Metabolism and DNA Binding of the Environmental Colon Carcinogen
6-Nitrochrysene in Rats

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ABSTRACT

The environmental contaminant 6-nitrochrysene (6-NC) has been shown to induce adenomas and adenocarcinomas in the colons of rats. The present study aimed at providing a better understanding of mechanisms that are responsible for this effect. Three female CD rats were injected i.p. with [3,4,9,10-\(^{3}\)H]6-NC ([\(\mu\)mol/rat (\(\mu\)Ci/rat)], and urine and feces were collected daily for 3 days. In the first 24 h, radioactivity corresponding to 1.3% of the dose was excreted in the urine, whereas 23.8% was recovered in the feces. After 3 days, the total excretions in urine and feces were 2.8% and 34.9% of the dose, respectively. Radioactivity measured in various organs 3 days after injection of [3,4,9,10-\(^{3}\)H]6-NC amounted to 24.8% of the administered dose. Fecal metabolites were identified, based on comparison of their chromatographic characteristics with those of standards, as \(\text{trans}-1,2\)-dihydro-1,2-dihydroxy-6-NC, chrysene, 5,6-quinone, and 6-aminochrysene (6-AC); the structure of the latter was further confirmed by mass spectrometry and UV spectral analysis. Metabolites identified in the urine were 6-AC, \(\text{trans}-1,2\)-dihydro-1,2-dihydroxy-6-NC, and \(\text{trans}-9,10\)-dihydro-9,10-dihydroxy-6-NC in free forms and also as glucuronide and/or sulfate conjugates. The \(^{32}\)P-postlabeling assay was used to determine the metabolic pathways that were leading to DNA adduct formation in the target (colon) and nontarget (liver, lung, and mammary tissues) organs of female CD rats injected with 6-NC under conditions identical to those of the bioassay (total, 14.8 \(\mu\)mol/rat; single i.p. injections on days 1, 8, 15, 22, and 29). Twenty-four h after the last carcinogen administration, the levels of the adduct derived from \(\text{trans}-1,2\)-dihydro-1,2-dihydroxy-6-NC were higher than those derived from \(N\)-hydroxy-6-AC in all organs examined; however, the highest levels of DNA adducts were found in the lung and not in the target organ, the colon. Although the role of each adduct in colon carcinogenesis needs to be determined, the results favor the ring oxidation and nitroreduction combination pathway as the primary contributor to the activation of 6-NC as a colon carcinogen in the rat.

INTRODUCTION

Humans are exposed to NO\(_2\)-PAH\(^1\) through cooked foods, combustion products, and polluted air (1–3). NO\(_2\)-PAH have been detected in tissues of lung cancer patients who were nonsmokers, implicating these NO\(_2\)-PAH as initiators of lung tumors in humans (4). 6-NC, one member of this class of compounds, has been shown to be mutagenic in both bacterial and mammalian cell systems (5, 6) and to be tumorigenic in several rodent model systems (7). Despite the fact that 6-NC is present in the environment at rather low levels, research on 6-NC has been inspired because of its remarkable carcinogenic activity in the newborn mouse lung adenoma assay (8, 9). 6-NC is the most potent lung tumorgen among all NO\(_2\)-PAH ever tested in this animal model, and its carcinogenicity approximates that of certain ultimate carcinogenic metabolites of PAH (8). In addition, intramammary injection of 6-NC induced mammary tumors in rats; 6-NC was more potent than the bay region diol epoxide derived from benz(a)pyrene (10, 11).

Injection of 6-NC (i.p.) into CD rats led to adenomas as well as adenocarcinomas in the colon (12). 6-NC and the food-borne heterocyclic aromatic amines are the only environmental agents known to induce colon cancer in rodents (13, 14). Because human tissues are capable of activating 6-NC to genotoxic metabolites (15, 16), 6-NC may play a role in the induction of colon cancer in humans. Therefore, it is essential to delineate the mechanism that is responsible for the colon carcinogenicity of 6-NC and similar agents in the human environment.

