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DNA adduct formation by the ubiquitous environmental pollutant 3-nitrobenzanthrone and its metabolites in rats☆

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Abstract

Diesel exhaust is known to induce tumours in animals and is suspected of being carcinogenic in humans. Of the compounds found in diesel exhaust, 3-nitrobenzanthrone (3-NBA) is an extremely potent mutagen and suspected human carcinogen forming multiple DNA adducts in vitro. 3-Aminobenzanthrone (3-ABA), 3-acetylaminobenzanthrone (3-Ac-ABA), and *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone (*N*-Ac-*N*-OH-ABA) were identified as 3-NBA metabolites. In order to gain insight into the pathways of metabolic activation leading to 3-NBA-derived DNA adducts we treated Wistar rats intraperitoneally with 2 mg/kg body weight of 3-NBA, 3-ABA, 3-Ac-ABA, or *N*-Ac-*N*-OH-ABA and compared DNA adducts present in different organs. With each compound either four or five DNA adduct spots were detected by TLC in all tissues examined (lung, liver, kidney, heart, pancreas, and colon) using the nuclease P1 or butanol enrichment version of the ^{32}P-postlabelling method, respectively. Using HPLC co-chromatographic analysis we showed that all major 3-NBA-DNA adducts produced in vivo in rats are derived from reductive metabolites bound to purine bases and lack an *N*-acetyl group. Our results indicate that 3-NBA metabolites (3-ABA, 3-Ac-ABA and *N*-Ac-*N*-OH-ABA) undergo several biotransformations and that *N*-hydroxy-3-aminobenzanthrone (*N*-OH-ABA) appears to be the common intermediate in 3-NBA-derived DNA adduct formation. Therefore, 3-NBA-DNA adducts are useful biomarkers for exposure to 3-NBA and its metabolites and may help to identify enzymes involved in their metabolic activation.

Keywords: 3-Nitrobenzanthrone; 3-Aminobenzanthrone; Diesel exhaust; DNA adducts; ^{32}P-postlabelling; Nitro-PAH; Metabolic activation

Environmental factors play an important role in the aetiology of many human cancers [1]. Epidemiological data have shown that occupational exposure to diesel exhaust is associated with an increased risk of lung cancer, and has led to the IARC classification of diesel exhaust as probably carcinogenic to humans (group 2A) [2]. Diesel exhaust consists of a gaseous phase together with a particulate phase that contains many absorbed chemicals such as polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs [3]. The predominant source of human exposure to nitro-PAHs is diesel exhaust matter in which 1-nitropyrene is the major component (~200 ppm) [3]. Lesser amounts of nitro-PAHs are also found in emission from gasoline engines, on the surface of ambient air particulate matter, river sediments, certain food products, and grilled food [4]. 3-Nitrobenzanthrone (3-NBA, 3-nitro-7H-benz[de]anthracen-7-one, Fig. 1), a member of this class of compounds, was recently identified in both diesel exhaust and ambient air particulate matter [5], but in much lower concentrations (0.6–6.6 ppm). However, 3-NBA is the most mutagenic

☆ *Abbreviations:* 3-NBA, 3-nitrobenzanthrone; 3-ABA, 3-aminobenzanthrone; *N*-OH-ABA, *N*-hydroxy-3-aminobenzanthrone; 3-Ac-ABA, 3-acetylaminobenzanthrone; *N*-Ac-*N*-OH-ABA, *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone; dA, 2′-deoxyadenosine; dG, 2′-deoxyguanosine; dAp, 2′-deoxyadenosine-3′-monophosphate; dGp, 2′-deoxyguanosine-3′-monophosphate; dGp-*N*-Ac-ABA, *N*-acetyl-3-amino-2-(2′-deoxyguanosine-3′-monophosphate-8-yl)benzanthrone; dAp-*N*-Ac-ABA, the structurally uncharacterised 2′-deoxyadenosine-3′-monophosphate adduct; TLC, thin layer chromatography; r.t., retention time.

