a level of 6.5 fmol/μg DNA, but less than 5% were positive (Vahakangas *et al.*, 1986). Recently, Synchronous fluorescence spectroscopy combined with HPLC was introduced for the detection of covalently bound BP residues in human DNA and hemoglobin (Weston *et al.*, 1989).

CARCINOGEN-PROTEIN ADDUCTS

In biological human monitoring, quantitation of carcinogen protein adducts from blood has been suggested as an alternate marker of genotoxic exposure to carcinogens. Measurements of hemoglobin adducts, for example, have advantages over DNA adduct measurements: 1) hemoglobin is present in larger quantities in blood (140-160 mg/ml) than white blood cell DNA (5-20 ug/ml), 2) the estimation of chronic exposure to low levels of chemical carcinogens may be possible over the lifespan of the erythrocyte (about 4 months in humans) due to the lack of repair mechanism in red blood cells, 3) hemoglobin is more readily available from human populations for routine screening than organ or tissue biopsies. Several studies have monitored human exposure to ethylene oxide (E0) (Calleman *et al.*, 1978: Tornqvist *et al.*, 1986) and 4-aminobiophenyl (Bryant *et al.*, 1987) by chemical measurement of hemoglobin adducts. The half life of albumin is 20 days in man and the predicted adduct level by chronic exposure is 30 times greater than that by 1 day exposure (Sabbioni *et al.*, 1987). In blood sera, albumin is a major protein (55-60 mg/ml) and has been shown to be highly modified by a number of carcinogens (Wild *et al.*, 1986: Lee and Santells, 1988). Due to the limitations of chemical approach to quantitate protein adduct, immunologic detection method needs to be developed.

In order to develop immunologic techniques for measuring benzo(a)pyrene (BP)-protein adducts, we previously developed two monoclonal antibodies which recognize protein modified by BPDE-I (Santella *et al.*, 1986). Another antibody, 8E11, developed from animals immunized with BPDE-I-guanosine coupled to albumin, was found to detect protein adducts with even greater sensitivity than those developed against the modified protein. However, competitive ELISA showed that all three antibodies had about tenfold lower reactivity for the modified protein compared to BPDE-I-tetraols, the hydrolysis products of BPDE-I. This was attributed to burying of the BP residue in hydrophobic regions of protein making the adduct inaccessible to the antibody. Since BPDE-I-tetraols can be released from BPDE-I modified protein by acid hydrolysis (Shugarts, *et al.*, 1985), a more sensitive assay for quantitation of adducts was developed, involving acid release of tetraols followed by their extraction and quantitation in an ELISA (Santella *et al.*, 1986). While this approach worked well on proteins modified *in vitro* with BPDE-I, it could not be used to determine adduct levels on globin (Gb) isolated from animals treated *in vivo* with [^{3}H]BP (Wallin *et al.*, 1987). Only about 2% of the total bound radioactivity could be released with acid treatment from the *in vivo* sample compared to about 40% from the *in vitro* sample. These results suggested that either the adducts formed *in vivo* were not diol epoxide adducts or diol epoxide adducts were formed but could not be acid hydrolyzed. Thus an alternate approach utilizing enzyme digestion of the protein before analysis by ELISA was developed. The use of enzyme allowed ELISA to quantitate protein adducts prepared by *in vitro* with high sensitivity.

To validate ELISA quantitation of protein adducts in biological samples, Gb was isolated from animals treated with [^{3}H]BP and enzymatically digested (Lee and Santella, 1988). The ELISA could detect 90-100% of the Gb adducts determined by

radioactivity over the range of BP doses tested. At all treatment levels, a good correlation was found between adduct levels in blood proteins and DNA adducts levels in tissues. This result suggests that both Gb and serum albumin could be used as surrogates for the measurements of specific tissue DNA adducts (Fig. 2). In addition, up to 200 μg/well of digested Gb from control animals did not interfere with the ELISA indicating that large amounts of protein from biological samples could be tested. To further improve the sensitivity of the ELISA an immunoaffinity technique was developed for enriching the sample in adduct before analysis. For application to human samples, the affinity column will probably be used on samples containing lower adduct levels than present in treated animals.

FACTORS AFFECTING ADDUCT FORMATION

Adduct formation by carcinogens is dependent on the bioavailability of ultimate carcinogen reached on target DNA or other macromolecules after bioactivation and detoxification of chemical carcinogens.