Studies in mice and \textit{in vitro} assays have indicated that 6-NC can be activated by two major pathways. The first pathway, involving the formation of \(N\)-OH-6-AC by simple nitroreduction, yields three major DNA adducts, \(N\)-(glycogenosyl-8-yl)-6-AC; \(N\)-(deoxyguanosin-8-yl)-6-AC; \(N\)-(dI-8-yl)-6-AC, \(N\)-(deoxyguanosin-8-yl)-6-AC; \(N\)-(dI-8-yl)-6-AC, \(N\)-(deoxyguanosin-8-yl)-6-AC; \(N\)-(dG-8-yl)-6-AC (17). The second pathway proceeds via the formation of the proximate tumorigen, 1,2-DHD-6-AC by a combination of ring oxidation and nitroreduction; it yields a single major DNA adduct resulting from the reaction with dG (18). The latter pathway seems to be the major contributor to the formation of DNA adducts in mice (19, 20). The present study examined the \textit{in vivo} metabolism, tissue distribution, and contribution of each pathway to the DNA binding of 6-NC in rats to provide insights into the mechanisms leading to colon cancer induction by 6-NC.

MATERIALS AND METHODS

Chemicals. \([3,4,9,10-\text{\(^{3}\)H}]6-NC\) (specific activity, 1.1 Ci/mmol) was purchased from Chemsyn Science Laboratories, Inc. (Lexena, KS) and further purified by HPLC (>95%). \(\beta\)-Gluconoridase, arylsulfatase, saccharic acid 1,4-lactone, micrococcal nuclease, spleen phosphodiesterase, and nuclease PI were obtained from Sigma Chemical Co. (St. Louis, MO). T4 polynucleotide kinase was procured from Amersham Co. (Arlington Heights, IL). \(\gamma\text{-}\text{\(^{32}\)P}\)ATP was synthesized from carrier-free \(\text{\(^{32}\)P}\)Piphosphate (ICN Biomedicals, Irvine, CA) as described (6). 1,2-DHD-6-NC was obtained from incubations of 6-NC with S9 liver fractions prepared as described previously (21).

\textit{In Vivo} Metabolism of 6-NC and Determination of Radioactivity in Tissues. Female CD rats [Crl:CD\(^{\text{\(\delta\)}}\)(SD)BR], purchased from Charles River Breeding Laboratories, Inc. (Kingston, NY), were fed NIH-07 diet and water ad libitum. Three rats were housed in cages (one rat per cage) designed to collect urine and feces and were kept in a light (12-h light/12-h dark)-, humidity (55 ± 15%), and temperature (22 ± 2°C)-controlled room. At 6 weeks of age, rats were given single i.p. injections of \([3,4,9,10-\text{\(^{3}\)H}]6-NC\) [9 \(\mu\)mol/rat (346 \(\mu\)Ci/rat)] dissolved in 300 \(\mu\)l DMSO. Twenty-four h-voids of urine (kept at 0–4°C) and feces were collected for 3 days at ambient temperature. Radioactivity in an aliquot of urine pooled from three rats was determined by a Tri-Carb 1900 CA liquid scintillation analyzer (Packard Instruments Co., Meriden, CT). Pooled fecal samples were pulverized and suspended in a 1:1 (v/v) mixture of ethyl acetate and ethanol (10 ml/g feces) and vortexed
for 20 min at 37°C to render homogeneous distribution of the activity. Following the complete evaporation of organic solvent, three aliquots of fecal samples were weighed and placed in Combust-o-cones (Packard Instruments Co.) and burned to completion (1-1.5-min burn time) in a Packard model 307 sample oxidizer. The titrated water resulting from the burning was collected with 10 ml Monophase S (Packard Instruments Co.) into glass vials for scintillation counting. The efficiency of recovery, determined with a tritium standard (Packard Instruments Co.), was 101.6 ± 6.4%.

In the same experiment described above, all major organs (liver, heart, kidneys, lungs, mammary glands, colon, small intestine, stomach, bladder, cecum, brain, pancreas, reproductive organs, connective tissues, and spleen) and contents (colon, small intestine, stomach, and cecum contents) were obtained 3 days after injection of [3,4,9,10-3H]6-NC. Each organ was minced, and the pieces were further chopped, crushed, and mixed well with a metal spatula. Similarly, organ contents were mixed thoroughly with a spatula. Connective tissues were frozen in liquid nitrogen and pulverized with a mortar and pestle. At least three weighed aliquots of these preparations of tissue or organ contents from each of the three treated rats were subjected to oxidative combustion using the Packard model 307 sample oxidizer. Radioactivity was determined as described above. Aliquots (100 μl) of whole blood were burned and counted. For all tissues, with the exception of the pancreas, the total radioactivity recovered in the organ was determined by multiplying the average μCi/g (μCi/ml for blood) by the total organ weight (total volume in the case of blood). In the case of the pancreas, the entire tissue was burned in multiple aliquots, and the recovered radioactivity was totaled.