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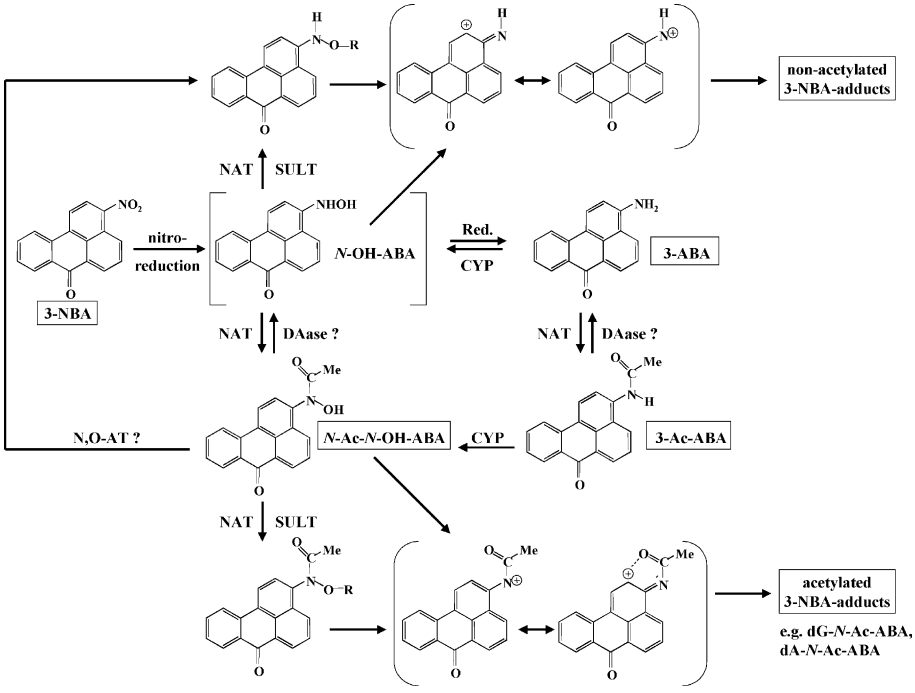

Fig. 1. Potential pathways of metabolic activation and DNA adduct formation of 3-NBA and its metabolites (3-ABA, 3-Ac-ABA, and *N*-Ac-*N*-OH-ABA). See text for details. Abbreviations are: NAT, N,O-acetyltransferase; N,O-AT, N,O-acetyltransfer (also catalysed by NATs); DAase, deacetylase; SULT, sulfotransferase; CYP, cytochrome P450; R = –$COCH_3$ or –SO_3H; Me = –CH_3.

mono-nitrated compound ever tested in the Ames *Salmonella* assay [5]. It also induces micronuclei in mouse and human cells and mutations in human cells [5,6]. Preliminary data also suggest that 3-NBA is carcinogenic in rats [7]. The genotoxicity of this suspected carcinogen was further documented by the detection of specific DNA adducts in vitro and in vivo in rats [8–11]. Recently Seidel et al. [12] demonstrated the uptake of 3-NBA in humans by the detection of 3-aminobenzanthrone (3-ABA, Fig. 1), a major metabolite of 3-NBA, in the urine of salt mining workers occupationally exposed to diesel emission.

Nitro-PAHs require metabolism to reactive electrophilic species prior to binding to DNA and exerting their genotoxic activity [13]. In incubations of rat lung alveolar type II cells with 3-NBA, 3-ABA was identified as the major metabolite, but small amounts of 3-acetylaminobenzanthrone (3-Ac-ABA, Fig. 1) were also observed [9]. Nitroreduction followed by O-acetylation and/or O-sulfonation seems to be the major pathway of bioactivation for 3-NBA [8,11,14]. In these studies, we showed that all in vivo 3-NBA-DNA adducts detected by ^{32}P-postlabelling are derived from reductive metabolites bound to the purine bases, deoxyguanosine (dG) and deoxyadenosine (dA). However, these adducts have not yet been structurally characterised. The only DNA adduct derived from 3-NBA which has been characterised spectroscopically was synthesised by reacting the activated ester of *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone (*N*-Ac-*N*-OH-ABA, Fig. 1), the *N*-acetoxy-*N*-acetylamino ester (Fig. 1), with dG. The adduct formed was an unusual dG C-8 adduct coupled through a C–C bond to the C-2 of benzanthrone, *N*-acetyl-3-amino-2-(2′-deoxyguanosine-8-yl) benzanthrone (dG-*N*-Ac-ABA) [15].

These studies have indicated that 3-NBA can be activated by two major pathways (Fig. 1). After nitroreduction to *N*-hydroxy-3-aminobenzanthrone (*N*-OH-ABA) the first pathway involves the formation of a nitrenium ion yielding non-acetylated 3-NBA-DNA adducts. The second pathway proceeds via the formation of *N*-Ac-*N*-OH-ABA and an *N*-acetyl-nitrenium ion yielding acetylated 3-NBA-DNA adducts. In order to gain insight into the metabolic pathways by which 3-NBA is activated in vivo, we have treated rats with 3-NBA, its known metabolites (3-ABA, 3-Ac-ABA) and *N*-Ac-*N*-OH-ABA and compared the DNA adducts formed in different organs.

Materials and methods

Synthesis of 3-NBA, 3-ABA, 3-Ac-ABA, and N-Ac-N-OH-ABA. 3-NBA and *N*-Ac-*N*-OH-ABA were prepared as described [14,15]. Syntheses of 3-ABA and 3-Ac-ABA will be described elsewhere. The authenticity of 3-NBA and its metabolites was confirmed by UV, mass, and high-field proton NMR spectroscopy.

