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Differences between human slow N-acetyltransferase 2 alleles in levels of 4-aminobiphenyl-induced DNA adducts and mutations

Jean Bendaly, **Mark A. Doll**, **Lori M. Millner**, **Kristin J. Metry**, **Ned B. Smith**, **William M. Pierce Jr.**, and **David W. Hein**
Department of Pharmacology & Toxicology, James Graham Brown Cancer Center, and Center for Environmental Genomics and Integrative Biology, University of Louisville School of Medicine, Louisville, Kentucky 40292 United States

Abstract

Aromatic amines such as 4-aminobiphenyl (ABP) require biotransformation to exert their carcinogenic effects. Genetic polymorphisms in biotransformation enzymes such as N-acetyltransferase 2 (NAT2) may modify cancer risk following exposure. Nucleotide excision repair-deficient Chinese hamster ovary (CHO) cells stably transfected with human cytochrome *P4501A1* (*CYP1A1*) and a single copy of either *NAT2*4* (rapid acetylator), *NAT2*5B* (common Caucasian slow acetylator), or *NAT2*7B* (common Asian slow acetylator) alleles (haplotypes) were treated with ABP to test the effect of NAT2 polymorphisms on DNA adduct formation and mutagenesis. ABP N-acetyltransferase catalytic activities were detectable only in cell lines transfected with NAT2 and were highest in cells transfected with *NAT2*4*, lower in cells transfected with *NAT2*7B*, and lowest in cells transfected with *NAT2*5B*. Following ABP treatment, N-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP) was the primary adduct formed. Cells transfected with both *CYP1A1* and *NAT2*4* showed the highest concentration-dependent cytotoxicity, *hypoxanthine phosphoribosyl transferase (hprt)* mutants, and dG-C8-ABP adducts. Cells transfected with *CYP1A1* and *NAT2*7B* showed lower levels of cytotoxicity, *hprt* mutagenesis, and dG-C8-ABP adducts. Cells transfected with *CYP1A1* only or cells transfected with both *CYP1A1* and *NAT2*5B* did not induce cytotoxicity, *hprt* mutagenesis or dG-C8-ABP adducts. ABP DNA adduct levels correlated very highly ($r > 0.96$) with ABP-induced *hprt* mutant levels following each treatment. The results of the present study suggest that investigations of NAT2 genotype or phenotype associations with disease or toxicity could be more precise and reproducible if heterogeneity within the “slow” NAT2 acetylator phenotype is considered and incorporated into the study design.

Keywords

N-acetyltransferase 2; Acetylator genotype; 4-aminobiphenyl; DNA adducts

Correspondence and reprints: David W. Hein, Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky 40292, USA. Tel: 502-852-5141; Fax: 502-852-7868; d.hein@louisville.edu.

1. Introduction

4-Aminobiphenyl (ABP) is present in both mainstream and side stream cigarette smoke [1] and recently was reaffirmed as a human urinary bladder carcinogen [2]. ABP-induced DNA adduct formation and mutagenesis require metabolic activation. P450s 1A1 and 1A2 catalyze the N-hydroxylation of arylamines in human tissues [3]. Although CYP1A2 is considered the primary isozyme for hepatic activation, CYP1A1 may be particularly important in urinary bladder, the target organ for ABP carcinogenesis in humans [4]. Cyp1a2 knockout mice do not differ in ABP DNA adduct levels in either urinary bladder or liver compared to wildtype mice [5,6]. Genetic polymorphisms in CYP1A1 have been shown to modify DNA adduct levels in normal breast tissues of women with breast cancer, especially in smokers [7]. As depicted in Figure 1, N-acetylation of ABP catalyzed by arylamine N-acetyltransferase 2 (NAT2) is generally considered a detoxification step since it competes with P450-catalyzed N-hydroxylation. Following N-hydroxylation, O-acetylation of N-hydroxy-ABP catalyzed by arylamine N-acetyltransferase 2 (NAT2) generates acetoxy-derivatives that are highly unstable, leading to the formation of an arylnitrenium ion that binds to DNA and ultimately leads to mutagenesis and carcinogenesis [3]. In humans, the major ABP-induced DNA adduct is N-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP) [8,9].

Epidemiological data on the role of NAT2 genetic polymorphism in susceptibility to various cancers suggest that the role of NAT2 genetic polymorphism varies both with carcinogen and organ site [10]. PubMed search (conducted April 2009) of “NAT2” and “cancer” yields over 500 literature citations. Because DNA adduct levels are a function of environmental exposure and polymorphism in genes involved in carcinogen metabolism, DNA adducts are an informative biomarker for investigations of genetic variation in carcinogen metabolism. There is a critical need to characterize genotype and associated phenotypic catalytic activities of NAT2 enzymes at physiological concentrations of co-factors and environmental exposure levels of carcinogen.

NAT2 genetic polymorphisms resulting in rapid and slow acetylator phenotypes are frequent in human populations [10]. The *NAT2*4* allele (haplotype) is associated with rapid acetylator phenotype, whereas the *NAT2*5B* and *NAT2*7B* alleles are associated with slow acetylator phenotype [10]. Relative to the *NAT2*4* reference allele, *NAT2*5B* possesses three single nucleotide polymorphisms (SNPs) in the *NAT2* open reading frame: T341C (I114T), C481T (synonymous) and A803G (K268R) whereas *NAT2*7B* possesses two polymorphisms in the NAT2 open reading frame: C282T (synonymous) and G857A (G286E) [10,11]. Epidemiologic studies suggest a role for NAT2 genetic polymorphism in susceptibility to various cancers related to arylamine exposures [10], but inconsistent results in these and other molecular epidemiology studies [11,12] suggest the need for laboratory-based experiments to support the biological plausibility and the conclusions inferred from these epidemiologic studies [13]. For example Ambrosone et al [14] reported that slow acetylator *NAT2* genotype increased risk for breast cancer in postmenopausal smokers but studies to replicate these findings have been inconsistent. Whereas some studies are supportive of this initial report [15–18], other studies [19–25] reported no appreciable difference between rapid and slow NAT2 acetylator smokers for breast cancer risk, while three others reported that *rapid* acetylator NAT2 smokers were possibly at higher risk [26–28].

