SHORT COMMUNICATION

DNA adduct formation and persistence in rat tissues following exposure to the mammalian carcinogen dibenzo[a,l]pyrene

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Dibenzo[a,l]pyrene (DBP), an environmentally significant polycyclic aromatic hydrocarbon (PAH), is one of the most potent carcinogens with greater carcinogenicity in rodent mammary glands and skin than 7,12-dimethylbenz[a]anthracene or benzo[a]pyrene, respectively. In this study, we have examined the formation and persistence of stable DNA adducts in rats administered a carcinogenic intramamillary (i.m.) dose of DBP (0.25 µmol/gland).

**Abbreviations:**

- B[a]P: benzo[a]pyrene
- CYP: cytochrome P450
- DBP: dibenzo[a,l]pyrene
- DBPDE: DBP-11,12-diol-13,14-epoxide
- DMBA: 7,12-dimethylbenz[a]anthracene
- DMSO: dimethyl sulfoxide
- i.m.: intramamillary
- i.p.: intraperitoneal
- PAH: polycyclic aromatic hydrocarbon
- PEI: polyethyleneimine
- RAL: relative adduct labeling
- TLC: thin-layer chromatography

**Results:**

DBP is highly reactive towards DBP metabolites as has been demonstrated for many other PAHs; and (iii) the peak binding of DBP with DNA was shifted beyond 14 days for the non-target tissues by i.m. route of exposure.

**Discussion:**

Bioactivation of DBP to DNA-reactive species involves metabolic activation to either diol-epoxides through cytochrome P450 (CYP)-dependent monooxygenases and epoxide hydrolases or to reactive metabolites by the one-electron oxidation pathway. DBP has been documented to produce mammary tumors in rats (2). Our previous studies with microsomal bioactivation showed that >60% of the total DNA adducts are adenine-derived and both syn- and anti-DBPDE are involved in DBP–DNA adduction (8).

In rat mammary and mouse skin models, DBP was found to be several fold more carcinogenic than either 7,12-dimethylbenz[a]anthracene (DMBA) or benzo[a]pyrene (B[a]P), respectively (2,3). This observation is consistent with the DNA adduct levels found in rat mammary epithelial DNA following intramamillary exposure to DBP, DMBA and B[a]P (12). Our previous studies with microsomal bioactivation showed that >60% of the total DNA adducts are adenine-derived and both syn- and anti-DBPDE are involved in DBP–DNA adduction (8).

In this study, we have examined the formation and persistence of stable DNA adducts in target and non-target tissues of rats treated with a carcinogenic dose of DBP by an intramamillary (i.m.) route.

Female Sprague–Dawley rats (60-days-old) were injected with DBP (0.25 µmol/gland/50 µl DMSO) by i.m. injection under the nipple region of third, fourth and fifth mammary glands on both the sides (12). This model was chosen since it has been documented to produce mammary tumors in rats (2). In a separate experiment, animals were also administered DBP (1.5 µmol/300 µl vehicle) by either intragastric (i.g.) or intraperitoneal (i.p.) routes. Control animals received either DMSO (i.m. and i.p.) or corn oil (i.g.).

**Conclusions:**

The DNA preparations were virtually free of RNA and protein. DNA were digested with micrococcal nuclease and spleen phosphodiesterase (enzyme:DNA, 1:5; 5 h, 37°C), followed by adduct enrichment with nuclease P1, as described (14). Enriched adducts were then labeled with molar excess of [γ-32P]ATP (100 µCi, <2 µM; <3000 Ci/mmol) and separated by multi-directional PEI–cellulose TLC using the following solvents: D1, 1.7 M sodium phosphate (pH 6.0); D3, 4 M lithium formate/7 M urea (pH 3.5); D4,
isopropanol:4 M ammonia (1:2:1); D5, 1.0 M sodium phosphate (pH 6.0) (8,12). DNA adducts were visualized and quantified by a Packard InstantImager, and adduct levels were determined by relative adduct labeling (14).

Intramamillary injection of DBP produced one predominant and at least six other adducts in the mammary epithelial DNA at all time points tested (Figure 1B). Various non-target tissues (lung, heart, pancreas and bladder) had a qualitatively identical adduct pattern (Figure 1C, E, F and G), which is chromatographically comparable with that found following metabolism of DBP by rat liver microsomes (8) and MCF-7 cells (10). Interestingly, the liver showed at least four additional adduct spots (Figure 1D, nos 8–11) which comprise 30% of the total adduct level. Adduct 6 was the major adduct in all tissues. Intensities of adduct spots 4 and 5, presumably derived from syn-DBPDE, in the liver were significantly lower than those in other tissues such as the heart and lung. This suggests that the ratio of metabolically formed syn- and anti-DBPDE is different or the syn-DBPDE adducts are more efficiently repaired in the liver as compared with the other tissues examined.