Animal experiments. Female Wistar rats (220–250 g) were treated with a single dose of 2 mg/kg body weight of 3-NBA (7.3 mol), 3-ABA (8.2 μmol), 3-Ac-ABA (7.0 μmol) or *N*-Ac-*N*-OH-ABA (6.6 μmol) by intraperitoneal injection. All compounds were dissolved in tricaprylin at a concentration of 0.5 mg/ml. Two control rats received tricaprylin only. The animals were killed 24 h after treatment. Six organs (lung,

liver, kidney, heart, pancreas, and colon) were removed and stored at −80 °C until DNA isolation by a standard phenol extraction method.

^{32}P-postlabelling analysis. ^{32}P-postlabelling analysis using nuclease P1 digestion, butanol extraction, and autoradiography were performed as described recently [14]. Chromatographic conditions for TLC on polyethyleneimine-cellulose (PEI-cellulose) were: D1, 1.0 M sodium phosphate, pH 6.0; D3, 4 M Li-formate, 7 M urea, pH 3.5; D4, 0.8 M LiCl, 0.5 M Tris, and 8.5 M urea, pH 8.0. DNA adduct levels (RAL, relative adduct labelling) were calculated from the adduct cpm, the specific activity of [γ-^{32}P]ATP, and the amount of DNA (pmol of DNA-P) used. Results were expressed as DNA adducts/10^8 nucleotides.

Preparation of reference compounds. Deoxyadenosine and deoxyguanosine 3′-monophosphates (dAp and dGp) were incubated with 3-NBA and xanthine oxidase performed as described recently [11]. Aliquots of the incubation were used directly for the butanol extraction-mediated ^{32}P-postlabelling procedure as described above. The authentic standards of the adducts, *N*-acetyl-3-amino-2-(2′-deoxyguanosine-3′-monophosphate-8-yl)benzanthrone (dGp-*N*-Ac-ABA) and the as-yet structurally uncharacterised related dAp-*N*-Ac-ABA, were synthesised essentially as described previously [10]. Briefly, *N*-Ac-*N*-OH-ABA (3 mg) was dissolved in 0.1 ml of 0.5 N NaOH. After addition of 2 μl acetic anhydride to the solution, the precipitate was filtered, and washed twice with 0.1 ml water. *N*-Acetoxy-*N*-acetyl-3-aminobenzanthrone, obtained as a yellow solid, was used without further purification for reaction with dGp or dAp. The solid was suspended in 0.2 ml acetonitrile and 0.1 ml of this suspension was treated with 1 mg dGp or dAp (10 mg/ml in water). Then 0.01 ml of 3 M sodium acetate (pH 5.2) was added to each reaction mixture, which was then incubated at 55 °C overnight. Addition of water, absorption onto a Waters C18 Sep-Pak, and elution with 3 ml 50% acetonitrile removed most of the unreacted nucleotide. The products were isolated by chromatography on a Jupiter ODS column (250 × 4.6 mm), eluted at 1 ml/min with 0.05 M ammonium formate–acetonitrile (1% to 46% in 45 min). The products were identified by their UV [10] and mass spectra; positive molecular ions were obtained at *m/z* 633 (dGp-*N*-Ac-ABA) and 617 (dAp-*N*-Ac-ABA). Aliquots of dGp-*N*-Ac-ABA and dAp-*N*-Ac-ABA were labelled directly by ^{32}P-postlabelling.

HPLC analysis of ^{32}P-labelled 3′,5′-deoxyribonucleoside bisphosphate adducts. Individual adduct spots detected by the ^{32}P-postlabelling assay, or the origin after D1 only, were excised from the TLC plates, extracted, and co-chromatographed with reference bisphosphate adduct essentially as described previously [11]. The dried extracts were redissolved in 100 μl methanol/phosphate buffer (pH 3.5) 1:1 (v/v). Aliquots (50 μl) were analysed on a phenyl-modified reversed-phase column (Luna 5 μ phenyl–hexyl, 150 × 4.6 mm, Phenomenex, UK) with a linear gradient of methanol (from 30% to 55% in 45 min) in aqueous 0.5 M sodium phosphate (pH 3.5) at a flow rate of 1 ml/min. Radioactivity eluting from the column was measured by monitoring Cerenkov radiation with a Flow Scintillation Analyzer (Packard, Dowers Grove, IL, USA).