We expected ABP-induced DNA adducts and mutants would be higher in slow than in rapid NAT2 acetylators. To test this hypothesis, we measured ABP-induced DNA adducts and mutants in nucleotide excision repair-deficient Chinese hamster ovary (CHO) cell lines transfected with human CYP1A1 and rapid or slow acetylator *NAT2* alleles.

2. Materials and methods

2.1 Cell Culture

The UV5-CHO cell line, a nucleotide excision repair-deficient derivative of the AA8 line [29], was obtained from the ATCC (Catalog number: CRL-1865). Since UV5-CHO lacks nucleotide excision repair due to a mutation in the XPD (ERCC2) gene [30], it is hypersensitive to bulky adduct mutagens and belongs to the excision repair cross complementation group 2. All cells were grown in alpha-modified minimal essential medium (Cambrex) without L-glutamine, ribosides, and deoxyribosides supplemented with 10% fetal bovine serum (Hyclone), 100 units/ml penicillin, 100 micrograms/ml streptomycin (Cambrex), and 2 mM L-glutamine (Cambrex) at 37 °C in 5% CO_2. Media were supplemented with appropriate selective agents to maintain stable transfectants.

2.2. Construction and characterization of UV5/CHO cell lines

Construction of UV5/CHO cells expressing human *CYP1A1* and either *NAT2*4* or *NAT2*5B* was recently reported and characterized [31]. An identical method was used for construction of UV5/CHO cells expressing *CYP1A1* and *NAT2*7B*. Briefly, the *NAT2*7B* open reading frame was amplified by polymerase chain reaction (PCR), digested with *Nhe*I and *Xho*I (New England Biolabs), and inserted into the similarly prepared pcDNA5/FRT vector (Invitrogen). The pcDNA5/FRT plasmid containing human *NAT2*7B* was co-transfected with pOG44, a Flp recombinase expression plasmid, into UV5FRT/CYP1A1 cells constructed and characterized previously [31]. Integration of the pcDNA5/FRT construct into the FRT site was confirmed by PCR. Geneticin-and hygromycin B-resistant clones containing the *NAT2*7B/CYP1A1* were expanded and assayed for CYP1A1 activity by measuring 7-ethoxyresorufin O-deethylase (EROD) activity as described previously [32]. *NAT2*7B/CYP1A1* clones with similar levels of EROD activity to the *NAT2*4/CYP1A1* and *NAT2*5B/CYP1A1* cell lines were selected for survival, mutagenesis, and DNA adduct studies.

Cell lysates from the UV5 and each of the transfected CHO cell lines were tested for sulfamethazine (SMZ; an NAT2-specific substrate) and ABP N-acetyltransferase activities as previously described [33,34].

2.3. Cell survival and mutagenesis

Assays for cytotoxicity and mutagenesis were carried out as described previously [32]. Briefly, cells were grown for 12 doublings, with selective agents in complete hypoxanthine-aminopterin-thymidine medium (30 μM hypoxanthine, 0.1 μM aminopterin, and 30 μM thymidine). Cells were plated at a density of 5×10^5 cells/T-25 flask and incubated for 24 h, after which media were changed and the cells were treated separately for 48 h under cover with - 1 to 4 μM ABP or vehicle control (0.5% dimethyl sulfoxide). Survival was determined by colony-forming assay and expressed as percent of vehicle control. The remaining cells were replated and subcultured. After 7 days of growth, cultures were plated for cloning efficiency in complete media and for *hypoxanthine phosphoribosyl transferase (hprt)* mutations in complete media containing 40 μM 6-thioguanine (Sigma). Dishes were seeded with 1×10^5 cells/100 mm dish (10 replicates) and incubated for 7 days; cloning efficiency dishes were seeded with 100 cells/well/six-well plate in triplicate and incubated for 6 days.

2.4. Identification and quantitation of ABP-DNA adducts

dG-C8-ABP and dG-C8-ABP-D5 adduct standard (>95% purity) were obtained from Toronto Research Chemicals. Cells grown in 15-cm plates were treated separately with ABP as described above for the cell survival and mutagenesis assays. Cells were harvested after 48 h of ABP treatment and DNA was extracted and quantified as previously described [32]. One-

tenth volumes each of proteinase K solution (20 mg/mL) and 10% SDS were added to the cell lysate, and the mixture was incubated at 37°C for 60 min. One volume of phenol, equilibrated with 10 mM Tris HCl (pH 8.0), was added to the mixture, which was then vortexed and centrifuged at 3,600 × g for 15 min. The aqueous layer was removed and added to 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1) saturated with 10 mM Tris HCl (pH 8.0), which was vortexed and centrifuged. The aqueous layer was removed and added to 1 volume of cold (−20°C) isopropanol, and the mixture was vortexed and centrifuged. The DNA pellet was washed with 70% ethanol and redissolved in 5 mM Tris HCl (pH 7.4) containing 1 mM $CaCl_2$, 1 mM $ZnCl_2$, and 10 mM $MgCl_2$. DNA was quantified by UV spectroscopy using $A_{260\ nm}$. DNA quality was monitored by UV spectroscopy using $A_{260/280\ nm}$ and this ratio was consistently above 1.9. DNA samples (200 µg) added to 1 ng (3.3 adducts per 10^6 DNA bases) deuterated internal standard (dG-C8-ABP-D5) were digested at 37ºC with 10 units DNAse I (US Biological) for 1 h followed by 5 units micrococcal nuclease (Sigma), 5 units nuclease P1 (US Biological), 0.01 units spleen phosphodiesterase (Sigma), and 0.01 units snake venom phosphodiesterase (Sigma) for 6 h followed by 5 units alkaline phosphatase (Sigma) overnight. Two volumes of acetonitrile were added to the digest, which was then filtered and concentrated to 100 µl in a speed vacuum.

Samples were subjected to binary gradient high performance liquid chromatography (HPLC) and introduced into a Micromass Quattro LC triple quadrupole mass spectrometer using a custom-built nanospray as described previously [32,35]. Samples were loaded onto a Inertsil C18 precolumn (5 mm x 300 m i.d., 5 µm; LC Packings) using Perkin Elmer ABI 140D syringe pumps and a Hewlett Packard 1100 Series autosampler. Multiple reaction monitoring (dwell time, 0.5 s; span, 0.4 Da) was used to measure the $[M+H]^+$ to $[(M-116) + H]^+$ (loss of deoxyribose) mass transition. Multiple reaction monitoring in the electrospray ionization-positive ion mode was carried out using argon as the collision gas. Capillary and cone voltages and collision energies were optimized for cleavage of the glycosidic bond. The dG-C8-ABP adduct was monitored using the transition from *m/z* 435 to *m/z* 319 and the deuterated internal standard (dG-C8-ABP-D5) was monitored using the transition from *m/z* 440 to *m/z* 324.