**Analysis of Urinary and Fecal Metabolites of 6-NC.** Twenty-four-hour voids of urine (5–8 ml) from the rats were extracted three times with an equal volume of ethyl acetate. The organic phase was dried (MgSO4) and analyzed by HPLC on a 10-μm Vydac C18 reverse-phase column (0.46 × 25 cm; Separations Group, Hesperia, CA) using two different elution conditions (16): system 1, a linear gradient from 100% water to 90% methanol in water in 100 min, followed by 100% methanol in 10 min at a flow rate of 1 ml/min; and system 2, a linear gradient from 45% methanol in water to 100% methanol in 100 min at a flow rate of 1.5 ml/min. Radioactivity was monitored with a Flo-one β-flow detector (Radiomatic Instruments and Chemical Co., Tampa, FL).

To analyze urinary conjugates, aliquots (8–15 ml) of urine that had been extensively extracted (three times) with ethyl acetate were treated with aryl-sulfatase in the presence of saccharic acid 1,4-lactone or with β-glucuronidase. In a typical experiment, either 63 units aryl-sulfatase or 20 mg saccharic acid 1,4-lactone or 10,000 units β-glucuronidase were used for each 10-ml urine sample. After incubation at 37°C for 6 h, enzymatically released metabolites were extracted with ethyl acetate and analyzed by HPLC as described above.

Feces collected for 24 h after administration of 6-NC were suspended in a 1:1 mixture of ethyl acetate and ethanol (10 ml/g feces) and vortexed for 20 min at 37°C as described above. The residue was filtered, and the filtrate was dried (MgSO4), reconstituted in tetrahydrofuran, and analyzed by HPLC under the conditions described above.

The identity of 6-AC was based on cochromatography and comparison of its UV and mass spectrometric spectra with those of the synthetic standard; other metabolites were identified on the basis of cochromatography with the standards.

**DNA Adduct Analysis.** Rats were treated with 6-NC under conditions identical to those of the bioassay described by Imaida et al. (12). Four female CD rat were injected i.p. with a total dose of 14.8 μmol 6-NC/rat; 15, 30, 60, 150, and 300 μmol DMSO, containing 6-NC at a concentration of 26.7 μmol/ml, were injected on days 1, 8, 15, 22, and 29, respectively. The four rats in the control group received DMSO only. Organs were harvested 24 h after the last injection with 6-NC or vehicle. DNA was isolated from the colon, liver, lung, and mammary fat pads by standard methods (22). The colon was washed with 0.9% NaCl, and the mucosal epithelium was collected on a glass slide prior to homogenization. Individual mammary fat pads were frozen in liquid nitrogen and pulverized before being homogenized. DNA was dissolved in 5 mM Tris-HCl buffer and 0.1 mM EDTA (pH 7.1), and its concentration was determined by A 260 nm. The A 260/A 280 ratio was approximately 1.8 for all DNA samples.

DNA (10 μg), isolated from tissues, was hydrolyzed to nucleoside 3'-monophosphates by incubating with micrococcal nuclease (0.012 units/μg DNA) and spleen phosphodiesterase (0.001 units/μg DNA) in 20 mM sodium succinate and 10 mM CaCl2 (pH 6.0) at 37°C for 3.5 h (23). Total nucleotides were determined as described by Gupta (23); the remainder of the digest was treated with nuclease P1 (0.54 units/μg DNA) for 1 h at 37°C (24), or it was extracted with normal butyl alcohol (23) and then 5'-32P phosphorylated at 37°C for 40 min with T4 polynucleotide kinase (0.35 units/μg DNA) and 200 μCi [γ-32P]ATP, and finally treated with potato apyrase (60 milliunits) for 30 min at 37°C to destroy the excess of [γ-32P]ATP (25). In the case of normal butyl alcohol extractions, one backwash of the extract with normal butyl alcohol-saturated water was used.