Results

DNA adduct analysis in rats

The formation of DNA adducts in various organs (lung, liver, kidney, heart, pancreas, and colon) of Wistar rats treated intraperitoneally with 3-NBA, 3-ABA, 3-Ac-ABA, or *N*-Ac-*N*-OH-ABA was analysed by ^{32}P-postlabelling. All four compounds induced essentially the same DNA adduct pattern (Fig. 2). Using butanol enhancement the pattern consisted of a cluster of four major adducts (spots 1, 2, 3, and 4) and one minor adduct (spot 5). Analyses using nuclease P1 enhancement resulted essentially in a cluster of four adducts (spots 1, 2, 3, and 6). Additionally, one minor adduct (assigned spot 8) was detected in kidney tissue after nuclease P1 digestion (Fig. 4), migrating slightly below adduct spot 3 in D3 direction. No DNA adducts were observed in DNA isolated from tissue of control animals treated with vehicle (tricaprylin) only (data not shown). Adduct spots 4 and 5 were detectable only after butanol extraction, which is characteristic for *N*-substituted aryl adducts bound to the C-8 position of guanine [16], whereas adduct spots 6 and 8 were observed only after nuclease P1 enrichment (Figs. 2 and 4). At present we can speculate only on the structure of these adducts but all major adduct spots detected in this study were chromatographically indistinguishable from adduct spots found in incubations with dAp (adducts 1 and 2) and dGp (adducts 3 and 4) generated by 3-NBA activated by xanthine oxidase [11]. Total DNA binding was highest after treatment with 3-NBA, followed by *N*-Ac-*N*-OH-ABA, and less with 3-ABA and 3-Ac-ABA (Fig. 3). The highest DNA binding by 3-NBA was found in the pancreas followed by colon and decreased in kidney > heart = lung > liver. For 3-ABA, 3-Ac-ABA, and *N*-Ac-*N*-OH-ABA there was less variation in the levels of DNA adducts in the tissues investigated. As shown for lung, liver, and kidney tissue (Fig. 4), irrespective of the tissue analysed but dependent on the enrichment procedures used, approximately the same relative amounts of individual adducts were found. In particular adduct spot 3 was the most abundant adduct found in each tissue (Fig. 4).

HPLC analyses of DNA adduct spots from rats treated with 3-NBA, 3-ABA, 3-Ac-ABA, or N-Ac-N-OH-ABA

We employed reversed-phase HPLC analysis as a second, independent chromatographic procedure to confirm identities of adduct spots formed by each of the four compounds. Areas containing individual adduct spots or the origin after D1 only from postlabelled samples were excised from the TLC plates and eluted with isopropanol/ammonia. Aliquots of the eluates were then analysed on HPLC (Fig. 5). The results thus obtained confirmed the findings from chromatography on TLC plates. Using the same approach as reported recently [11] we found that adduct spots 1 and 2 formed after treatment with 3-NBA, 3-ABA, 3-Ac-ABA, or *N*-Ac-*N*-OH-ABA eluted with the retention times (r.t.) of 42.0 and 34.0 min (Fig. 5), respectively, identical with the r.t. of the dA-derived adducts formed by treatment of dAp with 3-NBA after activation with xanthine oxidase. Likewise, spots 3 (r.t. 25.0 min) and 4 (r.t. 29.0) (Fig. 5) coeluted with the dG-derived adducts prepared in vitro.

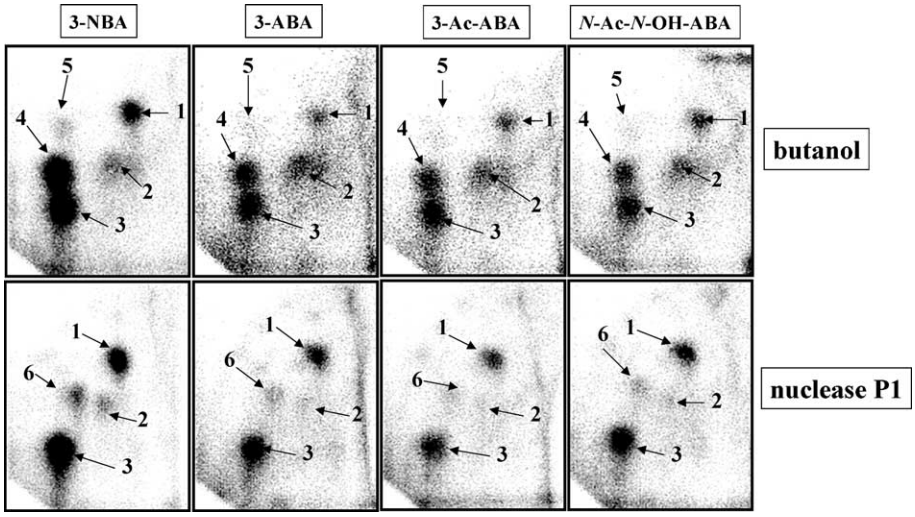

Fig. 2. Autoradiographic profiles of DNA adducts obtained from organs of Wistar rats treated with 3-NBA, 3-ABA, 3-Ac-ABA, or *N*-Ac-*N*-OH-ABA using the butanol (upper panels) or nuclease P1 (lower panels) enrichment version of the ^{32}P-postlabelling assay. Autoradiograms exemplify digests of lung DNA of rats.