Unlike genotoxic alkylating agents, BP, a precarcinogen, requires metabolic activation to become an electrophilic ultimate carcinogen which can interact with DNA and other cellular macromolecules. BP is biotransformed or biotransactivated by mixed function oxidase (MFO) system and other enzymes into a variety of metabolites such as phenols, quinones, dihydrodiols, epoxides, diol epoxides, triols, and tetraols. Among metabolites, (+)BPDE-I has been considered as the ultimate carcinogenic form of BP since it showed the highest tumorigenicity in animals (Buening *et al.*, 1978). BPDE-I covalently binds to DNA and perhaps thereby initiates tumorigenesis (Weinstein *et al.*, 1976). However, bioactivated BP metabolites can be detoxified by conjugation and excreted from the body. Epoxides and diol epoxides can be conjugated by glutathione-S-epoxide-transferase while other BP metabolites (phenol, dihydrodiol, triol, tetraol, and quinone) can be inactivated by conversion to glucuronides and sulfates (Selkirk *et al.*, 1986). Therefore, the bioavailability of the ultimate carcinogenic BPDE-I is significantly influenced by metabolism.

In addition, it must be also noted that adduct formation is affected by antioxidants (vitamin A, C, E, beta-carotene, etc.) which can interfere with the formation of adduct on DNA, RNA, and protein. Another important factor is repair in DNA. Once DNA adduct is formed, it can be repaired by various ways of repair mechanisms. Failure to repair or error-prone repair of DNA adducts, however, can lead to mutation, gene rearrangement and amplification, important steps in the development of malignant tumors. In summary, factors affecting adduct formation are bioactivation of carcinogens to become electrophiles (except for primary carcinogens), detoxification, bioavailability of ultimate carcinogens on macromolecules, repair (*e.g.* DNA) and external factors, like antioxidants.

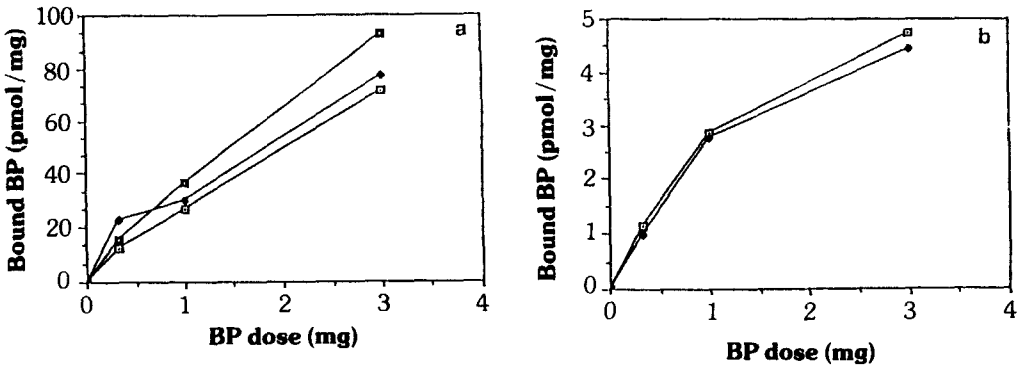

Fig. 2. Dose-response relationships for BP adduct (pmol/mg protein or DNA) formation in mice 24 h after i.p. treatment with [^{3}H]BP (mg/animal). The macromolecules were (a) erythrocyte lysate (Hb) (⊡), serum albumin (◆) and liver DNA (□) and (b) Gb (⊡) and Lung DNA (◆). Data are the means of two values (Reprinted from lee and Santella, 1988).

CANCER RISK ASSESSMENT

Risk assessment on cancer is to evaluate the probability that will produce carcinogenic effect or cancer under specified conditions. In general, the process for risk assessment includes risk characterization through hazard identification, dose-response relationship and exposure estimation and often relies on toxicologic and epidemiologic data (NAS/NRC, 1983).

Questions remain whether DNA adducts can serve as quantitative or qualitative predictors of cancer risk in humans. In animal study, there is a sufficient evidence that the ability of carcinogens to form adducts is well correlated with carcinogenic potency. For example, aflatoxin B1 can form more DNA adduct in the liver than any other carcinogen tested in rats (Lutz *et al.*, 1986). This suggests that the organotropic carcinogen-adduct formation may be used to predict the potency of its carcinogenicity on the target organ. In human cancer risk assessment, the best model carcinogen is ethylene oxide (E0) which has been used for sterilant in hospital equipment, fumigant and organic synthesizer. This alkylating agent directly binds to N-7 position of guanine in DNA to form N-7-(2-hydroxyl)guanine (Segerback *et al.*, 1983). In hemoglobin, histidine, N-terminal valine and cysteine are the major target amino acids hydroxylated by E0. By measuring E0-hemoglobin adducts in sterilization plant workers, the risk of 1 ppm.hr E0 exposure was estimated as a unit of radiation (Calleman *et al.*, 1978). The workers' average tissue dose of E0 was converted to the radiation risk equivalents. This radiation equivalent allowed a calculation of the excess risk of leukemia in the workers exposed to E0, which is similar to the result seen in follow-up epidemiological studies of that cohort (Hogstedt, 1986).

