Detection of DNA-Reactive Metabolites in Human Serum after 1,4-Dichlorobenzene Inhalation: Role of Human Biomonitoring

Hailin Tian,[a] Bathini Madhusree,[a] Morio Fukuhara,[a] Hiroshi Miyazawa,*,[a] Sumio Goto,[b] and Toshiaki Yoshida[c]

[a]Department of Pharmaceutical Sciences, [b]Department of Community Environmental Sciences, National Institute of Public Health, 4–6–1 Shirokanedai, Minato-ku, Tokyo 108–8638, Japan, and [c]Division of Industrial Health, Osaka Prefectural Institute of Public Health, 3–69 Nakamichi 1-chome, Higashinari-ku, Osaka 537–0025, Japan

(Received July 25, 2000; Accepted October 16, 2000)

Three healthy volunteers were exposed to 1,4-dichlorobenzene (*p*-DCB) vapor (2.4–2.8 ppm) for 1 hr, peripheral blood was taken before and after exposure. Serum was collected and incubated with calf thymus DNA to examine whether there are any DNA-binding metabolites persisting in human serum. Among the 3 subjects we detected 4, 2, and 0 DNA adducts respectively, no differences were found in adduct profiles before or after *p*-DCB inhalation. The result reflects that qualities and quantities of DNA-reactive metabolites in human sera are different, depending upon individual exposure to environmental carcinogens and their metabolism ability. There were no additional DNA-reactive metabolites found after inhalation exposure to *p*-DCB.

Key words —— 1,4-dichlorobenzene, DNA adduct, human serum, metabolite, mutagenicity, inhalation toxicity

INTRODUCTION

The chemical 1,4-dichlorobenzene (*p*-DCB) is a chlorinated aromatic compound, which has been used for several decades in consumer products, in pest control and as an intermediate in chemical industries. These uses result in different exposure patterns for human, the three most important routes being vapor exposure, oral exposure via contaminated foods and local contact.[1] *p*-DCB causes mitogenic stimulation of hepatocellular proliferation in rodents.[2] There are a number of reports on carinogenicity, mutagenicity in rats and mice, but data in humans are limited.[3,4] *p*-DCB showed major differences in the hepatic cytochrome P450-mediated biotransformation in different species and also in different rat strains, and is a selective carcinogen in these species.[5] Formation of DNA adducts is considered a crucial step in the initiation of carcinogenesis. The determination of macromolecular adducts in experimental animals and humans can contribute to better understanding of the mechanisms of chemical carcinogenesis. DNA adducts represent the end product of carcinogen disposition taking into account all the variable intraindividual factors of absorption, metabolism, excretion, and adduct repair, and thus, best represent the actual critical dose of a chemical measurable on an individual basis.[6,7] There are no available data on DNA adducts of *p*-DCB in laboratory animals or human beings. One of the difficulties is that human tissue DNA can not be obtained readily. The present study was undertaken to evaluate the use of blood serum as a source to detect reactive, adduct-forming metabolites of *p*-DCB by the ^{32}P-postlabeling method and as a potential novel approach to biomonitoring, which could be extended for use in humans due to the ready availability of human serum.

MATERIALS AND METHODS

Subject Information and *p*-DCB Inhalation —— Three volunteers, S1, S2 and S3, were selected for this experiment. All subjects inhaled *p*-DCB vapor (2.4–2.8 ppm) continually for 1 hr, approximately 10 ml of peripheral blood was collected at three timepoints: before inhalation (T1), just at the termination of inhalation (T2), and 1 hr after the termination of inhalation (T3). Concentration of *p*-DCB in each serum specimen was measured by GCMS-QP5050A (Shimadzu, Japan). Background information regarding the 3 selected subjects is shown in Table 1.

Reaction with DNA —— Six hundred μl of serum was mixed with 400 μl of 50 mM Tris–HCl buffer, pH 7.4, containing 300 μg of calf thymus DNA (Boehringer Mannheim, Gmbh, Germany) and incubated for 1 hr at 37°C with constant agitation.[8] Samples were extracted three times with water–satu-

*To whom correspondence should be addressed: Department of Pharmaceutical Sciences, National Institute of Public Health, 4–6–1 Shirokanedai, Minato-ku, Tokyo 108–8638, Japan. Tel.: +81-3-3441-7111, Ext. 282; Fax: +81-3-3446-4314; E-mail: miyazawa@iph.go.jp

Table 1. Background Information on the Subjects and Concentration of *p*-DCB in Each Serum

Volunteers		S1	S2	S3
Age		45	49	54
Smoker		No	Yes	Yes
Weight (kg)		57	70	77
Height (cm)		171	170	173
Exposure Conc. of *p*-DCB (ppm)		2.4	2.8	2.7
p-DCB absorption (mg)		2.4	4.7	3.3
Serum *p*-DCB (ng/ml)	T1	18.6	5	9.6
	T2	46.8	49.3	46.2
	T3	23.8	21	20.4

rated ethyl acetate to remove unreacted metabolites or the parent compound.[9] The aqueous phase was briefly evaporated to remove ethyl acetate.