An aliquot (7.7 μg DNA) of the labeling incubation mixture was spotted 12 cm from the bottom of a 20 × 10-cm Macherey-Nagel polyethyleneimine cellulose TLC plate (Alltech Associates, Deerfield, IL) with a Whatman 1MM wick (Whatman, Inc., Fairfield, NJ) attached. The plate was developed overnight with 0.65 m sodium phosphate (pH 6.8; D1). The wick and the top 6 cm of the plate were discarded. Development with 4.25 m lithium formate and 8.3 ml urea (pH 3.5) was carried out in the opposite direction to D1 (D2), followed by development at a 90° angle with 0.9 m sodium phosphate, 0.56 m Tris-HCl, and 7.6 m urea (pH 7.5; D3). After each development, plates were washed with deionized water. Plates were then exposed to Cronex X-ray film (DuPont, Wilmington, DE) for visualization of adducts. For use as adduct standards, calf thymus DNA, modified by [3,4,9,10-3H]v-0H-6-AC or a metabolite of [3,4,9,10-3H]1,2-DHD-6-AC formed in the presence of microsomes prepared from livers of 3-methylcholanthrene-induced rats, was prepared as described previously (17, 19). DNA was hydrolyzed, and adducts were 32P postlabeled and analyzed as described above. The adduct spots were identified and cut from the plates. Radioactivity was quantified by scintillation counting. Background radioactivity, determined from an approximately equivalent area of the same plate, was subtracted. Adduct levels in tissues were estimated by comparison with the corresponding adduct spot on a parallel plate on which DNA modified by [3,4,9,10-3H]v-0H-6-AC or a metabolite of [3,4,9,10-3H]1,2-DHD-6-AC was analyzed (26).

**RESULTS**

In Vivo Metabolism of 6-NC and Tissue Distribution of 6-NC and Its Metabolites. During the first 24 h, 23.0% of the dose administered was excreted in the feces, as determined by oxidative combustion, and 1.3% was eliminated in the urine. After 3 days, the excretions amounted to 34.9% and 2.8% of the dose in the feces and urine, respectively (Fig. 1).

To analyze 6-NC and its metabolites in the urine and feces by HPLC, aliquots of urine and feces collected over the 24-h period were extracted with ethyl acetate and a mixture of ethyl acetate and ethanol, respectively. In the case of fecal samples, the organic solvent extrac-
tion yielded much lower recovery than the oxidative combustion method; only 16% of the dose was recovered when organic solvent extraction was used, whereas oxidative combustion of fecal contents resulted in 23% of the dose recovered. Typical HPLC profiles of fecal and urinary metabolites are shown in Fig. 2. The major radioactive fraction found in the feces (amounts expressed as percentages of the dose based on recovery by organic solvent extraction) was that of unmetabolized 6-NC (15.61%), accounting for 98% of the radioactivity (Fig. 2A). In addition to unmodified 6-NC, 6-AC (0.19%), chrysene-5,6-quinone (0.12%), and 1.2-DHD-6-NC (0.08%) were detected in feces based on cochromatography with synthetic standards in two different HPLC systems. To confirm the structure of the major fecal metabolite, 6-AC, the peak corresponding to its retention time was collected, and its mass spectrometric and UV absorption spectra were found to be identical to those of the synthetic standard. The major ethyl acetate-extractable metabolites detected in the urine (amounts expressed as percentages of the dose) after 24 h were 6-AC (0.11%) and an unidentified compound eluting at 53 min (0.07%) in system 1 (Fig. 2B). Unmetabolized 6-NC (0.43%) was also detected in the ethyl acetate extract of urine. Fig. 3 shows HPLC profiles of urinary 6-NC metabolites released after enzyme hydrolysis. On the basis of chromatographic evidence, glucuronic acid and/or sulfate conjugates of 1.2-DHD-6-NC, 9,10-DHD-6-NC, and 6-AC were present in the urine at levels ranging from 0.01 to 0.06% of the administered dose. Other metabolites were detected but not identified. Polar metabolites other than glucuronides and sulfates accounted for 30% of the radioactivity in urine; they remained unidentified.

In the same experiment, radioactivity in various organs and organ contents was determined 3 days after i.p. injection of [3,4,9,10-3H]6-NC. A total of 24.8% of the administered dose was recovered in all organs and contents examined. The highest concentration of radioactivity was found in the pancreas (14.2% of the dose), followed by the connective tissues (8.8% of the dose). Radioactivity in all other major organs, including the colon, and organ contents ranged from 0.001 to 0.4% of the dose.

