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Functional effects of CYP1A2, NAT1, and NAT2 genetic variants in nucleotide excision repair-deficient human fibroblasts : implications for toxicological risk from environmental arylamines.

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FUNCTIONAL EFFECTS OF CYP1A2, NAT1, AND NAT2 GENETIC VARIANTS IN NUCLEOTIDE EXCISION REPAIR - DEFECIENT HUMAN FIBROBLASTS: IMPLICATIONS FOR TOXICOLOGICAL RISK FROM ENVIRONMENTAL ARYLAMINES

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M.S., Delaware State University, 2008

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DEDICATION

This thesis is dedicated to my parents Mr. Johnny L. Leggett and Mrs. Connie M. Leggett whose prayers and words of encouragement blessed me with the tenacity to persevere despite obstacles and disappointments.

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ABSTRACT

FUNCTIONAL EFFECTS OF CYP1A2, NAT1, AND NAT2 GENETIC VARIANTS IN NUCLEOTIDE EXCISION REPAIR - DEFECIENT HUMAN FIBROBLASTS: IMPLICATIONS FOR TOXICOLOGICAL RISK FROM ENVIRONMENTAL ARYLAMINES

Carmine S. Leggett

August 6, 2010

Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) catalyze the detoxification and/or activation of aromatic and heterocyclic amine carcinogens by two pathways. This metabolism reaction can lead to the detoxification by N-acetylation, or bioactivation by O-acetylation often preceded by CYP450 hydroxylation. Human NAT2 polymorphisms are characterized by rapid, intermediate, and slow acetylator phenotypes, thus resulting in differences in the rate of arylamine metabolism and consequently cancer risk. We have constructed nucleotide excision repair-deficient human cell model expressing human CYP1A2 and human NAT1and NAT2 in order to investigate carcinogen metabolism and cancer susceptibility in human cells.

In this study we introduce the utilization of SV40-transformed human fibroblasts (GM4429) to examine the functional effects of human NAT2 haplotypes, in the presence of human NAT1. Many carcinogens, such as 4-aminobiphenyl, are metabolized by both NAT1 and NAT2, consequently both isozymes have toxicologically significant functions in the metabolism of

arylamines. Furthermore, both NAT1 and NAT2 isozymes are expressed in human liver, where they are significant to carcinogen detoxification and/or activation consequences. With this model, we can examine the role of NAT1 and NAT2 carcinogen metabolism and genotoxicity in a NER-deficient human cell.

In this study, we investigate novel inhibitors of NAT1 and NAT2. Previous studies have identified approximately 150 NAT inhibitor candidates following computer-based *in silico* screening of approximately 20 million compounds. The inhibitory properties of these compounds were tested using a High Performance Liquid Chromatography assay specific for human NAT1 and NAT2. A novel compound was identified that is an effective inhibitor of NAT1 (100%) and NAT2 (90%). This inhibitor was selective for NAT1 (IC_{50} about 1 μM), compared to an IC_{50} of 82.2μM (SMZ) for NAT2. Further studies will determine potency and efficacy of NAT inhibitors to reduce DNA adduct formation and mutagenesis. We have also successfully constructed and characterized NER-deficient human fibroblast cells with stable expression of human NAT2 alleles (*NAT2*4, NAT2*5B,* or *NAT 2*7B*) and CYP1A2. These models are used to better understand the effects of NAT2 haplotypes on carcinogen metabolism and DNA adduct formation in human cells.

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CHAPTER I

INTRODUCTION

Susceptibility to chronic diseases, such as cancer, involves interactions between genetic variants of xenobiotic-metabolizing enzymes (XMEs) and the environment. However the effects of genetic variance on toxicological risk are not fully understood. Many environmental arylamine genotoxins are metabolized by arylamine N-acetyltransferases (NATs). The two isozymes, arylamine N-acetyltransferases 1 (NAT1) and 2 (NAT2), catalyze the detoxification and/or activation of aromatic and heterocyclic amines (Hein et al., 2000). These metabolic pathways can lead to the detoxification by N-acetylation, or bioactivation by O-acetylation, preceded by cytochrome P450 hydroxylation (Agudo et al 2009). The arylamine acetoxy products are capable of forming electrophilic species that covalently bind to DNA and proteins, thus causing DNA adduct formation (Figure 1.) and other toxicological insults.

NAT polymorphisms were first discovered after differences in toxicity were seen in patients treated with isoniazid for tuberculosis (Hein et al., 2000). These differences were attributed to single nucleotide polymorphisms (SNPs) in the NAT2 gene. Human NAT2 polymorphisms are characterized by rapid, intermediate, and slow acetylator phenotypes, thus resulting in differences in the rate of arylamine metabolism and consequently toxicological risk (Hein, 2009).

This family of enzymes has been studied for their functional roles in pharmacogenetics and toxicological susceptibility.

NAT1 and NAT2 genotypes may play an important role in cancer risk (Hein et al., 2000). Previous studies in our laboratory investigated the effects of NAT2 genetic variants on 2-amino-1-methyl-6-phenylimidazo[4,5b]pyridine (PhIP) induced DNA adduct formation and mutagenesis in nucleotide excision repair (NER) -deficient Chinese hamster ovary (CHO) cells that recombinantly express human CYP1A2 and human NAT2*4 or NAT2*5B alleles (Metry et al., 2007). This study provides evidence in support of the ability to study mutagenesis and DNA damage in a cell line stably transfected with human NAT2 and human CYP1A2. Our study will investigate the functional effects of altered enzymatic activity in CYP1A2 and NAT1 and genetic variants in the NAT2 gene on oxidative stress, DNA adduct formation and mutagenesis by exploiting the use of NER deficient Simian Virus 40 (SV40) - transformed human fibroblast (GM4429).

The utilization of SV40-transformed human fibroblasts to examine the functional effects of human NAT2 haplotypes in the presence of human NAT1 is a novel concept. Human fibroblast endogenously express NAT1, unlike any other cell model previously used. These cells have a deficiency in NER which inhibits the repair of bulky DNA lesions induced by UV or aromatic and heterocyclic amine genotoxins. The ability of this cell line to form bulky DNA lesions is pertinent to our study. GM4429 cells were derived from a Caucasian Xeroderma Pigmentosum (XP) patient exhibiting a delayed onset of neurological disease. These cells are widely used to study NER deficiency due to their variant

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complementation group A (XP-A) (States et al., 1996). Simian Virus 40, an oncogenic DNA virus, induces malignant transformation and cause a loss of cell cycle checkpoint control (States et al., 1996). The large T-antigen of the SV40 virus accomplishes abrogation of the cell cycle checkpoint by sequestering p53 which stops both G1 and G2 checkpoints (States et al., 2002). The use of this cell line to study NAT metabolism and genetic variance is an innovative and exciting addition to the previously used Chinese hamster ovary cell (Metry et al, 2007), COS-1 cell (Zang et al., 2007), yeast (Fretland et al., 2001), and *E. Coli* (Hein et al., 1994) models. We are able to utilize this cell line to study functional characterization of human CYP1A2, human NAT1 and human NAT2 phenotypic behavior in cells expressing these enzymes and genetic variants which is important to better understand the relationship between risk and genetic variance. Studies have indicated that synergistic effects caused by gene-gene interactions have been noted in increased risk to certain cancers (Cascorbi et al., 2001, Sanderson et al., 2007, and Taylor et al., 1998), further illustrating that the use of these cell lines expressing both human NAT isozymes and human CYP1A2 is beneficial for functional genomic studies of NAT and cancer risk. We believe the NER-deficient SV40-transformed human fibroblast cell lines will advance the study of NAT2 genetic variation and its effect on toxicological risk.