DNA Isolation —— DNA incubated with serum was treated with RNases and proteinase K to remove RNA and protein respectively and extracted with phenol, phenol : CIAA (chloroform : isoamyl alcohol = 24 : 1), and CIAA. DNA was recovered by ethanol precipitation and its concentration was determined spectrophotometrically.[10]

Analysis of DNA Adducts by the ^{32}P-Postlabeling Method —— DNA samples were analyzed by the ^{32}P-postlabeling method after enrichment of adducts with nuclease P1.[11] DNA was digested with micrococcal nuclease and spleen phosphodiesterase. Adducts were enriched by nuclease P1 and labeled by T4 polynucleotide kinase catalyzed phosphorylation in the presence of molar excess of [γ-^{32}P]ATP. Labeled adducts were resolved by multidirectional PEI-cellulose TLC using 1.0 M sodium phosphate, pH 6.0 for D1; 4.5 M lithium formate/8.5 M urea, pH 3.5 for D3; isopropanol:4 M ammonium hydroxide (1 : 1) for D4 and 1.7 M sodium phosphate, pH 5.7 for D5. The adducts were detected with a Bio-Image Analyzer (FLA-3000; Fuji Photo Film Co. Ltd. Tokyo, Japan) after exposing the TLC sheet to the Fuji imaging plate.

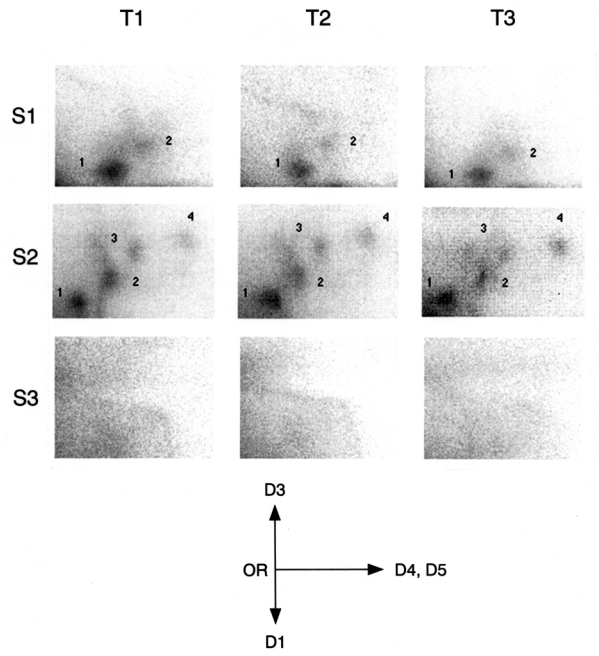

Fig. 1. [^{32}P]-DNA Adduct Maps Derived from Human Serum Exposed to Dichlorobenzene Vapor

DNA adduct was enriched by nuclease P1 treatment. Origin is at the left-down corner of each map. T1: before *p*-DCB inhalation; T2: termination of *p*-DCB inhalation; T3: 1 hr after termination of *p*-DCB inhalation. S1, S2, S3 represents different subjects.

RESULTS AND DISCUSSION

As shown in Fig. 1, each DNA adduct map was different. We detected 2 DNA adduct spots in serum from S1, 4 spots in that from S2, and no spots in that from S3. Comparing DNA adduct profiles at the three time-points: before inhalation, end of inhalation, 1 hr after ending of inhalation, almost the same patterns of qualitative and quantitative adducts were observed. These results demonstrate that after exposed to *p*-DCB vapor, there are no additional DNA-binding metabolites appearing in human serum compared with those before inhalation. Like other halobenzenes, such as chlorobenzene and bromobenzene, *p*-DCB is bioactivated only through oxidative metabolic steps.[12] Cytochrome P-450 dependent microsomal oxidase system and, to a much lesser extent, cytosolic GSH-transferases are involved in *p*-DCB metabolism.[5] *p*-DCB is mainly metabolized to 2,5-dichlorophenol.[13] Content of *p*-DCB in human serum decreased rapidly, only 1 hr after termination of inhalation, half of serum *p*-DCB has been metabolized and excreted as shown in Table 1. Although the *p*-DCB concentration in human serum before *p*-DCB inhalation was low, it was detectable in all three volunteers, suggesting that *p*-DCB pollution in the ambient environment is wide spread.