**Analysis of DNA Adducts Formed in Various Organs.** Fig. 4 shows autoradiograms of 32P-postlabeled digests of calf thymus DNA samples modified with either N-OH-6-AC or a microsomal metabolite of 1,2-DHD-6-AC. The DNA digests were treated with nuclease P1 or extracted with normal butyl alcohol prior to labeling with [γ-32P]ATP. Adducts 1, 2, and 3, derived from N-OH-6-AC, were identified as N-(dG-8-yl)-6-AC, N-(dI-8-yl)-6-AC, and 5-(dG-N2-8)-6-AC, respectively, as described in previous studies (17, 26). Adduct 1 was lost on nuclease P1 treatment, which is characteristic of a dG-C8 adduct (27, 28). Adduct 4, derived from 1,2-DHD-6-AC, was poorly recovered when the normal butyl alcohol extraction procedure was used. Although the structure of adduct 4 has not been characterized rigorously, earlier studies indicate that it seems to be a dG adduct (18), probably modified with 1,2-DHD-6-AC-3,4-epoxide (6, 18, 19, 21). Unidentified DNA adducts or adduct degradation products that develop during storage of DNA, which had been modified by the 6-NC metabolites, were also detected and are marked X.

DNA from the colons of rats that had been injected with DMSO or 6-NC was analyzed by the 32P-postlabeling assay using normal butyl alcohol extraction as well as the nuclease P1 enrichment method; adduct patterns are shown in Fig. 5. DNA from the colons of 6-NC-treated rats (Fig. 5, B and D) showed adducts derived from both N-OH-6-AC (adducts 1 and 3) and 1,2-DHD-6-AC (adduct 4). In addition, an unidentified putative DNA adduct (Fig. 5B, ?) and minor DNA adducts (Fig. 5B, XI and X2) were detected. The latter adducts are of unknown origin; they were also present in DNA from the colons of rats treated with DMSO alone (Fig. 5, A and C). DNA from the livers, lungs, and mammary fat pads of rats treated with 6-NC showed adduct patterns similar to those observed in the colons of 6-NC-treated rats (not shown). On the basis of the nuclease P1 enrichment method, the quantification of the DNA adduct levels in various organs obtained from 6-NC-treated rats showed that, irrespective of the organ examined, a dG adduct derived from 1,2-DHD-6-AC predominated.
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Fig. 4. Autoradiograms of 32P-postlabeled nuclease P1-treated (A and B) or normal butyl alcohol-extracted (C and D) enzymatic digests of calf thymus DNA modified with N-OH-6-AC (A and C) or a microsomal metabolite of 1,2-DHD-6-AC (B and D). Structures of adducts derived from N-OH-6-AC (adducts 1–3) are shown. The structure of adduct 4, a dG adduct derived from 1,2-DHD-6-AC, has not been characterized rigorously. X and ? unidentified DNA adducts or adduct degradation products. α, origin.

over those derived from N-OH-6-AC (Table 1). Only hepatic and colonic DNA were analyzed by the normal butyl alcohol extraction procedure. Adduct formation in the liver seemed to follow a similar trend as described above: adduct 3, 16 ± 6; adduct 4, 360 ± 106 adducts/10⁹ nucleotides (n = 4). Analysis of colonic DNA by the normal butyl alcohol extraction showed the presence of adducts 1 and 3 at levels of 27 ± 7 and 4 ± 2 adducts/10⁹ nucleotides (n = 4), respectively. However, adduct 4 was not detected due to low levels of DNA binding in the colon, combined with its poor recovery by the normal butyl alcohol extraction, as evident from the analysis of adduct standards (see Fig. 4).