Humans are exposed to many aromatic and heterocyclic amine environmental genotoxins that pose a risk upon metabolism. These risks include, but are not limited to, oxidative DNA damage, DNA adduct formation, and mutagenesis. NAT enzymes are recognized for their ability to metabolize

carcinogens and arylamine genotoxins such as 4-aminobiphenyl (ABP), 2-aminofluorene (AF), 2,6-dimethylaniline (2,6-DMA), 3,5-dimethylaniline (3,5-DMA), and 3-ethylaniline (3-EA). Carcinogens ABP and AF are metabolized by both NATs, illustrating that both NAT1 and NAT2 have an important role in the metabolism of arylamines (Grant et al., 1990). Exposure to ABP, which is present in tobacco smoke, has been associated with bladder cancer risk (Liu et al., 2008). AF, whose N-acetylated derivative was developed as an insecticide, is associated with mutagenesis (Sugamori et al., 2006). Although some arylamine substrates such as ABP and AF are metabolized by both NAT1 and NAT2, each of the NATs exhibit specificity (Liu et al., 2007). Studies show that when the relative capacities of human NAT1 and NAT2 to catalyze the metabolic activation of N-hydroxy-2-aminofluorene and N-hydroxy-4-aminobiphenyl were assessed, the rates were higher for NAT1 than NAT2 with both substrates (Hein et al., 1993). Capacities of human NAT1 to catalyze the metabolic activation of N-hydroxy-2-acetylaminofluorene and N-hydroxy-4-acetylaminobiphenyl via N,O-acetylation were lower than the metabolic rates of N-hydroxy-2-aminofluorene and N-hydroxy- 4-aminobiphenyl via O- acetylation (Hein et al., 1993).

Arylamine genotoxins 2,6-DMA and 3,5-DMA are present in cigarette smoke and pollution from industrial sources. The alkylanilines, 2,6-DMA ,3,5-DMA, and 3-EA, are associated with bladder cancer among the general population including non-smokers (Gan et al., 2004). Exposure to these alkylanilines (monocyclic arylamines) is of particular interest to toxicological risk studies because of the high distribution of these compounds in the environment. Monocyclic arylamines

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2,6-DMA , 3,5-DMA, and 3-EA are distributed widely in the environment, resulting in ubiquitous exposure (Skipper et al., 2010). Since these compounds are present in cigarette smoke, indoor spaces contaminated with cigarette smoke exhibit higher levels of alkylanilines than in uncontaminated spaces. Industrial sources also provide significant levels of exposure to these compounds (Skipper et al., 2010). It is important to note that in previous studies conducted in Italy, 2,6-DMA levels were higher among non-smokers than smokers warranting the conclusion that the environmental significance of monocyclic arylamine exposure is extensive and is not confined to groups such as active smokers or those subjected to occupational exposure (Skipper et al.,2010). Studies have indicated that diverse arylamine exposures are strongly associated with bladder cancer risk among nonsmokers (Gan et al., 2004). This suggests that environmental exposure is as important as active smoking exposure. The 5 arylamine compounds used in this study were chosen because of their possible impact on toxicological risk by NAT metabolism.

It is thought that monocyclic arylamines, to which humans are exposed, impose toxicological risk upon bioactivation by NATs. The activation of these compounds by metabolic hydroxylation by CYP and/or O-acetylation by NAT leads to the production of toxic N-arylhydroxyamines and N-acetoxyarylamines (Liu et al., 2007). These compounds are also N-acetylated by NAT which is considered the detoxification reaction. The N-acetylation reaction decreases substrate availability for metabolic hydroxylation of the primary amino group (Liu et al., 2007).

NAT1 and NAT2 have 87% nucleotide homology in the coding region, and despite their high protein homology, they differ in substrate specificity (Butcher et al., 1999). The substrate affinity for NAT1 or NAT2 will play a major role in arylamine metabolism and toxicological risk, therefore the affinity of the above mentioned arylamine genotoxins for NAT1 or NAT2 will be determined. They also exhibit differences in tissue distribution. Human NAT2 is expressed mainly in the liver and intestine, while human NAT1 has a widespread tissue distribution in adult tissues, including the liver (Russell et al., 2009). Moreover, they are both significant to carcinogen detoxification and/or activation consequences.

Oxidative DNA damage is an important toxicological response to arylamine genotoxin metabolism (Murata et al., 2001). Its implications have been noted in teratogenesis and neurodevelopmental deficits (Wells et al., 2010), pathological eye conditions, such as age dependent cataract formation (Dairou et al., 2005), chronic hepatitis, Fanconi's anemia, diabetes mellitus and *Helicobacter pylori* infections (Lodovici et al., 2000). Furthermore oxidative DNA damage is known as a risk factor for many diseases including many cancers such as breast cancer (Sova et al., 2010), renal cell carcinoma and squamous cell carcinoma (Tanaka et al., 2000). Reactive oxygen species (ROS) are produced by the metabolism of environmental arylamine genotoxins such as chemical carcinogens and environmental agents (Kim et al., 2003). ROS can lead to deleterious and extensive DNA damage including single strand breaks and DNA adduct formation. Modified bases such as 8-hydroxy-deoxyguanosine (8-OH-

dG) can be used as a marker to evaluate DNA damage caused by oxidative stress (Kim et al., 2003).

Oxidative stress has also been noted for its ability to regulate NAT1 activity. Reactive oxygen species, such as H_2O_2 at physiological concentrations, can reversibly inactivate the NAT1 enzyme due to oxidation of the catalytic cysteine residue in the active site (Rodrigues – Lima et al., 2004). This change in NAT1 enzymatic activity and function results in differences in potential toxicological consequences, further demonstrating the importance of understanding the relationship between oxidative damage following NAT metabolism and toxicological response. In this study we will utilize cell lines that express NAT2 alleles (*NAT2*4*, *NAT2*5B*, and *NAT2*7B*) and combinations of shRNA-lowered NAT1 and CYP1A2 enzymatic activity to assess how these changes affect levels of oxidative stress, DNA adduct formation, and mutagenesis following exposure of human cells to arylamines.

In order to elucidate ways to reduce or ablate toxicological risk caused by arylamine genotoxin metabolism, we will employ the use of a small molecule inhibitor. Studies provide evidence that NAT inhibitors have been beneficial in investigating the catalytic mechanism of NATs and are emerging as drug targets (Russell et al., 2009). Other studies explore NAT inhibitors as a means to alter biotransformation by NAT, consequently providing potential differences in toxicological consequences (Dairou et al., 2009). Our study introduces the use of novel and effective inhibitors of NAT1 to reduce levels of DNA adduct formation and mutagenesis. The selectivity of NAT1 inhibitors may be explained

by the NAT1 catalytic pocket comparison to that of NAT2. Both NATs possess a functional Cys-His-Asp catalytic triad that allows the transfer of an acetyl group for acetyl-coenzyme A in a ping-pong bi-bi reaction mechanism followed by acetyl group transfer to a substrate (Hein, 2009). The NAT1 catalytic pocket is 40% smaller than that of NAT2; this alludes to catalytic pocket size influence on substrate specificity. Substrate selectivity is also strongly influenced by three key active site loop residues F125, Y127, and R129, determined by site-directed mutagenesis of individual amino acids (Goodfellow et al., 2000).

There have been many reported associations between NAT1 genetic polymorphisms and increased risk of carcinogenesis by molecular epidemiological studies. NAT1 polymorphisms have been associated with individual risk to urinary bladder, colorectal, breast, lung, prostate, and pancreatic cancer (Walraven et al., 2008). The close association between NAT1 polymorphisms and risk for so many diseases indicates the potential clinical benefits of NAT1 inhibition. For example, in comparison with all other NAT1 genotypes the putative rapid acetylator genotype (*NAT1*10*) was significantly higher in prostate cancer cases than in controls (Hein et al., 2002). NAT1, which is present in most extrahepatic tissues, facilitates the activation of many carcinogens and arylamines by *O*-acetylation associating NAT1 with certain cancers (Hein, 2002). Taken together, this suggests that inhibition of NAT1 may be beneficial in reducing toxicological risk resulting from the activation of carcinogens and genotoxins by NAT1 *O*-acetylation.

The human NAT1 and NAT2 isozymes are similar in structure; therefore inhibition of NAT2 may be of concern considering the ability of NAT2 to metabolize many pharmaceutical agents. One may suspect that inhibition of NAT2 may lead to drug toxicity because NAT2 will be unable to facilitate the detoxification of its pharmaceutical targets. To address this concern, if the selective inhibitor is used as a drug, we are proposing overcoming this caveat by the use of site-directed administration of the potential inhibitor to extrahepatic target organs where NAT2 is not expressed. This will allow us to not only validate a NAT1 inhibitor as a tool to study NAT1 mechanistically, but to validate the potential use of the inhibitor as a drug to reduce NAT1 associated risk. Since NATs activate arylamine genotoxins to induce oxidative stress, DNA adduct formation, and mutagenesis, we will explore applications to reduce this risk with the use of a NAT1 inhibitor.