It is generally thought that the formation of DNA adducts by covalent interaction of electrophilic species of carcinogens with macromolecules, particularly DNA, is an essential first step in the multistage process of carcinogenesis. DNA adducts can be detected at a level of 1 adduct per 10^{10} nucleotides by ^{32}P-postlabeling technique.[14] Most of the known

chemical carcinogens are metabolized to reactive intermediary or ultimate metabolites which are capable of binding covalently to DNA. Human DNA can not be easily obtained from the target tissue of a specific chemical, therefore using human serum as a novel surrogate to overcome the need for tissues containing DNA for in the routine human biomonitoring assessment of exposure is a useful exploration. In practice, Arif *et al*. detected DNA reactive metabolites in serum and described the tissue distribution of these metabolites in mice exposed to carcinogens,[8] indicating that these approaches have implications for human biomonitoring using serum and carcinogenicity. In typical day-to-day life, normal human populations are exposed to various classes of carcinogenic substances, including PAHs, environmental tobacco smoke, diesel and gasoline exhausts, water pollutants, *etc*.[15] In this study, we detected 2 and 4 adduct spots in S1 and S2 respectively, indicating that S2 might have been exposed to more carcinogens than S1. There were no DNA binding metabolites persisting in serum from S3. Though the use of WBCs, PBLs and other cell systems has been applied in several studies regarding the ultimate toxicity of specific chemicals, these systems do not serve as a routine, simple and accurate measure for biomonitoring of exposure to carcinogens in humans. In conclusion, this detection approach, coupled with highly sensitive ^{32}P-postlabeling technique is capable of detecting early and relatively low dose exposure to genetic chemicals in humans.

Acknowledgements This work was supported by the Science and Technology Agency, Japan.

REFERENCES

1) Loeser, E. and Litchfield, M. H. (1983) Review of recent toxicology studies on *p*-dichlorobenzene. *Food Chem. Toxicol*., **21**, 825–832.
2) Eldridge, S. R., Goldsworthy, T. L., Popp, J. A. and Butterworth, B. E. (1992) Mitogenic stimulation of hepatocellular proliferation in rodents following 1,4-dichlorobenzene administration. *Carcinogenesis*, **13**, 409–415.
3) Haseman, J. K., Huff, J. E., Zeiger, E. and MaConnell, E. E., (1987) Comparative results of 327 chemical carcinogenicity studies. *Environ. Health. Perspect*., **74**, 229–235.
4) Barter, J. A. and Sherman, J. H. (1999) An evaluation of the carcinogenic hazard of 1,4-dichlorobenzene based on internationally recognized criteria. *Regul. Toxicol. Pharmacol*., **29**, 64–79.
5) Hissink, A. M., Oudshoorn, M. J., Van Ommen, B. and Van Bladeren, P. J. (1997) Species and strain differences in the hepatic cytochrome P450-mediated biotransformation of 1,4-dichlorobenzene. *Toxicol. Appl. Pharmacol*. **145**, 1–9.
6) Harris, C. C. (1991) Chemical and physical carcinogenesis: advances and perspectives for the 1990s. *Cancer Res. Suppl*., **51**, 5023s–5044s.
7) Perera, F. P. (1987) Molecular cancer epidemiology: a new tool in cancer prevention. *J. Natl. Cancer Inst*., **78**, 887–898.
8) Arif, J. M. and Gupta, R. C. (1996) Detection of DNA-reactive metabolites in serum and their tissue distribution in mice exposed to multiple doses of carcinogen mixtures: role in human biomonitoring. *Carcinogenesis*, **17**, 2213–2219.
9) Arif, J. M. and Gupta, R. C. (1997) Microsome-mediated bioactivation of dibenzo[*a*,*l*]pyrene and identification of DNA adducts by ^{32}P-postlabeling. *Carcinogenesis*, **18**, 1999–2007.
10) Gupta, R. C. (1984) Nonrandom binding of the carcinogen *N*-hydroxy-2-acetylaminofluorene to repetitive sequences of rat liver DNA *in vivo*. *Proc. Natl. Acad. Sci. U.S.A*., **81**, 6943–6947.
11) Reddy, M. V. and Randerath, K. (1986) Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts. *Carcinogenesis*, **7**, 1543–1551.
12) Macdonald, T. L. (1983) Chemical mechanisms of halocarbon metabolism. *Crit. Rev. Toxicol*., **11**, 85–120.
13) Hissink, A. M., Dunnewijk, R., Van Ommen, B. and Van Bladerne, P. J. (1997) Kinetics and metabolism of 1,4-dichlorobenzene in male Wistar rats: no evidence for quinone metabolites. *Chem. Biol. Interact*., **103**, 17–33.
14) Gupta, R. C. (1996) ^{32}P-postlabelling for Detection of DNA Adducts. In *Technologies for Detection of DNA Damage and Mutations* (Pfeifer, G. P., Ed.), Plenum Press, New York.
15) Beach, A. C. and Gupta, R. C. (1992) Human biomonitoring and the ^{32}P-postlabeling assay. *Carcinogenesis*, **13**, 1053–1074.