Results

The *CYP1A1-*, *CYP1A1/NAT2*5B-*, *CYP1A1/NAT2*7B-* and *CYP1A1/NAT2*4-* transfected cell lines catalyzed EROD activity at 100–120 relative fluorescence units (RFU)/ million cells that did not differ significantly ($p>0.05$) from each other. CYP1A1 EROD catalytic activity was not detected (<5 RFU/million cells) in the untransfected UV5 cells.

Transfection of *NAT2*7B* was confirmed by measurement of human NAT2-specific SMZ N-acetyltransferase catalytic activity. SMZ NAT2 activity in the UV5/CYP1A1/NAT2*7B cell line was similar to the UV5/CYP1A1/NAT2*4 cell line and over 12-fold greater ($p<0.001$) than the UV5/CYP1A1/NAT2*5B cell line (Fig. 2). SMZ *N*-acetyltransferase catalytic activity was not detected in the UV5 or UV5/CYP1A1 cell lines (<20 pmole/min/mg). Cell lysates from UV5 and each of the transfected CHO cell lines were tested for ABP NAT2 catalytic activity. ABP NAT2 catalytic activity in UV5/CYP1A1/NAT2*4 cells was about 2-fold higher ($p<0.01$) than in the UV5/CYP1A1/NAT2*7B cell line, which was about 7-fold higher ($p<0.01$) than the UV5/CYP1A1/NAT2*5B cell line (Fig. 2). ABP NAT2 activity was not detected in the UV5 and UV5/CYP1A1 cell lines (<10 pmol/min/mg protein).

As shown in Figure 3A, ABP treatment resulted in dose-dependent reductions in survival of the *CYP1A1/NAT2*4-* and *CYP1A1/NAT2*7B–*transfected CHO cell lines. Cell survival following 2–4 µM ABP treatment differed significantly ($p<0.001$) in the order UV5/CYP1A1/NAT*4 < UV5/CYP1A1/NAT2*7B < UV5, UV5/CYP1A1, and UV5/CYP1A1/NAT2*5B. As shown in Figure 3B, ABP treatment resulted in dose-dependent production of *hprt* mutants

in the *CYP1A1/NAT2*4*- and *CYP1A1/NAT2*7B*–transfected CHO cell lines. ABP -induced *hprt* mutants differed significantly in the order UV5/CYP1A1/NAT2*4 > UV5/CYP1A1/NAT2*7B > UV5, UV5/CYP1A1, and UV5/CYP1A1/NAT2*5B following 1 μM ($p<0.05$) and 2–4 μM ($p<0.001$) ABP treatment.

dG-C8-ABP standard was characterized by HPLC-tandem mass spectrometry (LC-MS/MS) and used to verify the identity of DNA adducts formed in cell culture. dG-C8-ABP corresponding to *m/z* 435, with a major aglycone ion of *m/z* 319 was identified in CHO cells treated with ABP (Fig 4). dG-C8-ABP was quantitated by comparison to dG-C8-ABP-D5 used as an internal standard. Identical patterns of fragmentation were observed for dG-C8-ABP and dG-C8-ABP-D5, except that the latter were shifted by 5 Da. As shown in Figure 5, dG-C8-ABP DNA adduct levels were dose-dependent only in the *CYP1A1/NAT2*4*- and *CYP1A1/NAT2*7B*–transfected cell lines. ABP-DNA adduct levels were not detected in UV5 CHO cell lines, nor in lines transfected with *CYP1A1* or *CYP1A1/NAT2*5B*. ABP-DNA adduct levels were significantly ($p<0.001$) higher in UV5/CYP1A1/NAT2*4 than UV5/CYP1A1/NAT2*7B cell lines after each ABP treatment. ABP DNA adduct levels correlated very highly ($r > 0.96$) with ABP-induced *hprt* mutant levels following each treatment. Transfection with NAT2 was required for significant detection of both adducts and mutants in the order *NAT2*4* ≫ *NAT2*7B* ≫*NAT2* 5B*.

Discussion

Our studies clearly show that metabolic activation of ABP can be catalyzed by CYP1A1 and that mutagenesis and DNA adduct formation require further metabolic activation by NAT2. Metabolic O-acetylation by NAT2 substantially increased levels of DNA adducts and mutants and rapid acetylator NAT2 catalyzed this activation to a substantially greater extent than slow acetylator NAT2. The primary DNA adduct formed was dG-C8-ABP, which is the primary adduct that has been identified in human urinary bladder [8,36,37], breast [38–40], lung [41] and pancreas [9] and is consistent with N-hydroxylation followed by O-acetylation pathway as depicted in Figure 1. The ABP concentration-dependent increases in dG-C8-ABP adducts and *hprt* mutants were similar and dG-C8-ABP adduct levels correlated very highly ($r > 0.96$) with *hprt* mutant frequency providing further evidence for this pathway. Taken together, these results strongly suggest that ABP-induced mutagenesis follows formation of the dG-C8-ABP adduct which in turn is dependent upon O-acetylation catalyzed by NAT2 as depicted in Figure 1.