CHAPTER II

SPECIFIC AIMS

The study of genetic variation in xenobiotic-metabolizing enzymes, such as arylamine N-acetyltransferases (NATs) and cytochrome P450s (CYPs) bestows an essential contribution to the understanding of consequences resulting from toxicological insult (e.g. oxidative stress, DNA adducts formation and mutagenesis). Functional genomic studies help to better understand relationships between genetic variation and toxicological risk. *It is our objective to study the effects of NAT2 genetic variants and altered enzymatic activity in xenobiotic-metabolizing enzymes (NAT1 and CYP) on oxidative stress, DNA adduct formation, and mutagenesis, by exploiting the use of nucleotide excision repair (NER) deficient SV40-transformed human fibroblast cells. We hypothesize that xenobiotic-metabolizing enzyme phenotypes (i.e., rapid or slow) associated with allelic variants (e.g. NAT2*4, NAT2*5B, NAT2*7B) will impact levels of oxidative stress, DNA adduct formation, and mutagenesis.* To test this hypothesis, we propose the following specific aims:

Aim 1: To construct and to characterize NER-deficient SV40-tranformed human fibroblast cell lines that recombinantly express human CYP1A2 and human NAT2 alleles *NAT2*4, NAT2*5B,* or *NAT2*7B. We hypothesize that alleles NAT2*5B and NAT2*7B will exhibit differences in arylamine metabolism as compared to the reference allele NAT2*4.* Previous studies successfully use a NER-deficient

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Chinese hamster ovary (CHO) cell model expressing human CYP1A2 and human NAT2 to investigate the effects of NAT2 genetic variants on carcinogen induced DNA adduct formation (Metry et al., 2007). In this study we introduce the utilization of SV40-transformed human fibroblasts (GM4429) to examine the functional effects of human NAT2 haplotypes on arylamine metabolism and toxicological risk. The construction of these cell lines will be accomplished using the Flp-In System (Invitrogen, Carlsbad, CA). We will complete the characterization by including the use of functional assays to evaluate CYP activity (ethoxyresorufin-o-deethylase (EROD) assay), NAT1 protein expression (Western blot assay), N- and O- acetylation activity *in vitro* (high-performance liquid chromatography *(HPLC); substrates:* p-aminobenzoic acid (PABA), Sulfamethazine (SMZ), 4-aminobiphenyl (ABP) , 2,6-dimethylaniline (2,6-DMA), 3,5-dimethylaniline (3,5-DMA), and 3-ethylaniline (3-EA), N-acetylation activity *in situ*, and NAT mRNA expression (Taqman assay). In addition, the genotype of NAT1 (Taqman assay) and CYP1A2 (sequencing) will be determined. We will also determine the affinity of arylamine genotoxins for NAT1 and/or NAT2 using kinetic studies (K_m, V_{max}) for each arylamine listed above.

Aim 2: To evaluate and to compare the effects of combinations of genetic variants in NAT2 (i.e. rapid or slow phenotypes) and high and low NAT1 and CYP1A2 enzymatic activities on oxidative stress, DNA adduct formation, and mutagenesis. *We hypothesize that cell lines with combinations of altered enzymatic activity associated with slow acetylator (NAT) and rapid hydroxylation (CYP) will present an increased incidence of oxidative DNA damage, while cell*

lines with rapid acetylator (NAT) and slow hydroxylation (CYP) will exhibit less oxidative DNA damage, but increased incidences of DNA adduct formation and mutagenesis. It has been reported that the N-hydroxy derivatives of arylamine genotoxins have the ability to cause oxidative stress (Murata et al., 2001). We will investigate oxidative stress in cell lines constructed in Aim 1, by measuring oxidized DNA, 8-hydroxy-2-deoxyguanosine (8-OH-dG), by Enzyme Linked Immunosorbent Assay (ELISA; Cayman Chemicals). We will utilize shRNA plasmids (SABiosciences) to achieve combinations of high or low enzymatic activity by significant gene knockdown of endogenous human NAT1 and/or stably transfected human CYP1A2. As noted in Aim 1, human NAT2 (NAT2*4, NAT2*5B, and NAT2*7B) will be stably transfected in these cells allowing the achievement of combinations with rapid and slow NAT2 phenotypes. Enzymatic activity will be measured using functional assays specific for NAT1 and NAT2 (HPLC) and CYP1A2 (EROD assay) to validate phenotypic behavior after shRNA knockdown. We will measure DNA adduct formation by HPLC–tandem mass spectrometry (LC-MS-MS) technology and mutagenesis by assays for mutagenesis at the hypoxanthine-guanine phosphoribosyltransferase (hrpt) locus.

Aim 3: To determine the effectiveness of a novel arylamine NAT1 small molecule inhibitors on reducing mutagenesis and DNA adduct formation. We hypothesize that the inhibition of NAT1 enzymatic activity will reduce mutagenesis and DNA adduct formation, but increase oxidative DNA damage. The inhibitory properties of compounds were tested using an enzymatic assay (HPLC) specific for human

NAT1 and NAT2. A compound was identified that is an effective inhibitor of NAT1. This inhibitor was also selective for NAT1. This study will determine potency and efficacy of this compound to reduce mutagenesis and DNA adduct formation in NER SV40-transformed human fibroblasts treated with compounds that have an affinity for NAT1. Additional compounds are currently being screened for their ability to inhibit NAT, after which their potency and efficacy to reduce toxicological risk will be evaluated.

CHAPTER III

METHODS AND MATERIALS

Aim 1: To construct and characterize NER-deficient SV40-tranformed human fibroblast cell lines that recombinantly express human CYP1A2 and human NAT2 alleles *NAT2*4*, *NAT2*5B*, or *NAT2*7B*. *We hypothesize that alleles NAT2*5B and NAT2*7B will exhibit differences in arylamine metabolism as compared to the reference allele NAT2*4.*

Stable Transfection and Cell Culture

We will construct stably transfected NER-deficient SV40-transformed human fibroblasts that express human CYP1A2 and NAT2 alleles (*NAT2*4*, *NAT2*5B*, or *NAT2*7B*) using the Flp-In system (Invitrogen) as described previously in CHO cells by Metry et al., (2007). NER-deficient SV40-transformed human fibroblasts (GM4429) were chosen because they endogenously express NAT1, they are immortalized by the SV40 oncogenic virus, and they poorly repair arylamine-induced DNA adducts. The pFRT/*lacZeo* plasmid will be transfected into the NER-deficient GM4429 cell, resulting in a single integrated FRT site. Cells will be split 24 hr after transfection and treated with medium supplemented with Zeocin (200 μg/mL) 48 hr after transfection, for selection according to the manufacturer's protocol. Zeocin – resistant clones will be picked with cloning cylinders. The cell lines with a single intergrated FRT site determined by real-

time polymerase chain reaction (PCR) and Southern blot screening will be selected for additional transfection. The pIRES plasmid containing cDNAs of human *CYP1A2* and NADPH-cytochrome P450 reductase gene, will be transfected into the newly constructed GM4429-FRT cell line using Effectene Transfecton Reagent kit (Qiagen). Geneticin -resistant clones will be picked using cloning cylinders. Human *NAT2* alleles (*NAT2*4*, *NAT2*5B*, or *NAT2*7B*) will be transfected using the pcDNA5/FRT plasmid and co-transfected with pOG44, a Flp recombinase expression plasmid, into GM4429-FRT-CYP1A2 cells (Figure 2.). Stably transfected cells will be selected by geneticin and hygromycin resistance. Cells will be grown in alpha-modified minimal essential medium (αMEM) supplemented with L-glutamine, 10% fetal bovine serum, 100 units/mL of penicillin, and 100 units/mL streptomycin; incubated at 37°C in 5% CO_2. Medium will be supplemented with selective agents (hygromycin and geneticin) to ensure maintenance of the stable transfection.