Measurement of adduct radioactivity revealed that the mammary DNA was most adducted (2640 ± 532 adducts/10⁹ nucleotides) after a single i.m. injection of DBP, while the non-target tissue DNAs were 10- to 65-fold less adducted (Figure 2). Adduct levels were the highest on day 14 in all tissues, except mammary tissue which showed a decline of ~40% as compared with the peak binding on day 5. The higher DNA binding in mammary tissue may be due to the direct injection of carcinogen at the target site, resulting in its trapping in the adipose tissue, which may eventually make it biologically more readily available to the mammary tissues (12,15). In the mammary gland, the cell turnover is generally reported to be higher than the other tissues, for example lung and liver (16). Thus, the decrease in the DBP–DNA adducts in mammary tissue on day 14 could be in part attributable to the increased cell turnover rate, in addition to DNA repair. On the contrary, the increase in the DBP–DNA adduction over a period of time in the non-target tissues could be due to gradual and continuous release of the trapped compound to the distant organs through systemic circulation (15). Recently, it has been reported that the majority of DBP–DNA adducts in mouse skin were found to adopt an intercalated conformation which render them less efficiently repaired than the external adducts (17). Furthermore, as a result of less efficient repair, DBP–DNA adducts can accumulate over a period of time which may eventually shift the peak binding time in the non-target tissues. Peak DNA bindings for several PAHs such as B[a]P, DMBA, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, and DBP in mouse skin and lung, have been reported to be between 1 and 4 days (18,19).

Administration of DBP by i.p. and i.g. routes resulted in essentially the same adduct patterns in the mammary and lung tissues examined (maps not shown), as formed by the i.m. route (Figure 1). However, quantitatively, the i.m. route resulted in significantly higher DNA adduction; the i.g. route was least effective (Table I).

Multiple isoforms of CYP have been suggested to be involved in the metabolism of a number of PAHs, including DBP in vitro (7,8,12,20). Our previous results showed that rat liver microsomal activation of DBP was almost completely abolished in the presence of α-naphthoflavone, a known inhibitor of CYP1A1 family (8). Furthermore, it was supported by another report where the recombinant human CYP1A1 was found most active in metabolizing DBP (7). However, in human tissues and MCF-7 cells, CYP1A1 has been reported to be virtually inactive in the absence of inducers (21). Recently, CYP1B1, a PAH-inducible isofrom found in several extrahepatic human tissues, has been suggested to play an important role in the metabolic activation of various PAHs,
including DBP (22–25). Studies with MCF-7 cells also revealed that CYP1A1 was not inducible with low doses of DBP and, thus, the DBPDE–DNA adducts resulted exclusively through the constitutive expression of CYP1B1 (25). Furthermore, the presence of highly polar DNA adducts in V79 cells expressing human CYP1A1, in contrast to the MCF-7 cells, suggested that CYP1B1 could be responsible for the metabolic activation of low doses of DBP in MCF-7 cells (24,26). It appears from several reports that CYP1A1 may be playing a major role in DBP metabolism by rat liver microsomes in vitro; however, in MCF-7 cells and in vivo systems, it could be preferentially metabolized by CYP1B1 (7,8,24).

Furthermore, CYP1A1 can metabolize DBP to at least three intermediate metabolites, namely 8,9-, 11,12- and 13,14-dihydrodiols (7). Recently, Nesnow et al. have shown that the 8,9-diol of DBP can be further metabolized to at least three ultimate metabolites (27) which can bind to DNA. In addition, several highly polar adducts have been observed (24,27). The present results along with our published data (12) revealed the presence of several liver-specific DBP–DNA adducts, which were chromatographically distinct from syn- and anti-DBPDE–DNA adducts. It is possible that these liver specific adducts are derived from further metabolism of the 8,9-dihydrodiol; however, it is unlikely that these adducts are highly polar since they did not migrate in D1 (1.7 M sodium phosphate).

Chromatographic comparison of DNA and individual nucleotide adducts formed by reaction with syn- and anti-DBPDEs showed that the in vivo adducts were both deoxyadenosine- and deoxyguanosine-derived, formed by interaction with both syn- and anti-DBPDEs, as published earlier (12). However, the adenine-derived adducts were found to be ~60–75% of the total adducts. Our results indicate that adenine in DNA is the major site of adduction with DBP in rat tissues and is in agreement with the published reports (8,10,12,17,26). The extensive binding of DBP to adenine residues in DNA, as reported earlier for other PAHs such as DMBa, benzo[๑]-phenanthrene and benzo[g]chrysene (28–31), may actually be responsible for its high carcinogenicity.

Our results suggest that DBP exposure can produce several lipophilic DNA adducts in target as well as non-target tissues of rats which can persist for a long period of time. Furthermore, abundance of adenine-derived adducts of DBP in the tissue DNA may eventually contribute to its exceptionally known high carcinogenicity.

### Table I. DBP–DNA adduct levels in mammary and lung tissues of rats treated by different routes

<table>
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<tr>
<th>Route of exposure</th>
<th>Total adducts/10⁹ nucleotides</th>
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<tr>
<td></td>
<td>Mammary</td>
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<tr>
<td>Intramammary (i.m.)</td>
<td>2640 ± 532b</td>
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<tr>
<td>Intraperitoneal (i.p.)</td>
<td>331 ± 136</td>
</tr>
<tr>
<td>Intragastric (i.g)</td>
<td>121 ± 46</td>
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*Animals were administered 0.25 μmol DBP/gland/50 μl DMSO by i.m. injection under the nipple region of the third, fourth and fifth mammary glands on both the sides, while 150 μmol DBP/50 μl DMSO or corn oil was given by i.p. and i.g. routes. The adduct levels were determined 5 days post dosing.

Values represent means ± SD (n = 4 or 5).

### Tissue distribution of DBP–DNA adducts

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### References


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