Previous studies have shown that the effect of NAT2 polymorphism on the levels of DNA adducts and mutations varies with carcinogen. Following treatment of human CYP1A1- or CYP1A2-transfected CHO cells with 0–2 μM 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine, additional transfection with rapid and slow acetylator NAT2 had little further effect [32,42]. In contrast, following treatment of human CYP1A1 transfected CHO cells with 0–3 μM 2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline [31], and of human CYP1A2-transfected CHO cells with 0–6 μM 2-amino-9H–pyrido[2,3-b]indole [43], additional transfection with rapid and slow acetylator NAT2 substantially increased levels of both DNA adducts and mutants. However, these arylamines are heterocyclic amine carcinogens which undergo N-acetylation very poorly [44–46]. Thus, it is not unexpected that O-acetylation of the N-hydroxy metabolites leads to higher levels of DNA adducts and mutants in rapid NAT2 acetylators. In contrast, ABP is an aromatic amine carcinogen that readily undergoes both N-acetylation and following N-hydroxylation, O-acetylation both catalyzed by NAT2 [47,48]. Thus, NAT2 could decrease or increase DNA adducts and mutants as depicted in Figure 1. Consistent with current literature and established theory, we expected ABP-induced DNA adducts and mutants would be higher in slow than in rapid NAT2 acetylators. However, our results clearly showed that they were both actually higher following transfection with

*NAT2*4*, the rapid acetylator allele. Our results are consistent with earlier studies reporting higher levels of DNA damage in hepatocytes from rapid NAT2 acetylator rabbits [49], and with urinary bladder DNA adduct levels in rapid NAT2 acetylator congenic Syrian hamsters [50] following exposure to another aromatic amine (2-aminofluorene). In addition, the results are consistent with higher levels of breast ABP-DNA adducts [40], and higher levels of bulky DNA adducts in urothelial [51] and white blood cells [52] of human rapid NAT2 acetylators.

Our most novel finding was the significant differences observed between *CYP1A1/NAT2*7B*-and *CYP1A1/NAT2*5B*–transfected CHO cells for both DNA adducts and mutants following ABP treatment. The differences were significant at all ABP concentrations (1–4 μM) tested but increased as the ABP concentration increased. This may reflect treatment concentrations below the affinity of ABP or N-hydroxy-ABP for human NAT2. An apparent Km of 26 μM for ABP [53] and 108 μM for N-hydroxy-ABP [54] have been reported for human NAT2. Although both *NAT2*4* and *NAT2*7B* are associated with “slow” NAT2 acetylator phenotype, previous studies have shown that recombinant expression of the *NAT2*5B* allele results in strikingly lower 2-aminofluorene N-acetyltransferase [55] and N-hydroxy-ABP O-acetyltransferase [56] catalytic activities than does recombinant expression of the *NAT2*7B* allele.

As recently reviewed [11,57], different mechanisms have been reported for the reductions in NAT2 activity resulting from SNPs within the *NAT2*5B* and *NAT2*7B* alleles. The T341C (I114T) polymorphism in *NAT2*5B* yields slow acetylator phenotype by enhanced protein degradation [58], whereas the G857A (G286E) polymorphism in *NAT2*7B* yields slow acetylator phenotype through altered affinity for some but not all substrates and acetyl coenzyme A cofactor [48]. Interestingly, G857A (G286E) has been shown to decrease apparent Km towards N-hydroxy-ABP from 108 to 50 μM [54]. Since single nucleotide polymorphisms effect changes via variant mechanisms, it follows that there may be heterogeneity within the “slow” acetylator phenotype [59] and such observations have appeared in the scientific literature. For example, isoniazid -induced hepatotoxicity is highest in “super slow” acetylators [60,61]. Individuals with slow acetylator NAT2 phenotype experienced more severe hepatotoxicity than rapid acetylators and less risk was observed in individuals possessing *NAT2*7* haplotypes [62]. English chemical dye workers with documented exposure to aromatic amine carcinogens showed a striking association between urinary bladder cancer and slow acetylator phenotypes [63]. The NAT2 phenotype data was not separated into two phenotypes (rapid and slow), but rather into eight phenotypes of metabolic ratios. Five of these ratios correspond to rapid acetylators, and the other three correspond to various degrees of slow acetylator phenotype. As reviewed previously [64], urinary bladder cancer risk increased as NAT2 metabolic ratio (phenotype) decreased and the risk was markedly increased in the “slowest” NAT2 phenotype. Four studies found that urinary bladder cancer risk was highest in individuals possessing *NAT2*5* haplotypes [65–68]. *NAT2*5* alleles also were associated with increased risk for breast cancer in women smokers [69–70]. These results suggest that NAT2 slow acetylator phenotype is not homogeneous, but rather that multiple slow acetylator phenotypes exist resulting from different mechanisms inferred by various SNPs and haplotypes.

An increased understanding of NAT2 genotype/phenotype relationships should lead to improved individualized risk assessments to disease and/or toxicities associated with carcinogen exposures. Lack of replication regarding the role of NAT2 genotype or phenotype on cancer risk following arylamine exposures may lead many to conclude that NAT2 genetic polymorphisms are not sufficiently biologically relevant to merit attention. The results of the present study suggest that the investigations of NAT2 genotype or phenotype associations with disease or toxicity could be more precise and reproducible if heterogeneity within the “slow” NAT2 acetylator phenotype is considered and incorporated into the study design.

