Tissue Distribution of DNA Adducts and Their Persistence in Blood of Mice Exposed to Benzene

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Chemicals combine with DNA, resulting in DNA damage, which could initiate carcinogenesis. To study whether benzene or benzene metabolites bind to DNA, DNA adducts in various tissues and their persistence in leukocytes were examined using the 32P-postlabeling assay. LACA mice were dosed ip with benzene at 500 mg/kg bw twice daily for 5 days. Two additional spots of DNA adducts are formed in bone marrow cells, liver cells, and peripheral blood compared with control mice. The relative adduct labeling values are 10.39, 11.32, and 13.77 adducts × 10−9 nucleotides in these tissues, respectively. DNA adducts in blood leukocytes were observed at 1, 4, 7, 14, and 21 days after exposure to benzene, but adduct levels decreased as a function of time. Relative adduct labeling of “adduct B” declined linearly but mildly, while “adduct C” displayed a stepwise decrease. The relative adduct labeling values of both these adducts at day 14 were 50% of those at day 1 after the last treatment. Both adducts were still detectable at day 21 after benzene exposure. These studies demonstrate that benzene could induce DNA adducts in bone marrow, liver, and white blood cells of mice dosed with benzene and that measurement of adducts in white blood cells may be useful as a biomarker to predict carcinogenic risk of benzene to workers exposed to benzene. — Environ Health Perspect 104(Suppl 6):1337–1338 (1996)

Key words: benzene, DNA adducts, relative adduct levels, leukocytes, 32P-postlabeling

Introduction

The relationship between benzene and leukemia is well known. Benzene also induces solid tumors in various tissues (7). Benzene was listed as a human carcinogen by the International Agency for Research on Cancer (IARC) (2). There are a large number of workers exposed to benzene or benzene-containing compounds (3). Although the mechanism of benzene poisoning has been studied for a long time, the mechanism of carcinogenesis is uncertain. The combination of chemical carcinogen and DNA might initiate the process of chemical carcinogenesis (e.g., the chemical may undergo a reaction with DNA) (4). Over the past decade, the development of techniques in molecular biology, particularly the P1-enhanced 32P-postlabeling method (5), has made it possible to detect DNA adducts in a low molecular weight chemical carcinogen. Hence, it is now possible to detect DNA adducts in vivo without radioisotopic labeling of chemicals. Whether benzene induces DNA adducts and whether such adducts could be used as biomarkers in human monitoring have been of great interest to many investigators.

Materials and Methods

Female LACA mice, 18–22 g, were divided into five per group. In experiment 1, mice were dosed ip with benzene at 500 mg/kg bw twice daily for 5 days. Control mice were treated with peanut oil. Blood, liver, and bone marrow were collected at 24 hr after the last benzene injection. Experiment 2 was the same as experiment 1 except blood was collected at 1, 4, 7, 14, and 21 days after the last benzene injection.

Blood samples were collected from postocular veins and pooled from five mice of each group. Liver (0.2 g) and bone marrow (single femur) were selected after sampling blood. DNA was isolated by a procedure involving solvent extraction and enzymatic digestion of protein and RNA.

DNA adducts were analyzed by the nuclease P1-enhanced version of 32P-postlabeling assays. Briefly, 10 μg DNA was digested with micrococcal nuclease and spleen phosphodiesterase into 3'-mononucleotides. The modified nucleotides were first enriched by nuclease P1-catalyzed dephosphorylation of normal nucleotides, then labeled by (r-32P)ATP and T4-polynucleotide kinase, and finally separated by multidimensional thin-layer chromatography. Adducts were detected by autoradiography and quantified by scintillation counting.

Results

Two DNA adducts are formed in all three tissues (liver, bone marrow, and blood). Spots B and C were identified as shown in Figure 1. The number of DNA adducts were counted as counts per minute using scintillation counter by scraping the spots in PEI-cellulose, comparing the corresponding area in PEI-cellulose in the control group, and calculating the relative adduct labeling (RAL) value. The results are shown in Figure 2. The number of DNA adducts in the tissues are 10.39, 11.32, and 13.77 × 10−8 in blood, liver cells, and bone marrow cell, respectively. RAL values in order were therefore bone marrow > liver > blood.

As shown in Figure 1, two additional spots (B,C) were noticed on the chromatograms of mice treated with benzene compared with controls at 1, 4, 7, 14, and 21 days after the last exposure to benzene.
Levels of these two adducts decreased as a function of time (Figure 3). Levels of adduct B declined linearly but mildly while adduct C displayed a stepwise decrease. The RAL values of both adducts at 14 days were 50% of those at 1 day after the last exposure. Both adducts were still detectable at 21 days after benzene exposure.

Discussion

Bone marrow is the target organ for benzene and benzene is metabolized in liver tissue. Peripheral blood is easy to sample and can be available for human monitoring compared with bone marrow and liver cells. As shown in Figure 2, the level of DNA adducts are highest in bone marrow, lowest in peripheral blood, and medium in liver tissue. This reveals the different function of various tissue in regard to benzene metabolism. Bone marrow could accumulate benzene and its metabolites. For this reason, there is more opportunity for benzene metabolites to react with DNA in bone marrow cells and to form DNA adducts in this tissue. Crewet et al. (6) detected radioactivity in various tissues and reported similar results for DNA adduct levels: bone marrow > liver > peripheral blood. Bodell et al. (7) found three DNA adducts in human bone marrow cells cultured with benzoquinone using the 32P-postlabeling assay. Snyder et al. (8) detected DNA adduct peaks in rats treated with benzene, and DNA adducts were measured in 500- to 9000-g samples of liver cells in rabbits dosed with benzene (9). DNA adducts present in peripheral blood could come from DNA adducts formed in bone marrow or formed during the period of transport of benzene metabolites, which could react with DNA of white blood cells from liver to bone marrow. Although levels of adduct in blood are lower, blood is easy to sample. Blood sampling is of significance in predicting the risk from benzene carcinogenesis to human.

DNA adducts rose the first day after exposure ended and stayed at this level about 4 days. Benzene is absorbed and metabolized quickly after ip injection. Therefore, benzene metabolites react spontaneously with DNA. The level of DNA adducts reflect a dynamic equilibrium in the body between formation of DNA adducts and the repair function of DNA. A trend of declining RAL is shown after the first 4 days. This may relate to repair function of DNA. The persistence of DNA adducts illustrates not only the carcinogenicity of benzene and its metabolites but it also provides a scientific basis for sampling time of biomonitoring to humans.

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