However, many factors are influencing on cancer risk assessment. The major pro-

blem in estimating cancer risk is extrapolation from high dose data to low dose. Probably, adducts produced by low dose exposure to carcinogens may not be detected by available technology. It is of concern due to the fact that genetoxic carcinogens such as BP and EO have no threshold in theory of cancer initiation. For the accuracy of cancer risk assessment, more sensitive methods to detect carcinogen adducts need to be developed. Other factors are individual susceptibility and synergistic interaction with other biological or physical agents.

FUTURE DIRECTION AND CONCLUSION

Research on DNA and protein adducts has been progressed at a rapid speed in monitoring humans exposed to chemical carcinogens. The measurement of cross-sectional carcinogen adduct can provide a biological effective dose, which considered an important biomarker in tumorigenesis at the late stage. This is based on the current understanding of chemical carcinogenesis mechanism. However, it has not been known that which type of DNA adduct, for example, is critical for the tumor formation. If a specific DNA adduct (*e.g.* BPDE-I-dG) or gene damage is involved in tumor formation, it will be more proper to monitor it rather than whole DNA damage.

Furthermore, carcinogen adducts represent only initiation markers in the multistages of chemical carcinogenesis. For the complete monitoring of human carcinogenesis, we have to search for promotion and progression markers. Depending on the specificity and sensitivity of biomarkers, the use of mutiple biomarkers could be advantageous in predicting cancer risk at different stages. Progress in this field, however, will be able to answer questions raised and contribute to elucidate cancer etiology and prevention in the future.

REFERENCES

Bryant, M.S., Skipper, P.L. and Tannenbaum, S.R. (1987): Homoglobin adducts at 4-aminobiphenyl in smokers and nonsmokers, *Cancer Res.*, **47,** 602-608.

Buening, M.K., Wislock, P.G., Levin, W., Yagi, H., Thakker, D.R., Akagi, H., Koreeda, M., Jerina, D.M. and Conney, A.H. (1978): Tumorigenecity of the optical enantiomers of the diastereomeric benzo(a)pyrene 7,8-diol-9,10-epoxides in newborn mice:Exceptional activity of (+)-7,8-dihydroxy-9,10-epoxy-7,8,9.10-tetrahydrobenzo(a) pyrene, *Proc. Natl. Acad. Sci. (USA),* **7,** 5358-5361.

Calleman, C.J., Ehrenberg, L., Jansson, B., Osterman-Golker, S., Segerback, D., Svensson, K. and Wachtneister, C.A. (1978): Monitoring and risk assessment by means of alkyl groups in hemoglobin in persons occupationally exposed to ethylene oxide, *J. Environ. Path. & Toxic.*, **2,** 427-442.

Calleman, C.J. (1986): Monitoring of background levels of hydroxyethyl adducts in human hemoglobin, *Prog. Clin. Biol. Res.*, **209**B, 261-270.

Dunn, B.P. and Stich, H.F. (1986): ^{32}P-Postlabeling analysis of aromatic DNA adducts in human oral mucosal cells, *Carcinogenesis,* **7,** 115-1120.

Ehrenberg, L., Hiesche, K.D. Osterman-Golkar, S. and Wennberg, I. (1974): Evolution of genetic risks of alkylating agents: Tissue doses in the mouse from air contaminated with ethylene oxide, *Mutation Res.*, **24,** 82-203.

Everson, R.B., Randerath, E., Santella, R.M., Cefalo, R.C., Avitts, T.A. and Randerath, K. (1986): Detection of smoking-related convalent DNA adducts in human placenta, *Science*, **231,** 54-57.

Everson, R.B. Randerath, E., Santella, R.M., Avitts, T.A., Weinstein, I.B. and Randerath, K. (1988): DNA damage in human placenta and maternal smoking and birth weight, *J. Natl. Cancer Inst.*, **80,** 567-576.