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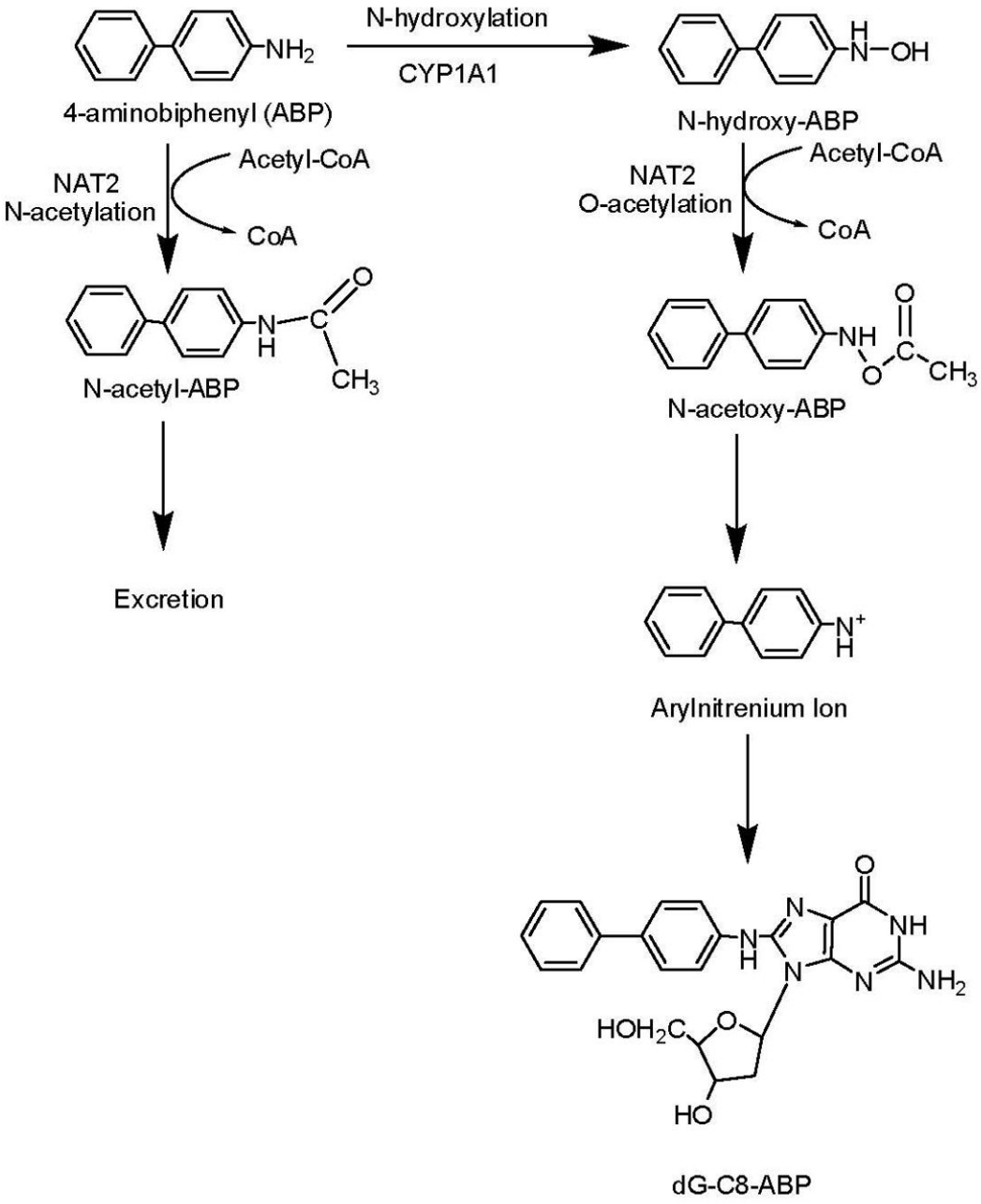


Fig. 1.