Cytochrome P450 (CYP1A2) Activity

Different clones will express different levels of CYP1A2 catalytic activity. To ensure that all cell lines have comparable CYP1A2 enzymatic activity whole cell samples will be assayed for 7-ethoxyresorufin oxidation activity by fluorimetric determination as described by Bendaly et al., (2007). This reaction will be conducted in a 96-well plate, containing 1 million cells in 10 µl of PBS with 5mM glucose and 5µM 7-ethoxyresorufin. The reaction will be incubated at 37°C for 10 minutes. Resorufin formation will be measured by fluorescence at emission

wavelength 585 nm and excitation wavelength of 530 nm. Cell lines from clones exhibiting 7-ethoxyresorufin oxidation activity comparable to the parent cell line (GM4429) will be utilized for further characterization and study.

NAT Protein Expression (Western Blot Assay)

NAT1 protein expression will be evaluated using western blot assays as described by Stanley et al., (1996). Approximately 20 µg of cell lysate will be mixed with 2x loading buffer, boiled for 5 min, and resolved by 12% SDS-PAGE gel and then transferred by semi-dry electroblotting to polyvinylidene fluoride (PVDF) membrane. The membrane will be probed with the primary polyclonal antibody ES195 (1:1000, provided by Edith Sim, University of Oxford, UK) and the HRP-conjugated secondary mouse anti-rabbit IgG antibody (1:10,000, provided by Pierce Protein Research Products, Rockford, IL.). ECL plus chemiluminescence will be used to detect the signal (Amersham Biosciences, Piscataway, NJ).

N-acetylation Activity (in vitro)

N-acetylation of PABA, SMZ, and selected arylamine genotoxins (2,6-DMA, 3,5-DMA, 3-EA) will be evaluated in vitro using reverse phase high performance liquid chromatography (HPLC) (Fretland et al., 2001). Reactions will be prepared with acetyl- coenzyme A (300µM), selective substrates (300µM) p-aminobenzoic acid (NAT1), sulphamethazine (NAT2), or selected arylamine genotoxins, and incubated at 37°C. Reactions will be stopped using 1M acetic acid and protein

centrifuged at 10,000 xg for 10 minutes. Reaction products in the resulting supernatant will be separated by reverse-phase HPLC. HPLC separation for sulphamethazine NAT2 assays will be achieved on a C18 LiChrospher® 125x4mm column using a gradient 91:9 sodium perchlorate (pH 2.5) / acetonitrile to 71:29 sodium perchlorate (pH 2.5) / acetonitrile over 5 min at 260 nm. HPLC separation for p-aminobenzoic acid NAT1 reactions will be achieved using a gradient 96:4 sodium perchlorate (pH 2.5)/ 88:11acetonitrile at 280nm. HPLC separation for 2,6-DMA, 3,5-DMA, and 3-EA will be achieved using a gradient 50:50 acetonitrile and 0.01M potassium phosphate buffer (pH 5) at 1 ml/min at 210 nm (Martin et al., 1996).

O-acetylation Activity (*in vitro*)

O-acetylation catalytic activity will be evaluated using reverse phase HPLC instrumentation as described above. Reactions will be carried out as described by Bendaly et al., (2007). Briefly, reaction mixtures containing cell lysate, 1 mg/ml deoxyguanosine, substrate (100 μM N-hydroxy-ABP) and (300 μM) acetyl coenzyme A will be incubated at 37°C and stopped with ethyl acetate. Reactions will be centrifuged for 10 minutes and the organic phase will be transferred, allowed to dry, and re-suspended in 100 μM of 10% acetonitrile. HPLC separation will be achieved using a gradient of 50:50 sodium perchlorate (pH 2.5)/ acetonitrile over 3 minutes to 80:20 sodium perchlorate (pH 2.5)/acetonitrile over 2 minutes at a wavelength of 300 nm. Measurements will be adjusted according to baseline measurements using lysates of GM4429/CYP1A2 cell line.

N-acetylation Activity (*in situ*)

N-acetylation activity *in situ* will measured in whole cell samples. Human fibroblast cell lines, grown to confluency, will be treated with medium supplemented with PABA, SMZ, ABP, AF, 2,6-DMA, 3,5-DMA, and 3-EA in varying doses including 10, 50, and 300 μM. Medium will be collected at various time points (2, 6,8,10 h). Existing protein will be precipitated out of solution using 1 mM acetic acid; the reaction products and substrates will be subjected to HPLC for separation. Measurement of acetylated PABA, SMZ, ABP, AF, 2,6-DMA, 3,5-DMA, and 3-EA will be carried out as described above.

Kinetic Studies

We will measure kinetic parameters K_m and V_{max} of acetyl CoA and arylamine genotoxins such as 2,6-dimethylaniline (2,6-DMA), 3, 5-dimethylaniline (3,5-DMA), and 3-ethylaniline (3-EA) for NAT1 or NAT2. We will determine acetyl CoA K_m and V_{max} with varying acetyl CoA concentrations (0- 400 μM) and fixed substrate concentrations (300 μM). The substrate K_m and V_{max} will be determined with varying substrate concentrations (0 – 1mM) and a fixed acetyl CoA concentration of 100 μM. The apparent K_m and V_{max} constants will be calculated using linear regression of Eadie-Hoftsee plots. These values (apparent K_m and V_{max}) will allow us to select arylamines for further study based on arylamine affinity for either NAT1 or NAT2.

NAT mRNA expression (Taqman Quantitative RT-PCR Assays)

TaqMan assays will be used to assess level of *NAT1 and NAT2* mRNA expressed in each cell line. TaqMan Universal Master Mix (Applied Biosystems, Foster City, California, USA), primers and probes, 96 well optical plates and caps will be used. Synthesis of first strand cDNA will be carried out using Superscript III ReverseTranscriptase (Invitrogen) following the manufacturer's protocol using 1 μg of DNase-treated total RNA. PCR with 1x final concentration of TaqMan Universal Master Mix, 300 nM of each primer and 100 nM of probe in 20 μl will be performed using Applied Biosystems StepOnePlus Real-Time PCR Systems (Applied Biosystems). For quantitative RT-PCR of *NAT1* (Husain et al, 2007a) and *NAT2* (Husain et al., 2007b), forward primer and reverse primer will be used with TaqMan probe.

NAT1 Genotyping

DNA will be extracted from human fibroblast cell samples using the "Qiagen DNA Purification kit" (Qiagen, Valencia. CA). NAT1 genotype will be determined as previously described (Doll and Hein, 2002). Briefly, *NAT1* amplification will be carried out in an Applied Biosystems StepOnePlus Real-Time PCR Systems with two initial hold steps (50 °C for 2 min., followed by 95 °C for 10 min.) and 40 thermocycles of a two-step PCR: 92°C for 15 s, 60° C for 1min. The fluorescence intensity of each sample will be measured at temperature change to monitor amplification of the NAT1 gene. Controls are run to ensure that contaminated

DNA is not amplified. Primers, probes, and DNA are added to final concentrations of 300 nM, 100 nM, and 0.5 -2.5 ng/µl, respectively

Aim 2: To evaluate and compare the effects of combinations of genetic variants in NAT2 (i.e. rapid or slow phenotypes) and high and low NAT1 and CYP1A2 enzymatic activities on oxidative stress, DNA adduct formation, and mutagenesis. *We hypothesize that cell lines with combinations of altered enzymatic activity associated with slow acetylator (NAT) and rapid hydroxylation (CYP) will present an increased incidence of oxidative DNA damage, while cell lines with rapid acetylator (NAT) and slow hydroxylation (CYP) will exhibit less oxidative DNA damage, but increased incidences of DNA adduct formation and mutagenesis.*

NAT1 and CYP1A2 gene knockdown by RNA interference

We will utilize Dharmacon SMART pool siRNA (Thermofisher) formatted to target the open reading frame of human CYP1A2 and the 3' untranslated region (3'UTR) of human NAT1 mRNA to achieve knock down of the expression of CYP1A2 and NAT1. SMART pool siRNA products contain four highly functional duplexes that target different regions of the target gene, guaranteeing 75% knockdown of genes. The open reading frame region of CYP1A2 will be targeted because only the complete coding region sequence of CYP1A2 is present in the pIRES plasmids used for initial transfection. The 3' UTR of NAT1 will be targeted

because the cell lines also express human NAT2, and the vast difference between the 3'UTRs of both isozymes will prevent targeting of both NAT genes. The siRNA transient transfection will be achieved according to manufacturer's instructions. GM4429 cells will be seeded 48 hours before the transfection at $3x10^4$ cells/well in order to have 60-70% confluency at the day of transfection. In a microcentrifuge tube 20 µM siRNA will be mixed with 25 µl of siRNA diluents and 10 µl serum-free medium. In another tube, 5 µl of transfection reagent will be diluted with 25 µl serum-free medium. The two tubes will be mixed and incubated for 15 minutes at room temperature. The mixture will be added to a 6-well plate, which will contain 930µl of serum-free medium. After incubating the mixture for 3-4 hours, the medium will be changed. Seventy-two hours after the application of the smart pool, the cells will be harvested for qRT-PCR to evaluate the levels of knockdown. Enzymatic activity will be measured using functional assays for NAT (HPLC) and CYP (EROD assay) as earlier described to initially test transfection efficacy.