Gan, L.S., Skipper, P.L., Peng, X., Groopman, J.D., Chen, J., Wogan, G.N. and Tannenbaum, S.R. (1988): Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: correlation with aflatoxin B1 intake and urinary excretion of aflatoxin M1, *Carcinogenesis*, **9,** 1323-1325.

Harris, C.C., Vahakangas, K., Newman, J.M., Trivers, G.E., Shamsuddin, A., Sinopoli, N., Mann, D.L. and Wright, W.E. (1985): Detection of benzo[a]pyrene diol epoxide-DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers, *Proc. Natl. Acad. Sci.* (USA), **82,** 6672-6676.

Haugen, A., Becher, G., Benestad, C., Vahakangas, K., Trivers, G.E., Newman, J.J. and Harris, C.C. (1986): Determination of polycyclic aromatic hydrocarbons in the urine, benzo[a]pyrene diol epoxide-DNA adducts in lymphocyte DNA and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in work atmos, *Cancer Res.*, **46,** 4178-4183.

Hogstedt, C. (1986): Future perspectives, needs and expectations of biological monitoring of exposure to genotoxicants in prevention of occupational disease, *in Monitoring of Occupational Genotoxicants* (M. Sora and H. Norppa, (Eds.) Allan R. Liss, Inc., New York, p. 231-243.

IARC, (1987): IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Supplement 7, Overall evaluations of carcinogenicity: An updating of IARC monographs, International Agency for Research on Cancer, Lyon.

Lee, B.M. and Santella, R.M. (1988): Quantitation of protein adducts as a marker of genotoxic exposure: immunologic detection of benzo(a)pyrene-globin adducts in mice, *Carcinogenesis*, **9,** 1773-1777.

Lee, B.M., Baoyun, Y., Herbert, R., Hemminki, K., Perera, F.P., Santella, R.M. (1990): Immunologica measurement of polycyclic aromatic hydrocarbon-albumin adducts in foundry workers and roofers, (*in preparation*).

Lutz, W.K. (1986): Quantitative evaluation of DNA binding data for risk estimation and for classification of direct and indirect carcinogens, *J. Cancer Res. Clin. Oncol.*, **112,** 85-91.

Mark, J.L. (1989): How DNA viruses may cause cancer, *Science*, **243,** 1012-1013.

NAS/NRC. (1983): Risk assessment in the Federal Government: managing the process (National Academy Press, Washington, D.C.), p. 8-15.

Perera, F.P., Poirier, M.C., Yuspa, S.H., Nakayama, J., Jaretzki, A., Curnen, M.M., Knowles, D.M. and Weinstein, I.B. (1982): A pilot project in molecular cancer epidemiology: determination of benzo[a]pyrene-DNA adducts in animal and human tissues by immunoassay, *Carcinogenesis,* **3,** 1405-1410.

Perera, F.P., Santella, R.M., Brenner, D., Poirier, M.C., Munshi, A.A., Fischman, H.K. and VanRyzin, J. (1987): DNA Adducts, Protein Adducts and SCE in Cigarette Smokers and Nonsmokers, *J. Natl. Cancer Inst.*, **79,** 449-456.

Perera, F.P., Hemminiki, K., Young, T.L., Santella, R.M., Brenner, D. and Kelly, G. (1988): Detection of Polycyclic Aromatic Hydrocarbon-DNA Adducts in White Blood Cells of Foundry Workers, *Cancer Res.*, **48,** 2288-2291.

Phillips, D.H., Hewer, A. and Grover, P.L. (1986): Aromatic DNA adducts in human bone marrow and peripheral blood leukocytes, *Carcinogenesis,* **7,** 2071-2075.

Phillips, D.H., Hemminki, K., Alhonen, A., Hewer, A. and Grover, P.L. (1988): Monitoring occupational exposure to carcinogens: detection by ^{32}P-postlabelling of aromatic DNA adducts in white blood cells from iron foundry workers, *Mutation Res.*, **204,** 531-541.

Poirier, M.C. (1981): Antibodies to carcinogen-DNA adducts, *J. Natl. Cancer Inst.*, **67,** 515-519.

Poirier, M.C., Reed, E., Ozols, R.F. and Yuspa, S.H. (1986): DNA adduct formation and removal in human cancer patients, in *Biochemical and Molecular Epidemiology of Cancer,* (Harris, C.C. (Ed.), Alan R. Liss, Inc., New York, pp. 303-311.