Metabolic pathways for 4-aminobiphenyl.

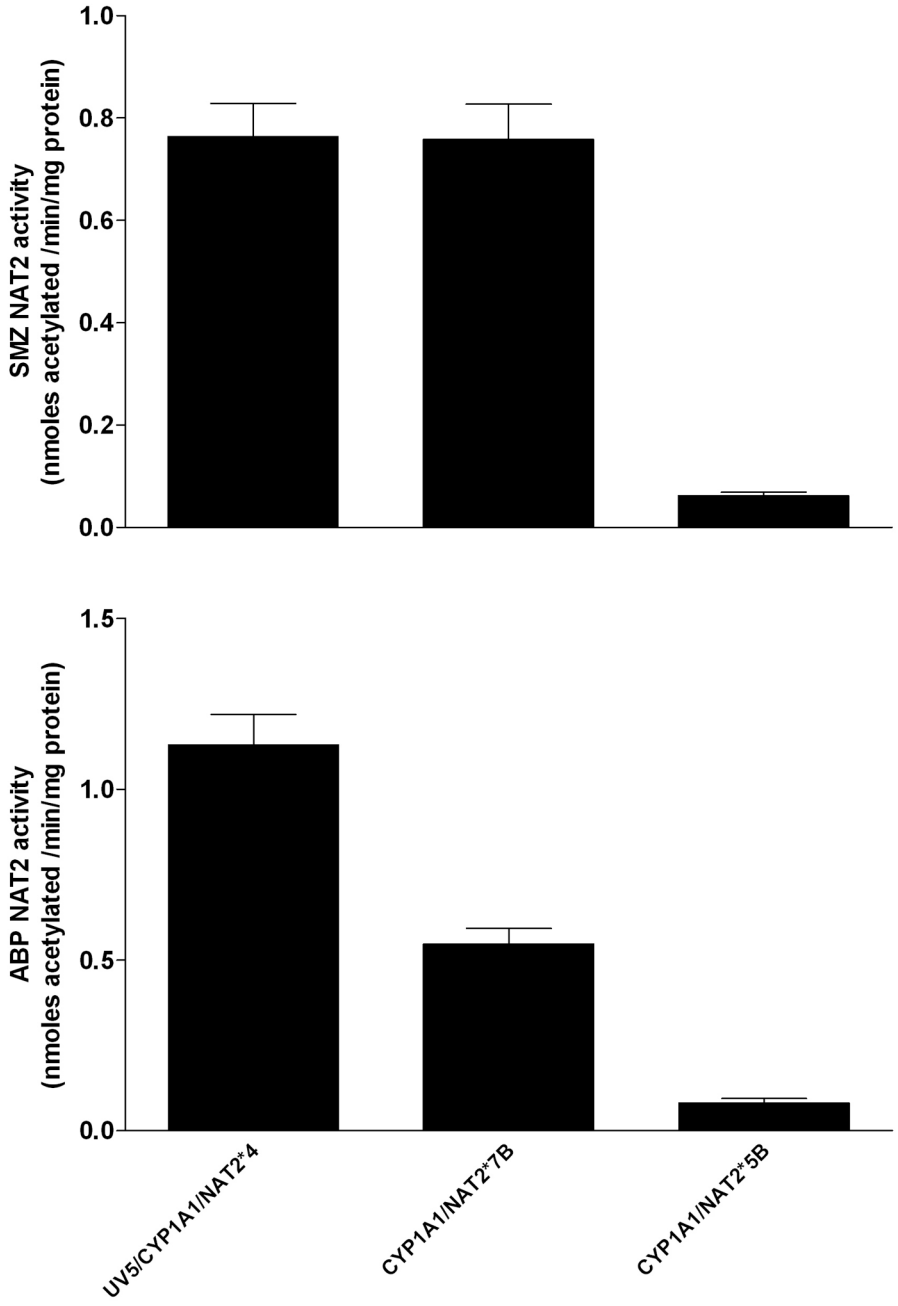
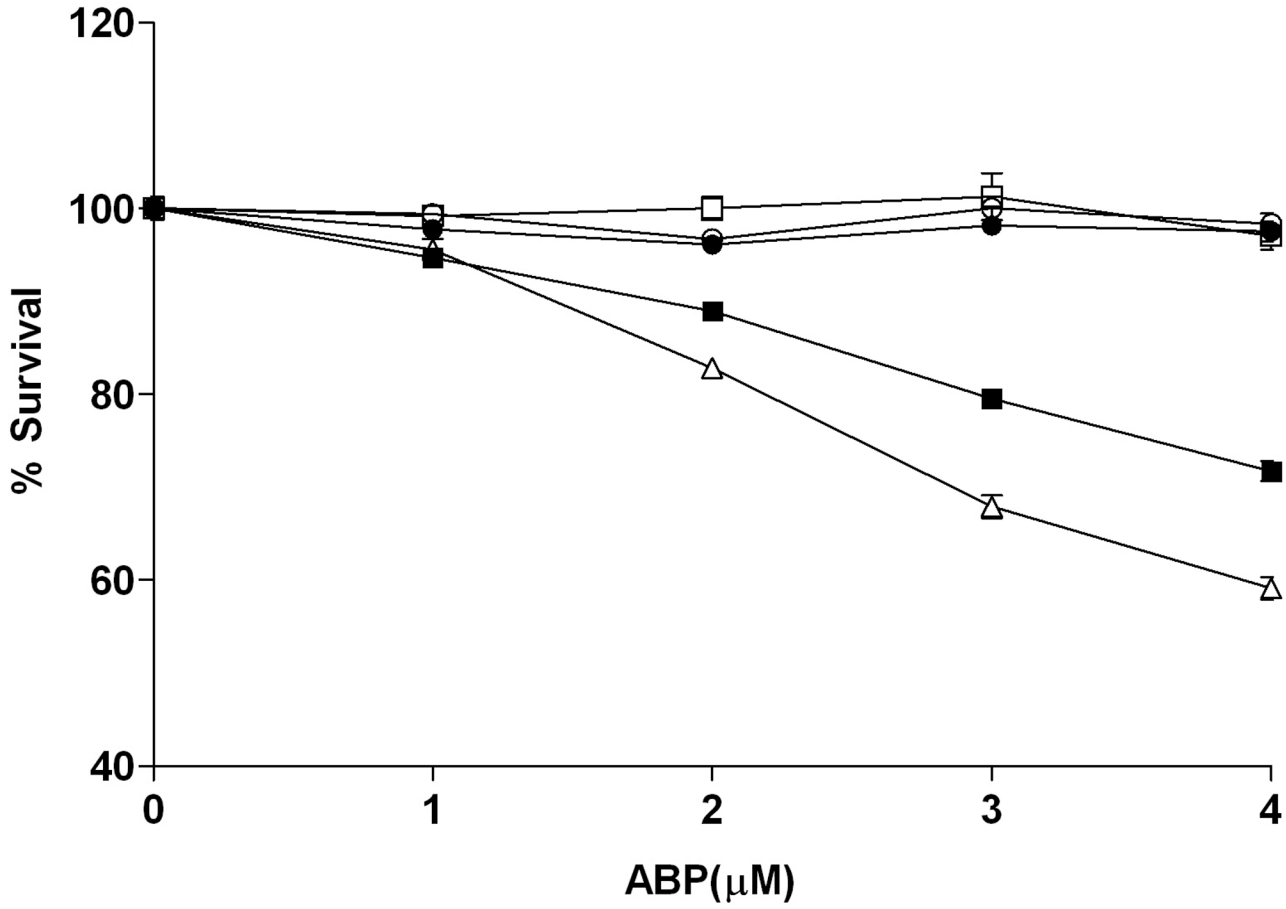