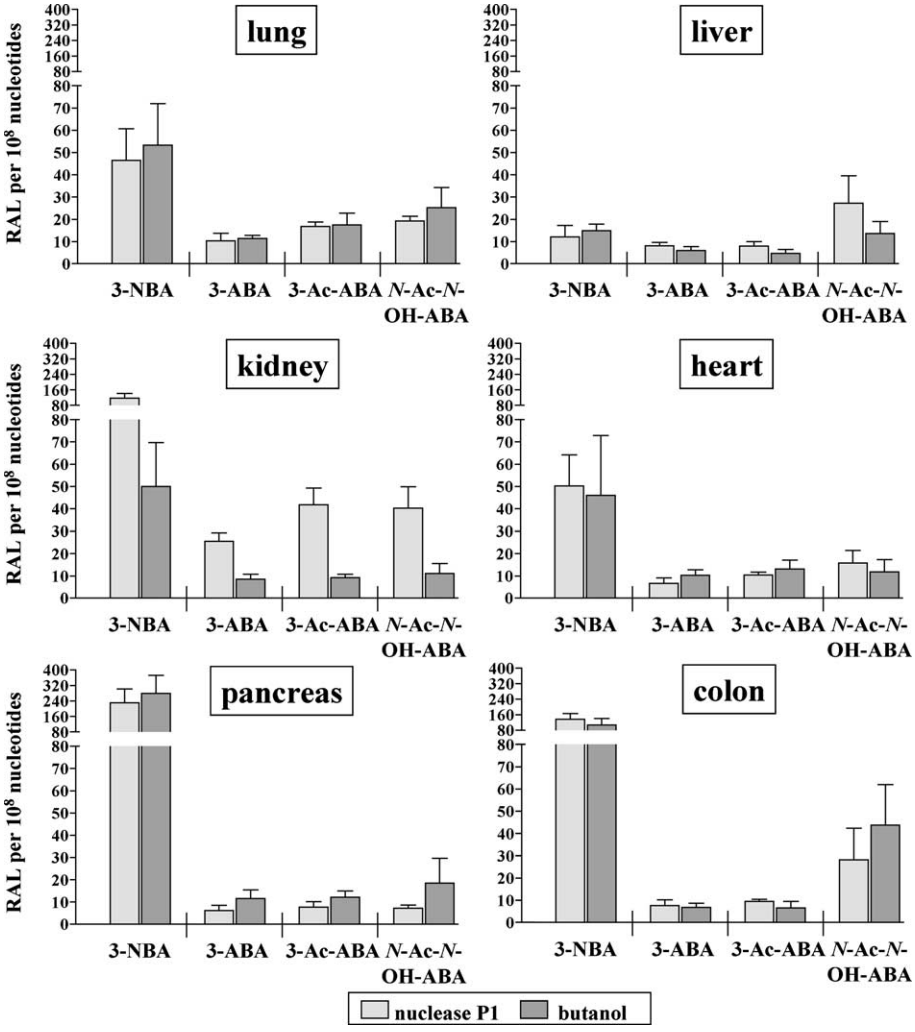

Fig. 3. RAL (relative adduct labelling) of total DNA adducts in various organs of rats treated with 3-NBA, 3-ABA, 3-Ac-ABA, or *N*-Ac-*N*-OH-ABA. Values represent means ± SD from three treated rats, the DNA from each organ being postlabelled twice.