Table 1 DNA adduct levels, quantified by the nuclease P1 enrichment method, in rats treated with multiple doses of 6-NC

<table>
<thead>
<tr>
<th>Adduct/10⁹ nucleotides</th>
<th>Colon</th>
<th>Liver</th>
<th>Mammary gland</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adduct 1</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Adduct 2</td>
<td>10 ± 2</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Adduct 3</td>
<td>67 ± 28</td>
<td>244 ± 69</td>
<td>190 ± 87</td>
<td>413 ± 192</td>
</tr>
<tr>
<td>Adduct 4</td>
<td>66 ± 33</td>
<td>28 ± 2</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

DISCUSSION

The results of this study clearly indicate that the major route of excretion of 6-NC, when given to rats by i.p. injection, is via the feces. The major metabolite (6-AC) detected in both the urine and feces is derived from simple nitroreduction of 6-NC. The particular significance of this investigation, however, lies in the identification of an adduct that was derived from a combination of ring oxidation and nitroreduction in the target organ (the colon) as well as in nontarget organs. DNA adducts derived from simple nitroreduction were also detected in all organs examined but at much lower levels than the adduct derived from a combination of ring oxidation and nitroreduction pathways.

The excretion of 6-NC in female CD rats after i.p. injection was comparable to that observed in mice (29); a total of 37.7% of the dose administered was excreted after 3 days, with the feces being the major route of excretion. A previous study had indicated that when 6-AC, the major metabolite of 6-NC, was administered i.p. to rats, a similar
The extent of metabolism of 6-NC in rats was extremely limited; unmetabolized 6-NC was the major component found in the feces after the first 24h, accounting for 98% of the radioactivity. This finding contrasts with that observed in mice. When mice were administered 6-NC i.p., only 1.1% of total radioactivity in the feces represented unchanged 6-NC, indicating extensive metabolism of 6-NC (29). This difference in the metabolic capacities of rats and mice has also been observed in studies with the colon carcinogen PhIP (31, 32). The presence of urinary and fecal metabolites such as 6-AC, 5,6-dihydrodiol-6-NC, 1,2-DHD-6-NC, and 9,10-DHD-6-NC supports the involvement of both nitroreduction and ring oxidation in the metabolism of 6-NC in vivo in rats. The major urinary and fecal metabolite of 6-NC was 6-AC. As is the case with mice (29), nitroreduction seems to be a major metabolic pathway of the i.p.-administered 6-NC in rats.

6-AC was present in the urine in the free form as well as conjugated as glucuronides and sulfates, according to enzymatic hydrolysis. In colon and bladder carcinogenesis, N-glucuronidation has been recognized as an important metabolic process, playing a dual role. N-glucuronidation of N-hydroxy derivatives of aromatic amines such as 2-naphthylamine and 3,2'-dimethylyl-4-aminobiphenyl is believed to be involved in the bioactivation pathway, providing a means of transport of the reactive N-hydroxypyrenes to extrahepatic tissues (33), whereas N-glucuronidation of PhIP and N-hydroxy-PhIP may play a key role in detoxification (34). Consistent with the present study, the formation of N-glucuronide of 6-NC has been observed in the bile of rats treated with 6-AC (30). Although it is an uncommon metabolic process, N-sulfation of aromatic amines and the food-borne heterocyclic aromatic amines has been reported. Boyland et al. (35) have observed the urinary excretion of sulfamate derivatives (N-sulfates) in rats and rabbits dosed with aniline or 1- or 2-naphthylamine. The formation of N2-sulfamate was shown to be the major pathway of 2-amino-3-methylimidazo[4,5-f]quinoline (36) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline metabolism (37, 38) in rats. Monkeys and humans also detoxify 2-amino-3-methylimidazo[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline via sulfamation (39-41). Unlike the N-sulfate of 6-AC, however, the sulfamates mentioned above were reported to be resistant to enzymatic hydrolysis. It seems that, depending on the substrate, sulfamates vary in their sensitivity toward enzyme hydrolysis.

Glucuronides and/or sulfate conjugates of the dihydrodiol 1,2-DHD-6-NC and 9,10-DHD-6-NC were found in rat urine following the administration of 6-NC. Similar findings were reported in the literature for several PAH. Following treatment with phenanthrene, trans-9,10-dihydro-9,10-dihydroxyphenanthrene was excreted in the form of glucuronide and sulfate conjugates in rats and rabbits (42). Glucuronides of benzo(a)pyrene diols have been detected in rats (43) as well as humans (44-46). Glucuronides and/or sulfates of the K-region diol trans-4,5-dihydro-4,5-dihydroxy-1-nitropyrene have been identified in the urine of germ-free rats (47) and as rat biliary metabolites (48). The formation of glucuronic acid and sulfate conjugates of trans-1,2-dihydoro-1,2-dihydroxy-5-methylchrysene, trans-7,8-dihydro-7,8-dihydroxy-5-methylchrysene, and trans-1,2-dihydro-1,2-dihydroxy-6-nitro-5-methylchrysene in mouse skin has also been reported (49, 50).