Fig. 2.

NAT2 catalytic activities in cell lysates of *CYP1A1/NAT2*4*, *CYP1A1/NAT2*7B*- and *CYP1A1/NAT2*5B*-transfected CHO cells. Each bar represents Mean ± S.E.M. for three determinations. NAT2 activities in the UV5 and UV5/CYP1A1 cell lines were not detected (<20 pmole/min/mg). Sulfamethazine (SMZ) NAT2 catalytic activities (top) were significantly lower ($p<0.001$) in the *CYP1A1/NAT2*5B*- transfected cell line. ABP NAT2 catalytic activities (bottom) differed significantly ($p<0.01$) between all NAT2-transfected cell lines. ABP NAT2 activity was not detected in the UV5 and UV5/CYP1A1 cell lines (<10 pmol/min/mg protein).



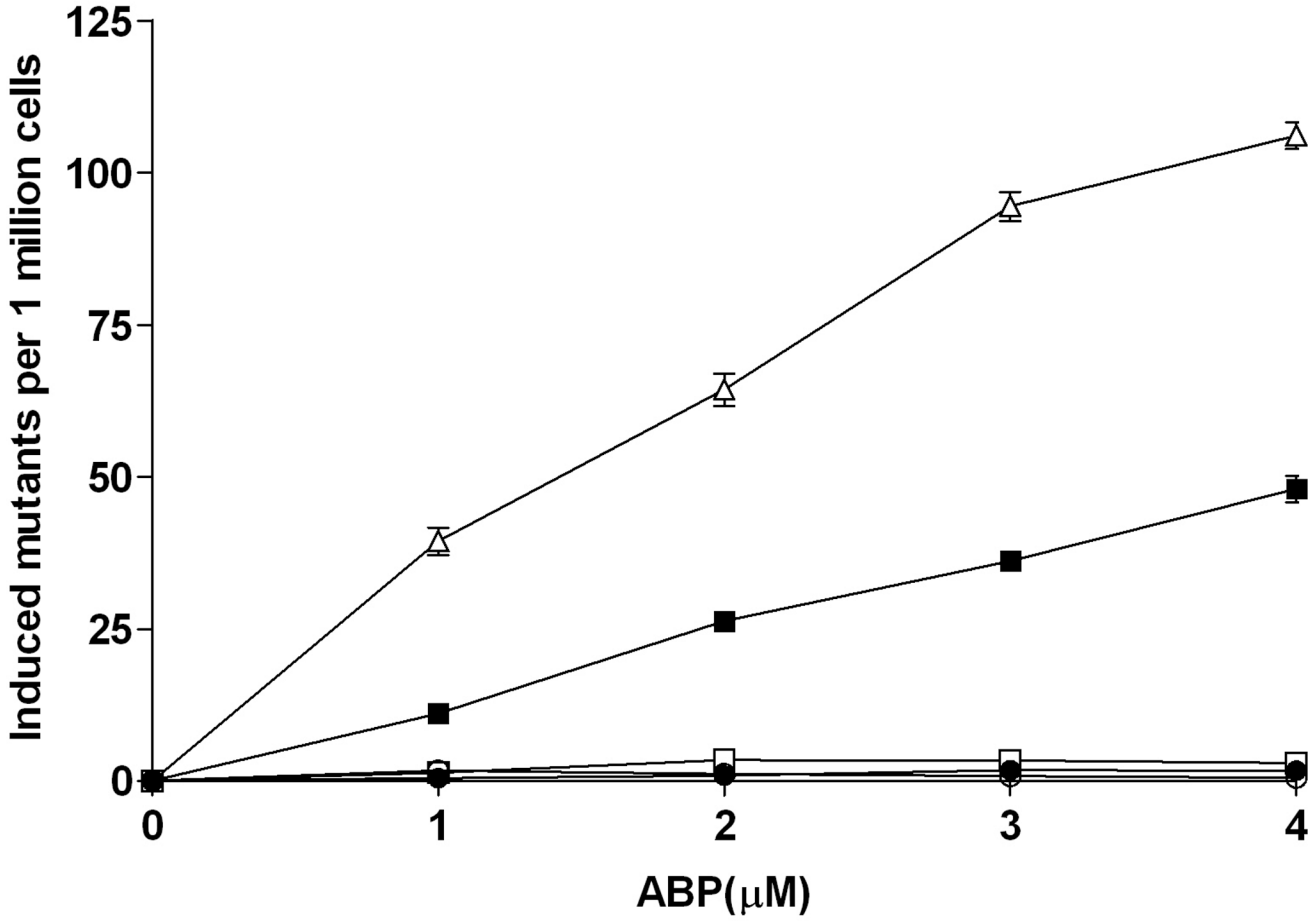


Fig. 3.

A: Cell survival in UV5 CHO cell lines following treatment with ABP. Percent survival on the ordinate is plotted versus ABP treatment concentration on the abscissa. Each data point represents Mean ± S.E.M. for three to six experiments in UV5 (open circles), UV5/CYP1A1 (closed circles), UV5/CYP1A1/NAT2*5B (open squares), UV5/CYP1A1/NAT2*7B (closed squares), and UV5/CYP1A1/NAT2*4 (open triangles) cell lines. Cell survival differed significantly ($p<0.001$) in the order UV5/CYP1A1/NAT*4 < UV5/CYP1A1/NAT2*7B < UV5, UV5/CYP1A1, and UV5/CYP1A1/NAT2*5B following 2–4 μM ABP treatment.

B: ABP -induced *hprt* mutants in UV5 CHO cell lines. ABP-induced *hprt* mutants are plotted on the ordinate versus treatment concentration on the abscissa. Each data point represents Mean ± S.E.M. for two to five experiments in UV5 (open circles), UV5/CYP1A1 (closed circles), UV5/CYP1A1/NAT2*5B (open squares), UV5/CYP1A1/NAT2*7B (closed squares), and UV5/CYP1A1/NAT2*4 (open triangles) cell lines. ABP -induced *hprt* mutants differed significantly in the order UV5/CYP1A1/NAT*4 > UV5/CYP1A1/NAT2*7B > UV5, UV5/CYP1A1, and UV5/CYP1A1/NAT2*5B following 1 μM ($p<0.05$) and 2–4 μM ($p<0.001$) ABP treatment.