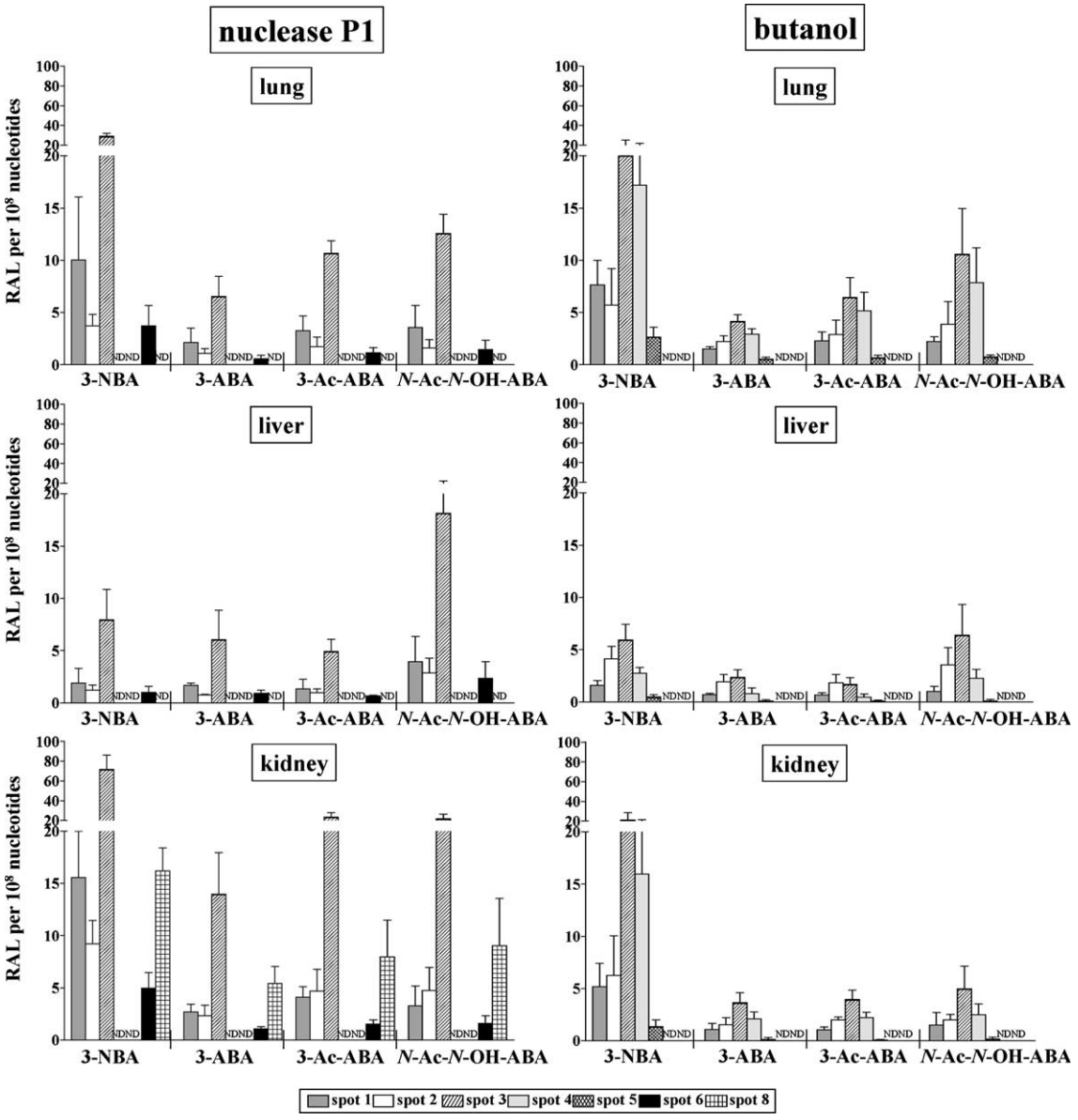

Fig. 4. RAL (relative adduct labelling) of individual DNA adducts in various organs of rats treated with 3-NBA, 3-ABA, 3-Ac-ABA, or *N*-Ac-*N*-OH-ABA. Values represent means ± SD from three rats, the DNA from each organ being postlabelled twice. ND, not detected.

Comparison of DNA adducts found in vivo with dGp-N-Ac-ABA and dAp-N-Ac-ABA adduct standard

As reported before, labelling of the dGp-*N*-Ac-ABA standard resulted in one major adduct spot (spot S1) and one minor adduct spot (spot S2) [11], the latter most likely representing a degradation product. On HPLC spots S1 and S2 eluted with a r.t. of 24.0 and 20.5 min, respectively. Comparative chromatographic analyses on TLC showed that dGp-*N*-Ac-ABA was not detected in rats treated with 3-NBA, 3-ABA, 3-Ac-ABA, or *N*-Ac-*N*-OH-ABA. Labelling of dAp-*N*-Ac-ABA resulted in one major adduct spot (spot S3) on TLC, migrating similarly to adduct spot 1 obtained with DNA from rats treated with 3-NBA or its metabolites. However, comparative chromatographic analysis on TLC showed that both spots were distinguishable (data not shown). To confirm this finding with a second, independent chromatographic procedure, spot S3 was subjected to HPLC. It eluted with a r.t. of 40.0 min, different from those of the adducts formed in vivo.

Discussion

3-NBA is an extremely potent mutagen and suspected human carcinogen identified in diesel exhaust and airborne particulate matter [5,7,12]. Previous work has shown that 3-NBA induces a specific DNA adduct pattern in vitro using different activating systems [8]. In