Evaluation of the Effect of Altered Enzymatic Activity and Genetic Variance on Toxicological Outcomes

Cell lines with combinations of CYP1A2 and/or NAT1 knock down and variant NAT2 alleles will be used to evaluate the effects of altered enzymatic activity of expressed XMEs on toxicological risk. Newly generated cell lines will be treated with arylamine genotoxins and toxicological outcomes will be measured as described below.

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Oxidative DNA Damage

Oxidative DNA damage will be evaluated by enzyme immunoassay (EIA) to measure 8-hydroxy-2-deoxyguanosine (8-OH-dG), according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI) . Cell lysates will be collected and DNA will be purified and digested (Qiagen) according to manufacturer's instructions. Fifty µl of sample will be applied to a 96 well plate containing 100 µl of EIA buffer or 50 µl of 8-OH-dG EIA standard. Fifty µl of 8-OH-dG acetylcholinesterase tracer will be added to each well except the total activity and blank wells, followed by 50 µl of 8-OH-dG EIA monoclonal antibody. The plate will be covered and incubated for 18 hr at 4°C. Two hundred µl of reconstituted Ellman's reagent will be added to each well and 5 µl of tracer to the total activity wells. The plate will be read by Spectra Max Gemini XS plate reader (Molecular Devices, Sunnyvale, CA) at a wavelength between 405 and 420 nm and 8-OH-dG concentration will be quantified.

DNA Isolation and DNA Adduct Quantitation

DNA adduct levels will be measured as previously described in our laboratory (Metry et al., 2007). DNA will be isolated from cells grown in 15 cm plates and treated with ABP, AF, 2,6-DMA, 3,5-DMA, or 3-EA. Our laboratory has extensive experience with ABP adduct level measurement studies in other cell lines, therefore ABP will be used as a positive control in the present study. Cells will be harvested after 48 hr and the pellet suspended in 500 µl of 50 mM Tris HCl and

10 mM EDTA. Cells will be incubated in 50 µl of 10% SDS and 20 µg/ml proteinase K for 45 min at 37 °C. DNA will be extracted by phenol:chloroform:isoanyl alcohol (25:24:1) and precipitated using cold isopropanol and centrifuged for 1 min. Supernatant will be removed and the pellet washed with ethanol, dried, and resuspended in buffer containing 5mM Tris HCl, 1mM $CaCl_2$, 1mM $MgCl_2$, and 1mM $ZnCl_2$. DNA will be quantified at A_{260}. DNA samples will be treated with 10 units DNAase I (US Biological, Swampscott, MA) for 1 hour at 37°C and nuclease P1 (US Biological) for six hours, followed by overnight treatment with 5 units of alkaline phosphatase (Sigma). Two volumes of acetonitrile will be added to stop the reaction. Samples will be filtered using a 5,000 nominal molecular weight limit centrifugal filter device (Millipore, Bedford, MA) and will be evaporated to 50 µL. Samples will then be reconstituted in 5% acetonitrile to a final volume of 100 µL. Samples will be used for LC-MS-MS analysis performed by Biomolecular Mass Spectrometry laboratory of Dr. William Pierce. Samples used for quantitative analysis will be spiked with one ng deuterated internal standard before any sample treatment. Samples will be loaded into the Inertsil C18 precolumn using Perkin Elmer ABI 140D syringe pumps and a Hewlett Packard 1100 Series Autosampler. Samples will be injected into a Micromass Quattro LC triple quadrupole mass spectrometer using nanospray with 20 µm i.d. fused silica tubing inserted through 125 µm i.d. PEEK tubing as previously described (Neale et al., 2008).

Assays for Mutagenesis at the *hprt* Locus

To evaluate mutagenesis at the *hprt* locus we will employ methods previously used in our lab (Metry et al., 2007). Cells will be grown for 12 doublings, with selective agents in complete HAT medium (30 µM hypoxanthine, 0.1 µM aminopterin, and 30 µM thymidine). Survival will be determined by colony forming assay and expressed as percent of vehicle control. The remaining cells will be re-plated and sub-cultured. After 7 days of growth, cultures will be plated for cloning efficiency in complete media and for mutations in complete medium containing 40 µM 6-thioguanine (6-TG) (Sigma). Dishes will be seeded with 1 x 10^5 cells /100 mm and incubated for 7 days; cloning efficiency dishes will be seeded with 100 cells/ well/ 6-well plate and incubated for 6 days. In order to distinguish between effects of DNA damage by activated arylamine versus oxidative damage we will employ a Cu(II) specific chelator, bathocuproine, to ablate mutagenesis occurring as a result of oxidative damage.

Aim 3: To determine the effectiveness of a novel arylamine NAT1 small molecule inhibitor on reducing mutagenesis and DNA adduct formation. *We hypothesize that the inhibition of NAT1 enzymatic activity will reduce mutagenesis and DNA adduct formation, but increase oxidative DNA damage.*

In order to identify novel inhibitors of NAT a two-stage screening process will be utilized. The first stage included previous studies by Dr. John Trent, University of Louisville, that identified approximately 150 NAT inhibitor candidates by computer-based *in silico* screening of approximately 20 million compounds.

Initial screening of inhibitory properties of the candidate compounds will be evaluated using NAT1 (PABA)- and NAT2 (SMZ)- specific enzymatic assays that recombinantly express human NAT1 or NAT2 in yeast. Reactions will be prepared with acetyl- coenzyme A (1 mM), selective substrates p-aminobenzoic acid (NAT1) or sulphamethazine (NAT2) (300 µM), and potential inhibitors at varying concentrations between 0 – 1 mM. Control reactions will substitute the vehicle (DMSO) for inhibitor. The reactions will be stopped using 1M acetic acid and protein will be centrifuged at 10,000 x g for 10 minutes. Reaction products in the resulting supernatant will be separated by reverse-phase HPLC as previously described. We will evaluate the effectiveness of promising inhibitors on reducing mutagenesis and DNA adduct formation. Cells will be treated with varying doses of inhibitor, followed by measurements of oxidative damage, *hprt* mutagenesis, and DNA adduct levels as described above. IC_{50} values of the most promising NAT1 inhibitors will be determined by evaluating the inhibition of ABP, PABA, and SMZ *N*-acetylation activity in lysates of yeast that recombinantly express human NAT1 or NAT2. Activity will be determined using 100 µM acetyl CoA and 10 µM ABP, PABA, or SMZ with varying concentrations of inhibitor (0- 100µM). Screening of additional compounds will be completed to determine their ability to inhibit NAT.

Statistical Analysis

Quantitative analysis of differences between various test groups will be done using one-way analysis of variance (ANOVA), with the Bonferroni posttest. We

will use the Student's t test to compare differences between an experimental group and a control group.

CHAPTER IV

RESULTS AND DISCUSSION

We have successfully constructed NER-deficient SV40- transformed human fibroblast cells that stably express human NAT2 alleles (*NAT2*4*, *NAT2*5B*, or *NAT 2*7B*) and human CYP1A2. This was accomplished using the Flp-In system (Invitrogen, Carlsbad, CA). The CYP1A2-, CYP1A2/*NAT2*4*-, CYP1A2/*NAT2*5B* and CYP1A2/*NAT2*7B*- transfected cells exhibited EROD when assessed using fluorimetric determination of 7-ethoxyresorufin oxidation. No statistical differences ($p > 0.05$) in CYP1A2 EROD catalytic activity were observed between GM4429/CYP1A2 cells and the cell lines further transfected with *NAT2* alleles (Figure 3.).