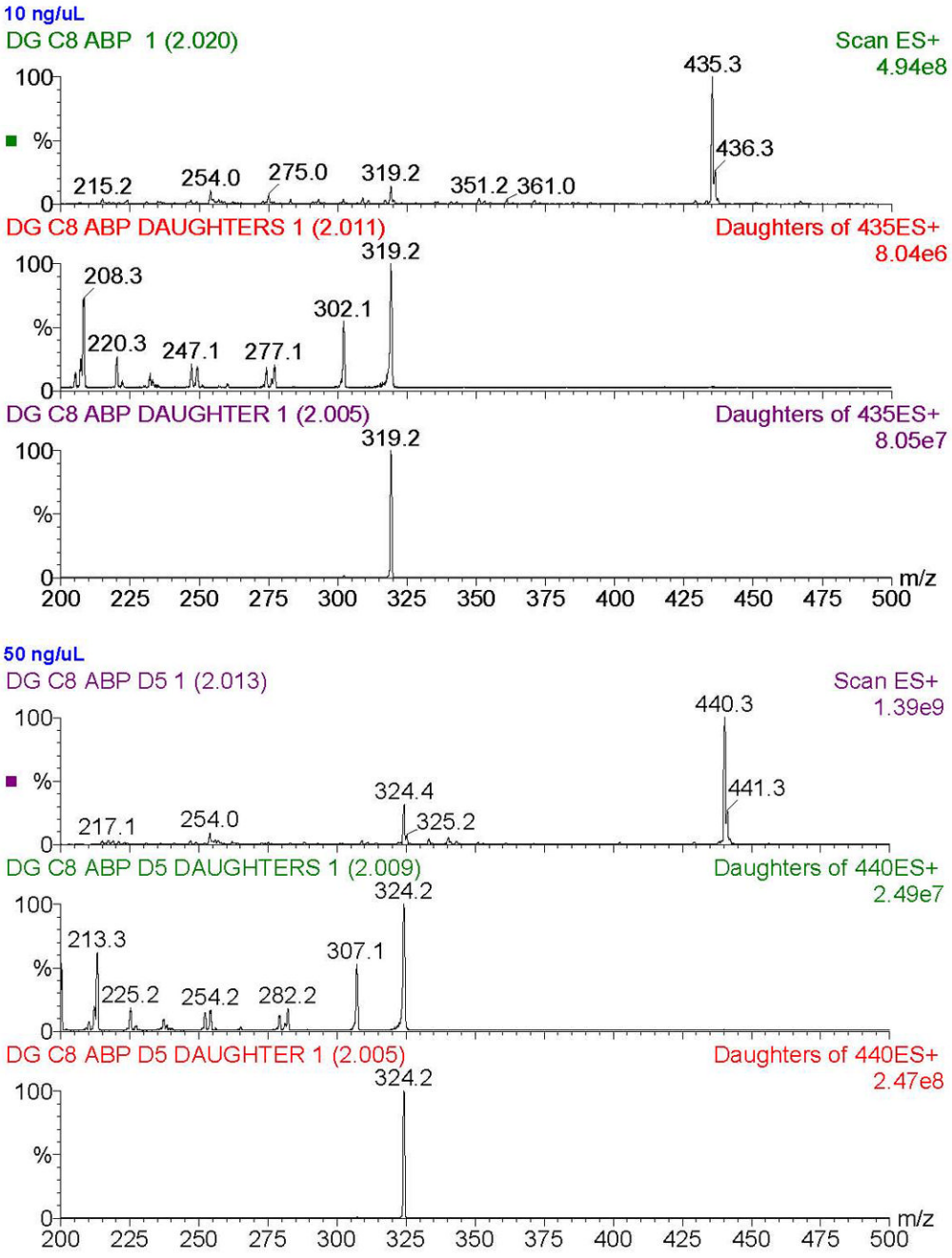


Fig. 4.

Electrospray ionization spectra of dG-C8-ABP and dG-C8-ABP-D5. Collision induced dissociation fragmentation of dG-C8-ABP and dG-C8-ABP-D5 at −50 V collision energy (middle panels) shows fragmentation of the ABP and guanosine moieties dominated by the major fragment produced from loss of deoxyribose: the aglycone ion of *m/z* 319 or *m/z* 324 for dG-C8-ABP and dG-C8-ABP-D5, respectively. The collision energy was −25 V for multiple reaction monitoring transitions used during quantitation (lower panels).

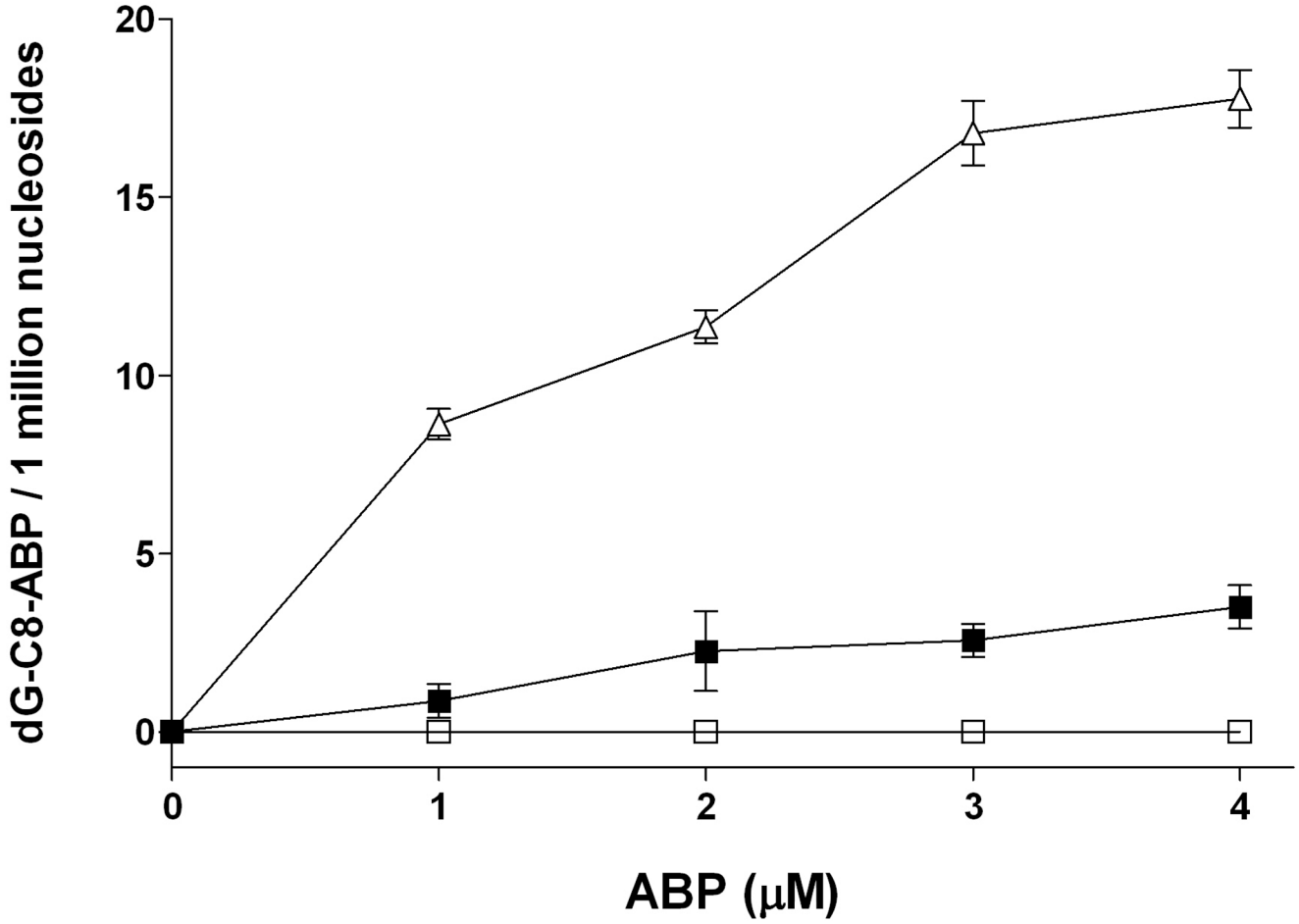


Fig. 5.

ABP -induced dG-C8-ABP adduct levels in UV5 CHO cell lines. Adduct levels are plotted on the ordinate versus ABP treatment concentration on the abscissa. Each data point represents Mean ± S.E.M. for three experiments (the S.E.M. sometimes falls within the symbol) in UV5/CYP1A1/NAT2*5B (open squares), UV5/CYP1A1/NAT2*7B (closed squares), and UV5/CYP1A1/NAT2*4 (open triangles) cell lines. dG-C8-ABP DNA adduct levels were not detected in UV5 CHO cell lines, nor in *CYP1A1*- or *CYP1A1/NAT2*5B*- transfected cell lines (data not shown). dG-C8-ABP DNA adduct levels were significantly ($p<0.001$) higher in UV5/CYP1A1/NAT2*4 than UV5/CYP1A1/NAT2*7B cell lines at each ABP treatment concentration.