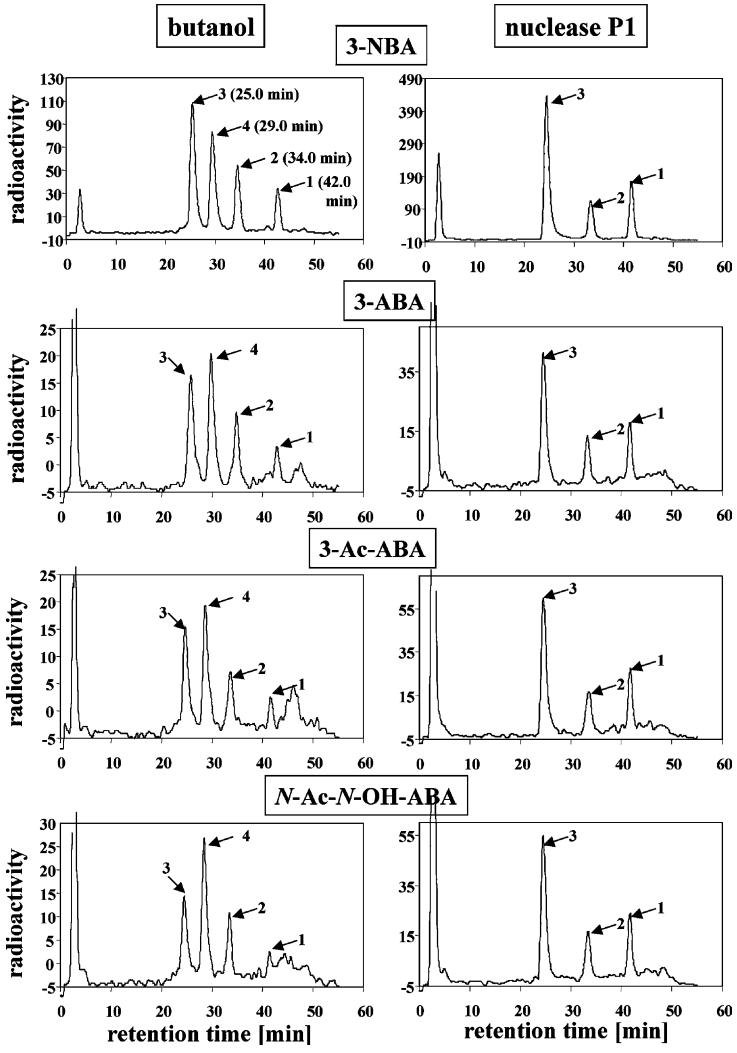

Fig. 5. Separation of ^{32}P-labelled nucleoside 3′,5′-bisphosphate adducts on a phenyl-modified reversed-phase column. HPLC autoradiograms show digests of lung DNA of rats. Origins after D1 were excised and extracted from the TLC plates, dissolved, and injected. Chromatographic conditions are described in Materials and methods.

the present study we compared DNA adduct formation by 3-NBA and its metabolites (3-ABA, 3-Ac-ABA, and *N*-Ac-*N*-OH-ABA) in vivo in rats given by intraperitoneal injection to assess the role of potential metabolites in 3-NBA-derived DNA adduct formation. Tissues were collected after 24 h treatment to allow sufficient time for metabolism.

^{32}P-postlabelling analyses of DNA of several organs from rats revealed a qualitatively similar adduct pattern with each compound (Fig. 2), indicating a common ultimate reactive species leading to the formation of specific DNA adducts. The highest DNA binding for 3-NBA was observed in the pancreas, followed by colon (Fig. 3). In this context, it is interesting to note that pancreas exhibits high nitroreductase activity and that nitro-PAHs are considered a possible risk factor for pancreatic cancer in humans [17]. Substantial adduct formation was also found in rat kidney, heart, lung, and liver (Fig. 3), indicating that 3-NBA and its metabolites are distributed systemically via the bloodstream. The formation of 3-NBA-DNA adducts in various tissues is in line with the results of a recent study in which DNA adducts by 3-NBA were observed in numerous rat tissues (forestomach, glandular stomach, lung, liver, kidney, bladder, and small intestine) after short-term (4 h) oral treatment with 3-NBA [11]. Similar DNA damage by 3-NBA and its metabolites was found in rat lung and heart (Fig. 3). It is of note that similarly high levels of smoking-related DNA adducts were observed in human lung and heart tissue [18]. Using two independent chromatographic systems (TLC and HPLC) we clearly showed that all four compounds induced the same DNA adducts (Figs. 2 and 5). The adduct pattern was similar to that obtained from in vitro reaction of DNA with 3-NBA after activation with xanthine oxidase, a mammalian nitroreductase [8,11]. Moreover, we showed that

all four major adducts (adducts 1, 2, 3, and 4) are products derived from simple nitroreduction bound to dA (adducts 1 and 2) or dG (adducts 3 and 4) that do not possess an *N*-acetyl group. On the basis that treatment with each of the four compounds results in the formation of the same DNA adducts and considering the different chemical nature of 3-NBA and its metabolites, it seems that different metabolic pathways lead to the same ultimate reactive species.