Human NAT2-specific SMZ *N*-acetylation activity was measured to validate successful production of stable transformants expressing *NAT2*4*, *NAT2*5B* and *NAT2*7B* (Figure 4.). All NAT2 transfected cells had significantly higher SMZ *N*-acetylation activity than the non-transfected cell lines. Lysates collected from cells transfected with *NAT2*5B* and *NAT2*7B* showed significantly lower SMZ *N*-acetylation activity when compared to the reference allele NAT2*4 using one-way ANOVA analysis followed by Bonferroni posttest ($p < 0.001$). Activity in GM4429 cells not transfected with NAT2 were below the level of detection for SMZ *N*–acetyltransferase (<0.15nmoles/min/mg).

As shown in Figure 5., cells were tested for PABA *N*-acetyltransferase activity in lysates of NER-deficient SV40 transformed GM4429 cells stably transfected with human CYP1A2 and human NAT2 alleles. No significant difference in PABA *N*-acetylation activity was observed among all GM4429 cells lines using one-way ANOVA analysis ($p > 0.05$).

The identification of NAT inhibitors in a two stage screening method is previously described. Based on our intial *in vitro* screening we determined that the compound ethyl[1-(3-ethoxycarbonylpropanoyloxy)-10-dioxo-2-anthryl]-butanedioate (compound 10) was most promising in its ability to inhibit acetylation activity (Table 1.). Compound 10 inhibition of PABA and ABP *N*-acetylation activity in lysates of yeast that recombinantly express human *NAT1*4* was determined to be 100% (Figure 6.). IC_{50} values were calculated and determined to be 0.75 µM (PABA) and 0.97 µM (ABP) in yeast lysate that recombinantly express human *NAT1*4* (Figure 6.). In yeast lysate that recombinantly express human *NAT2*4* IC_{50} values were determined to be 41.3 µM (ABP) and 82.2 µM (SMZ) (Figure 7.). In CHO cells stably transfected with human *NAT1*4* IC_{50} values for compound 10 were determined to be 8 µM (ABP) and 118 µM (PABA) (Figure 8.).

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DISCUSSION

Our results show that we have successfully constructed and characterized NER-deficient human fibroblast cell lines that stably express human NAT2 alleles (*NAT2*4*, *NAT2*5B*, or *NAT 2*7B*) and CYP1A2. These cell lines can be used to study NAT metabolism. Future studies include the investigation of functional effects of genetic variations in NAT2, in the presence of human NAT1, on arylamine metabolism and toxicological risk. The cell lines used in this study are most beneficial because they endogenously express human NAT1, therefore are practical in studying not only substrate specificity, but combinations of altered enzymatic activity in human CYP1A2, human NAT1 and genetic variance in human NAT2.

This study also introduces the use of novel small molecule NAT1 inhibitors to reduce DNA adduct formation and mutagenesis. The applications of these compounds are novel. We expect that inhibiting NAT1 catalytic activity will reduce mutagenesis and DNA adduct formation resulting from NAT1 *O*-acetylation. We expect to see an increase in oxidative stress because inhibition of NAT activity will increase substrate availability for metabolic hydroxylation leading to an increased production of reactive oxygen species, consequently increasing oxidative DNA damage. The results of this investigation provide insight into functional genomic studies of NAT2 in human cells. We expect that

future studies will allow us to determine more details of the relationship between genetic variations and toxicological risk from genotoxic environmental arylamines.
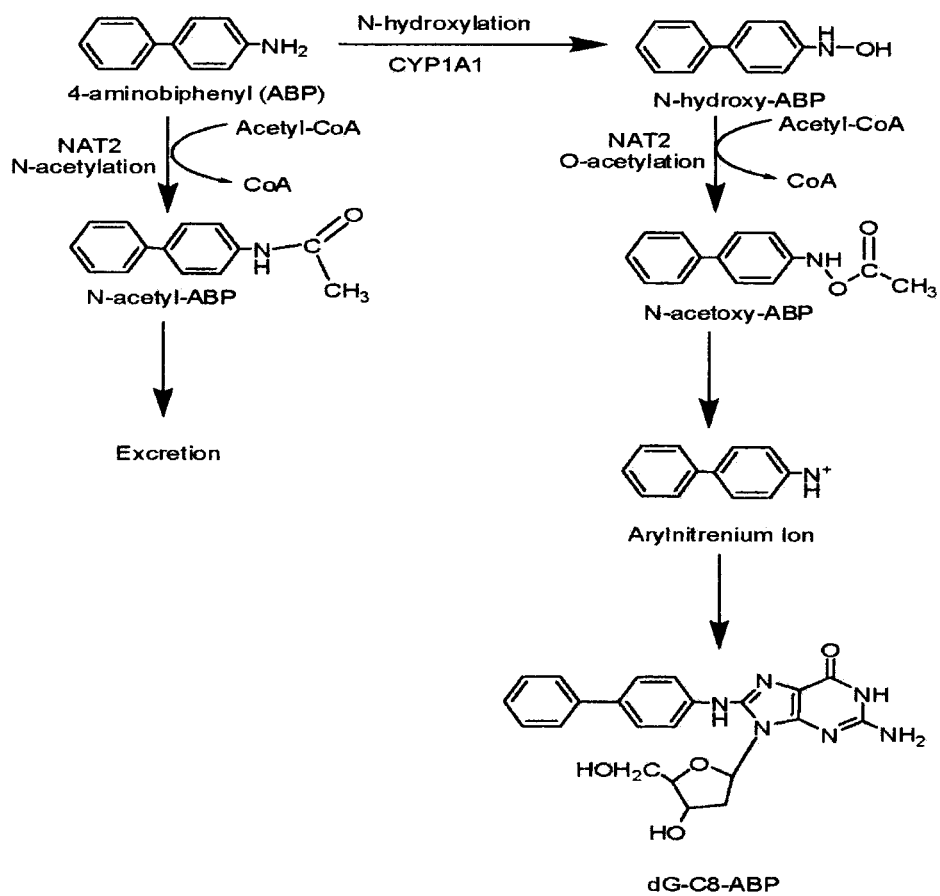
Figure 1. Metabolic pathways of 4-aminobiphenyl by NAT.
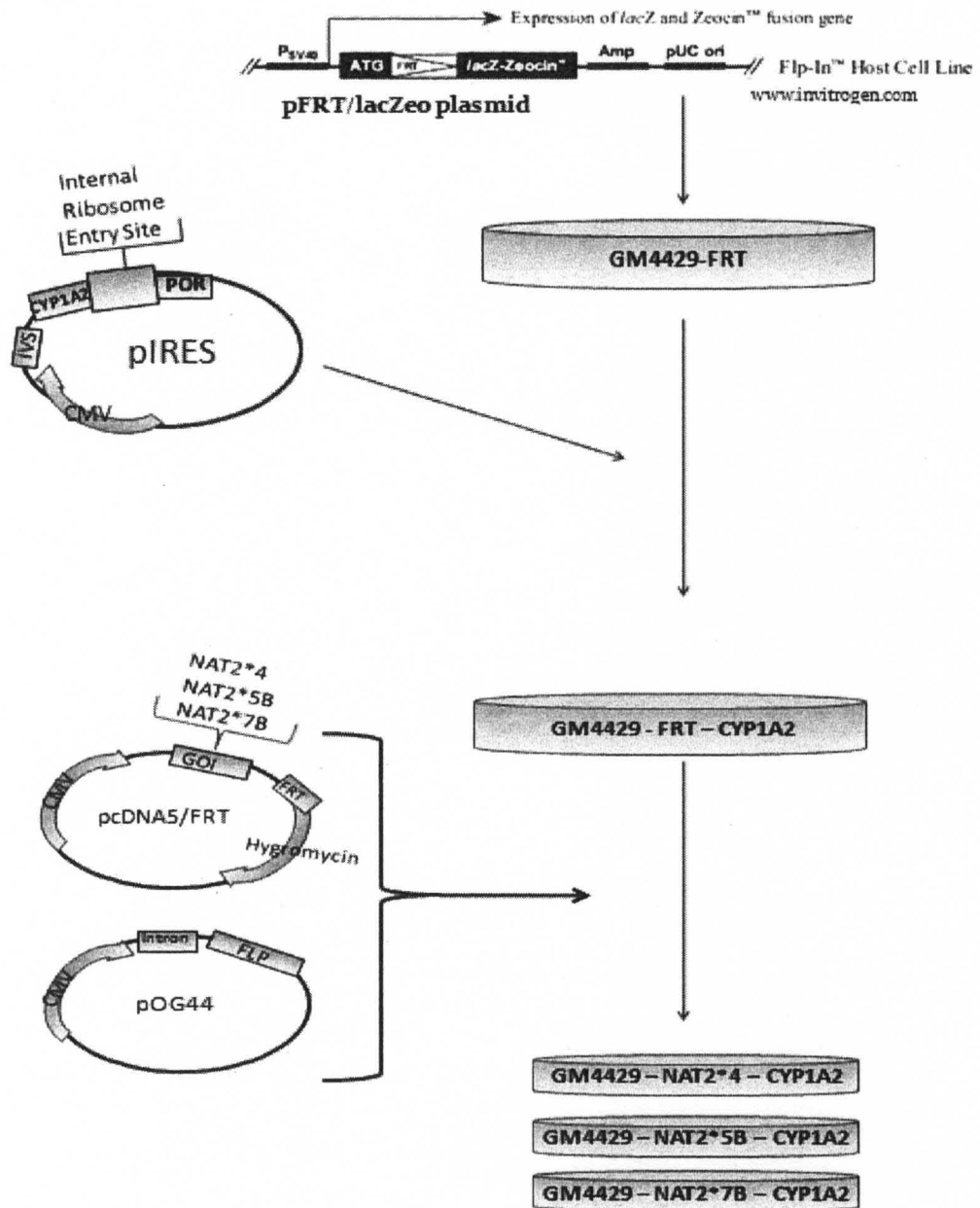
Adapted from Bendaly et al., 2009.