Pott, P. (1775): Chirurgical observations relative to the cataract, the polypus of the nose, the cancer of the scrotum, the different kinds of ruptures, and the mortification of the toes and feet, in Chirurgical Works (Hawes, L., Clark, and Collins, R., (Eds.), London, p. 63-68.

Rahn, R.O., Chang, S.S., Holland, J.M. and Shugart, L.R. (1982): A fluorometric-HPLC assay for quantitating the binding of benzo[a]pyrene metabolities to DNA, *Bio. Biophys. Res. Comm.*, **109,** 262-268.

Reddy, M.V. and Randerath (1986): Nuclease P1-mediated enhancement of sensitivity of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts, *Carcinogenesis,* **7,** 1543-1551.

Sabbioni, G., Skipper, P.L., Buchi, G. and Tannenbaum, S.R. (1987): Isolation and characterization of the major serum albumin adduct formed by aflatoxin B1 *in vivo* in rats, *Carcinogenesis,* **8,** 819-824.

Santella, R.M., Lin, C.D., Cleveland, W.L., and Weinstein, I.b. (1984): Monoclonal antibodies to DAN modified by a benzo[a]pyrene diol epoxide, *Carcinogenesis,* **5,** 373-377.

Santella, R.M., Lin, C.D. and Dharmaraja, N. (1986): Monoclonal antibodies to a benzo[a]pyrene diolepoxide modified protein, *Carcinogenesis,* **7,** 441-444.

Santella, R.M., Gasparo, F.P., and Hsieh, L.L. (1987): Quantitation of Carcinogen-DAN Adducts with Monoclonal Antibodies, *Progress in Experimental Tumor Research,* **31,** 63-75.

Santella, R.M., Weston, A., Perera, F.P., Trivers, G.T., Harris, C.C., Young, T.L.,

Nguyen, D., and Lee, B.M., Poirior, M.C. (1988): Interlaboratory comparison of antisera and immunoassays for benzo(a)pyrene-diol-epoxide-I-modified DNA, *Carcinogenesis*, **9,** 1265-1269.

Segerback, D. (1983): Alkylation of DNA and hemoglobin in the mouse following exposure to ethene and ethene oxide, *Chem. Biol. Inter.*, **45,** 139-151.

Shugart, L. (1985): Quantitating exposure to chemical carcinogens: *in vivo* alkylation of hemoglobin by benzo[a]pyrene, *Toxicology*, **34,** 211-220.

Selkirk, J.K., Merrick, B.A., Schaeffer, E.L., Mann, R.C., and Mansfield, B.K. (1986): The role of metabolism in benzo(a)pyrene carcinogenesis, *Prog. Clin. Biol. Res.*, **209A,** 483-493.

Tornqvist, M., Mowrer, J., Jensen, S., and Ehrenberg, L. (1986): Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degration method, *Anal. Biochem.*, **154,** 255-266.

Vahakangas, K., Haugen, A. and Harris, C.C. (1985): An applied synchronous fluorescence spectrophotometric assay to study benzo[a]pyrene-diolepoxide-DNA adducts, *Carcinogenesis*, **6,** 11-9-1116.

Wallin, H., Jeffrey, A.M. and Santella, R.M. (1987): Investigation of Benzo-[a]pyrene-globin Adducts, *Cancer Letter.*, **35,** 139-146.

Weinstein, I.B., Jeffrey, A.M., Jennette, K.W., Blobstein, S.H., Harvey, R.G., Harris, C., Autrup, H., Kassai, H. and Nakanishi, K. (1976): Benzo(a)pyrene diol epoxides as intermediates in nucleic acid binding *in vitro* and *in vivo*, *Science*, **193,** 592-595.

Weinstein, I.B. (1982): Carcinogenesis as a multistage process-experimental evidence, *in* Host Factors in Human Carcinogenesis, (Armstron, B. and Bartsch, H., Eds.) IARC Sci. Publ. Lyon, Vol. **39,** p. 9-24.

Weston, A., Rowe, M.L., Manchester, D.K., Farmer, P.B., Mann, D.L. and Harris, C.C. (1989): Fluorescence and mass spectral evidence for the formation of benzo(a)pyrene anti-diol-epoxide-DNA and -hemoglobin adducts in humans, *Carcinogenesis*, **10,** 251-257.

Wild, C.P., Umbenhauer, D., Chapot, B., Montesano, R. (1986): Monitoring of individual human exposure to aflatoxins (AFB) and Nitrosamines (NNO) by immunoassays, *J. Cell. Biochem.*, **30,** 171.