Nitroreduction to *N*-OH-ABA followed by O-acetylation and/or O-sulfonation seems to be the major pathway of activation for 3-NBA (Fig. 1) [8,11,14]. In mammalian tissue, both cytosol and microsomes contain enzymes that catalyse the reduction of the nitro group [13]. Xanthine oxidase is a cytosolic enzyme already shown to be capable of activating 3-NBA. Preliminary data from experiments using human hepatic microsomes also indicate an important role for microsomal NADPH:P450 reductase in the bioactivation of 3-NBA (Arlt, Stiborova, Schmeiser, Phillips, unpublished data). *N*-Hydroxy-arylamine intermediates can be further metabolised by N,O-acetyltransferases (NATs) or sulfotransferases (SULTs) [19,20], leading to reactive *N*-acetoxy- or *N*-sulfooxyesters that undergo heterolysis of the N–O bond to produce electrophilic nitrenium ions capable of reacting with DNA. Recombinant human NAT2, and to a lesser extent recombinant human NAT1 and SULT1A1, strongly increase DNA adduct formation by 3-NBA [14]. Thus, the formation of reactive esters (*N*-acetoxy- or *N*-sulfooxyesters) after initial nitroreduction may be important for the in vivo metabolism of 3-NBA (Fig. 1).

Cytochrome P450 (CYP) enzymes are involved in the metabolic activation of several aromatic and heterocyclic amines due to N-oxidation. For instance, human CYP1A2 catalyses metabolic activation of the human bladder carcinogen 4-aminobiphenyl (ABP) [21]. Therefore, it seems likely that N-hydroxylation of 3-ABA to *N*-OH-ABA by CYP enzymes is involved in the bioactivation of 3-ABA (Fig. 1). Preliminary data from our laboratory indicate that recombinant human CYP1A2 activates 3-ABA, leading to the formation of the same 3-NBA-derived DNA adducts (Arlt, Glatt, Schmeiser, Phillips, unpublished data). Consistent with recent data [14] our in vivo data suggest that *N*-Ac-*N*-OH-ABA is readily deacetylated to *N*-OH-ABA (Fig. 1). An important role for microsomal deacetylase in adduct formation by *N*-hydroxy-2-acetylaminofluorene (*N*-OH-AAF) was demonstrated [22,23]. On the other hand *N*-Ac-*N*-OH-ABA may be detoxified by pathways such as glucuronidation explaining why DNA binding by *N*-Ac-*N*-OH-ABA is lower than that by 3-NBA (Fig. 3). NATs also catalyse, to a limited extent, N,O-acetyltransfer reactions [19]. Thus, it may be possible that *N*-Ac-*N*-OH-ABA is converted to the corresponding *N*-acetoxyester by N,O-acetyltransfer, which can react directly with DNA (Fig. 1). Comparative chromatographic analyses with dGp-*N*-Ac-ABA, an identified adduct standard, and dAp-*N*-Ac-ABA, a related, but structurally not yet fully characterised dA adduct, revealed that these adducts are not detected in rats treated with 3-NBA or its metabolites, indicating that the reactive ester *N*-acetoxy-*N*-acetyl-3-aminobenzanthrone does not contribute to 3-NBA-derived adduct formation in vivo. Unlike other metabolites, 3-Ac-ABA accumulated in rat lung alveolar type II cells treated with 3-NBA [9], indicating that the formation of 3-Ac-ABA is a late event in the metabolism of 3-NBA, resulting from N-acetylation of 3-ABA, the major metabolite formed (Fig. 1). N-oxidation of 3-Ac-ABA by CYP enzymes could lead to the formation of *N*-Ac-*N*-OH-ABA (Fig. 1). Alternatively, microsomal deacetylases could convert 3-Ac-ABA back into 3-ABA. 3-ABA was recently identified in urine samples of salt mining workers occupationally exposed to diesel emission [12]. Moreover, 3-ABA was the main metabolite in rat lung alveolar type II and human fetal bronchial cells treated with 3-NBA [9]. 3-ABA was capable of forming the same 3-NBA-derived DNA adducts. Therefore, 3-ABA and 3-NBA-derived DNA adducts may be useful internal biomarkers of exposure to diesel emission. 3-NBA-derived DNA adducts may also serve as a predictive biomarker of lung cancer risk in humans exposed to environmental sources containing 3-NBA.

In summary, the specific DNA adduct formation detected in this study indicates that 3-NBA and its metabolites (3-ABA, 3-Ac-ABA, and *N*-Ac-*N*-OH-ABA) are subjected to several biotransformation reactions including nitroreduction, N-oxidation, O-esterification, O-sulfonation, deacetylation, and/or N,O-transacylation before becoming bound to DNA and forming the same DNA adducts. Our results indicate that *N*-OH-ABA is an important precursor for the formation of the electrophilic arylnitrenium ions capable of reacting with DNA (Fig. 1). Many genes of enzymes (CYPs, NATs, and SULTs) metabolising 3-NBA and its metabolites are known to exist in variant forms or show polymorphisms resulting in different activities of the gene products and are expressed differently in tissues [1]. Our study demonstrates that 3-NBA-derived DNA adducts are useful biomarkers of exposure to 3-NBA and its metabolites which can be used to identify enzymes involved in their activation and to determine their catalytic specificities.

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