Figure 2. Schematic of stable transfection using the Invitrogen Flp-In System
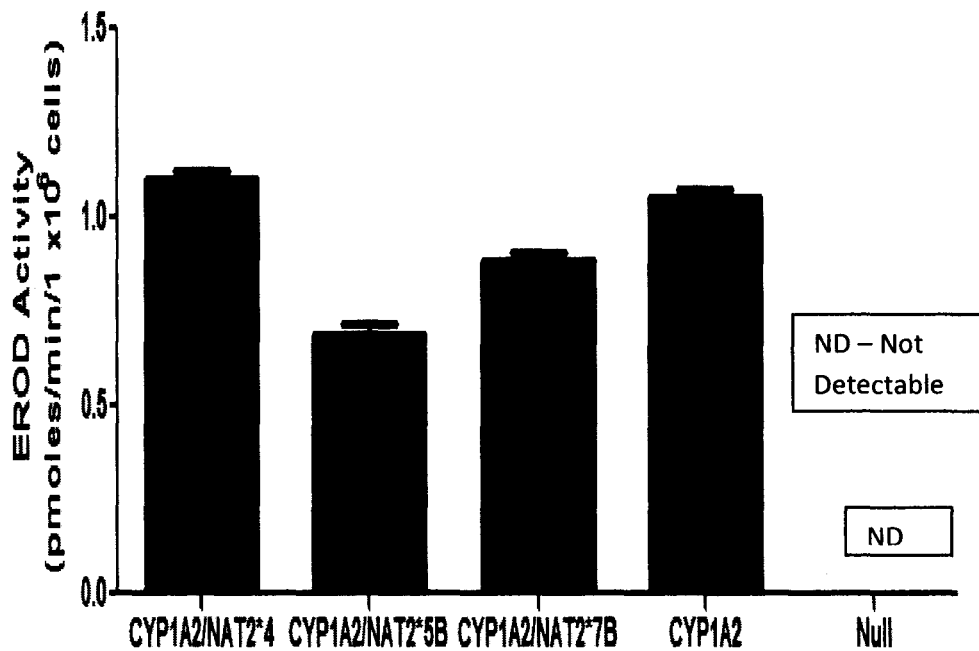
Figure 3. CYP1A2 EROD activity in NER-Defiecient SV40-transformed GM4429 cells.Each bar represents mean ± SEM for 3 experiments determining 7-ethoxyresorufin-O-deethylase (EROD) activity (pmoles/min/1 x 10^6 cells) in NER-deficient SV40-transformed GM4429 cells stably transfected with human CYP1A2 and human NAT2 alleles. No significant difference in EROD activity was observed among transfected cells using one-way ANOVA analysis (p > 0.05) EROD activity in non-transfected cells was below the limit of detection (<0.05 pmoles/min/1 x 10^6 cells).
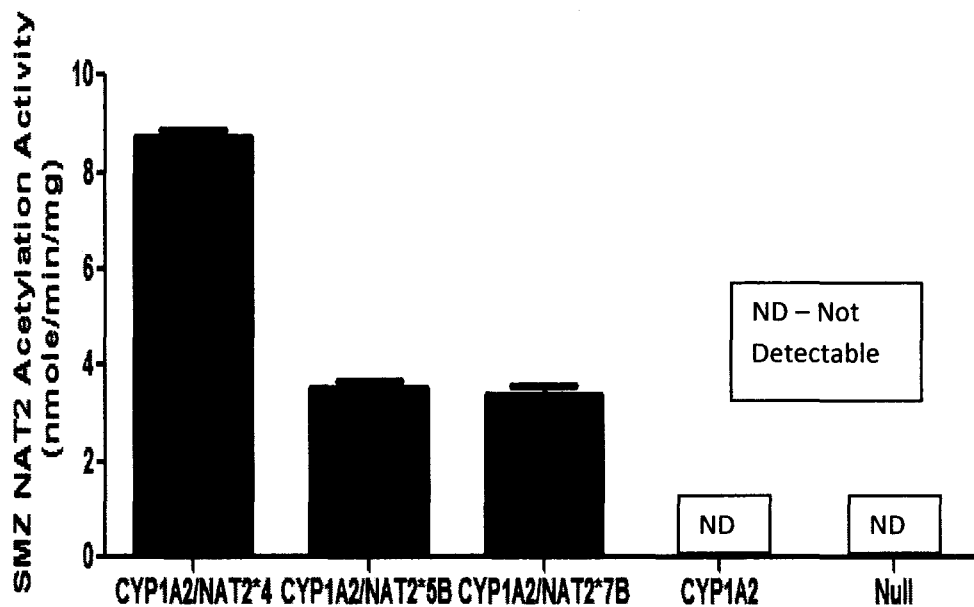
Figure 4. SMZ *N*-acetyltransferase activity in cell lysates of NER-deficient SV40-transformed GM4429 cells stably transfected with human CYP1A2 and human NAT2 alleles. All NAT-transfected cells had significantly higher SMZ *N*-acetylation activity than the non-transfected cells. Lysates collected from cells transfected with NAT2*5B and NAT2*7B showed significantly lower SMZ *N*-acetylation activity when compared to extracts of cells transfected with the reference allele NAT2*4 using one-way ANOVA analysis followed by Bonferroni posttest ($p < 0.001$). SMZ *N*-acetyltransferase activity of non-transfected GM4429 cells was below the level of detection (<0.15nmoles/min/mg protein).
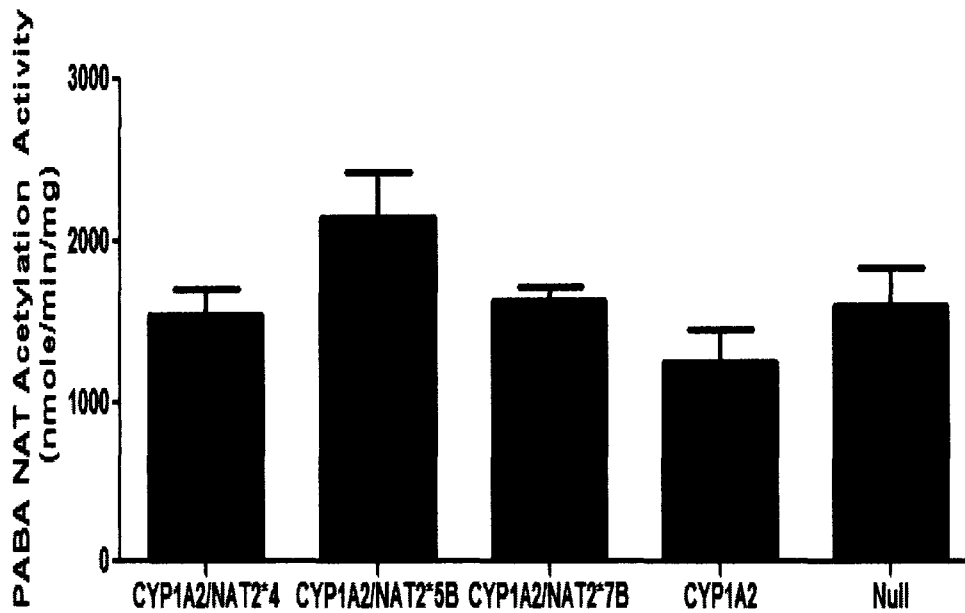
Figure 5. PABA *N*-acetyltransferase activity in cell lysates of NER-deficient SV40 transformed GM4429 cells stably transfected with human CYP1A2 and human NAT2 alleles. PABA *N*–acetylation was determined with 1 mM acetyl CoA and 300 µM PABA. No significant difference in PABA *N*-acetylation was observed among all GM4429 cells lines using one-way ANOVA analysis ($p > 0.05$).