It has been proposed that colon cancer induction by aromatic amines in laboratory animals may involve the formation of N-glucuronimides of the reactive N-hydroxy derivatives formed in the liver, followed by their transport, via the bile, to the intestinal tract, where the N-hydroxy derivatives could be liberated by mammalian or bacterial β-glucuronidase. These highly reactive species may readily bind to colonic DNA and initiate the neoplasm. Alternatively, the ultimate carcinogenic metabolites may be formed directly in the intestinal tract. Microsomes prepared from the intestinal tracts of laboratory animals and humans have been shown to mediate the metabolic activation of aromatic amines to mutagenic agents (51, 52). The presence of the cytochrome P450 enzymes in the colonic mucosa of rats, rabbits, and humans has been reported (52-56). In particular, McKinnon et al. (52, 56) have demonstrated the expression of cytochrome P4503A4 in the human colonic epithelium that had been shown to be responsible for the activation of 6-NC (16, 57, 58).

Transport of ultimate carcinogens from the liver to the target tissue via the blood stream is also conceivable in view of a recent study by Kaderlik et al. (34), who presented evidence for this with the colon carcinogen PhIP. In the current investigation, adducts derived from N-OH-6-AC and 1,2-DHD-6-AC were detected in the target organ, the colon. Whether these metabolites were formed there or were transported to the colon is not investigated. It would be important in future studies to examine the biliary metabolites of 6-NC as well as the ability of colonic microsomes to activate 6-NC and to elucidate the role of biliary and circulatory transport of those metabolites in DNA adduct formation in target tissues.

Metabolic activation pathways of 6-NC in rats seem to involve both nitroreduction and a combination of ring oxidation and nitroreduction. DNA adducts derived from both pathways were detected in the target organ, the colon, as well as in nontarget organs such as the liver, lung, and mammary tissues, with the adducts derived from the combination pathway being predominant. These results are similar to those obtained in previous studies in mice (20, 29). The D(G) adduct derived from 1,2-DHD-6-AC was predominant in mice, and in some cases, one of the N-OH-6-AC-derived adducts, N-(dl-8-y1)-AC, was detected as a minor adduct (20). It is likely that the high sensitivity of the 3P-postlabeling technique used in this study allowed detection of the N-OH-6-AC-derived adducts [N-(dG-8-y1)-AC and N-(dl-8-y1)-AC] formed at levels lower than those detectable by HPLC and radioactive activity analysis (20, 29).

The lung contained the highest level of DNA adducts, followed by the liver, when rats were administered 6-NC under conditions identical to those reported for the induction of colon cancer in a bioassay (12). The observation that levels of DNA adducts formed by critical metabolites of carcinogens in various organs do not correlate with their degree of carcinogenicity is not limited to our study with 6-NC. For instance, although dietary PhIP induced colon cancer in F344 rats (59, 60), the highest level of DNA adducts was found in the heart (61) and the pancreas (34) but not in the colon. Thus, both the levels and nature of DNA adducts discussed above could not explain the tissue susceptibility of 6-NC tumorigenesis to the colon. In the case of the colon, rapid cell turnover relative to that in other tissues probably plays a major role in determining adduct loss (62). On the other hand, the high rate of cell division and DNA synthesis taking place in the colon may promote fixation of an initiated DNA lesion. In addition, the rate of DNA repair undoubtedly accounts for some of the differences in adduct levels in various organs and needs to be examined.

The results of this study indicate that the activation of 6-NC to a colon carcinogen in rats seems to occur primarily through a combination of ring oxidation and nitroreduction. However, the role of the simple nitroreduction pathway in activation should not be underestimated, because the adducts derived from N-OH-6-AC were also found in the colons of rats. Studies in mice had also suggested the involvement of both pathways in the induction of lung tumors by 6-NC (20, 26, 29). The relative contributions of DNA adducts derived from 6-NC via the combination and nitroreductive pathways to the induction of adenoma and adenocarcinoma in the colons of rats need to be investigated.
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REFERENCES


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