Figure 6. Compound 10 inhibition of PABA and ABP *N*-acetylation activity in lysates of yeast that recombinantly express human NAT1. Each point represents mean ± SEM for 3 experiments determining compound 10 inhibition of PABA and ABP *N*-acetylation activity in lysates of yeast that recombinantly express human NAT1.
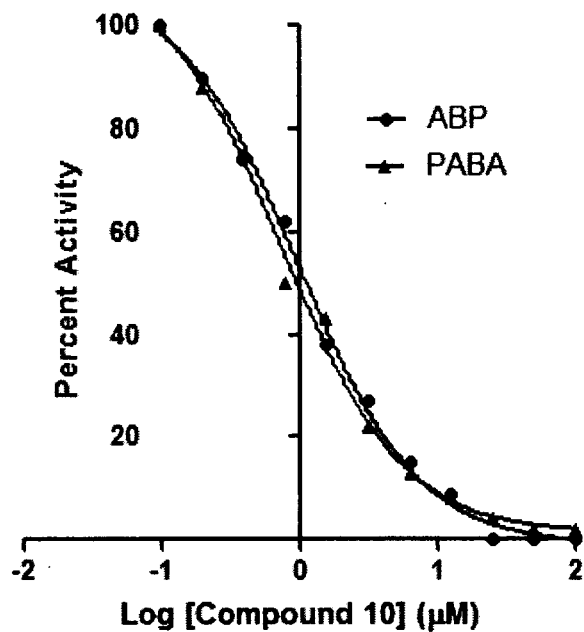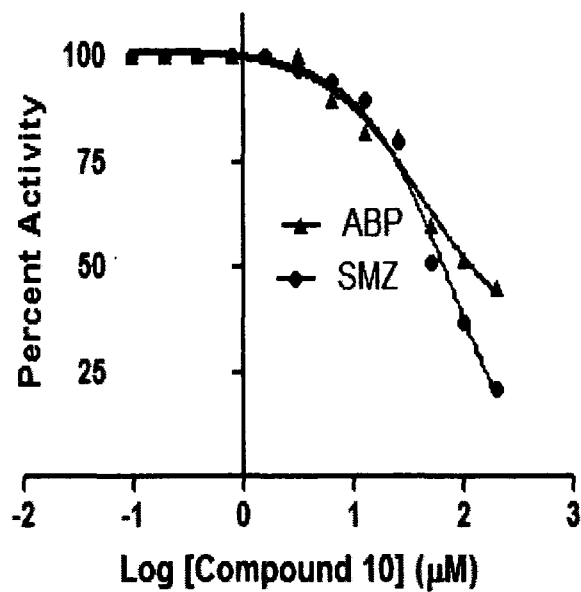
Figure 7. Compound 10 inhibition of ABP and SMZ N-acetylation activity in lysates of yeast that recombinantly express human NAT2. Each point represents mean ± SEM for 3 experiments determining compound 10 inhibition of ABP and SMZ N-acetylation activity in lysates of yeast that recombinantly express human NAT2.

Figure 8. Compound 10 inhibition of ABP and PABA *N*-acetylation activity in CHO cells that recombinantly express human NAT1. Each point represents mean ± SEM for 3 experiments determining compound 10 inhibition of ABP and PABA *N*-acetylation activity in CHO cells that recombinantly express human NAT1.
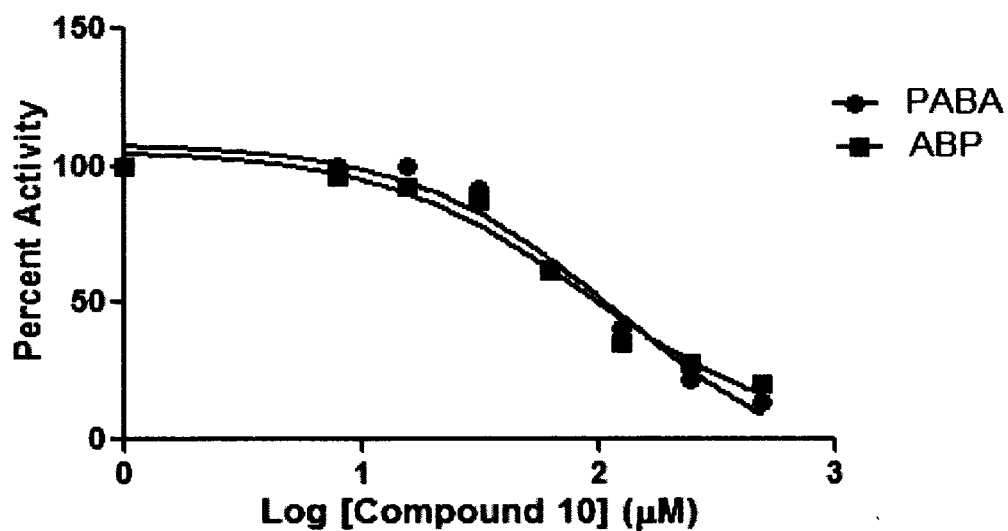
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Table 1. Target NAT isozyme, priority number, and percent inhibition of screened compounds

Entry		Priority number	% inhibition of NAT2	% inhibition of NAT1
1	NAT2	6	4	0
2	NAT2	9	49	7
4	NAT1	14	3	0
5	NAT1	19	0	30
6	NAT2	7	7	0
7	NAT2	11	0	25
8	NAT1	3	0	0
9	NAT1	20	7	14
10	NAT1	23	100	100
11	NAT1	40	88	95
12	NAT1	67	23	8
13	NAT1		0	3
14	NAT1		0	7
15	NAT2	24	9	5
16	NAT2	23	5	75
17	NAT1	27	0	0
18	NAT2	25	0	0
19	NAT1	47	2	16
20	NAT2	28	2	16
21	NAT1	50	9	2
23	NAT2	26	21	5
24	NAT2	27	22	15
25	NAT2	30	37	2
26	NAT1	26	0	31
27	NAT2	46	0	0
28	NAT2	57	26	0
30	NAT1	49	7	0
31	NAT2	52	14	27
32	NAT1	54	30	43
33	NAT2	53	0	7
34	NAT2	33	10	14
35	NAT2	22	2	0
36	NAT2	41	0	0
37	NAT2	32	4	17
38	NAT2	60	13	21
39	NAT2	67	0	5
40	NAT2	58	0	3
41	NAT2	56	2	0
42	NAT2	49	0	14
43	NAT2	55	27	0
44	NAT2	68	10	13
45	NAT2	51	0	20
46	NAT2	38	15	21
47	NAT2	44	14	5

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Graduate Research Assistant, BNL Medical Department
Radiotracer Chemistry and Instrumentation Biological Imaging
Graduate Research Internship Program (GRIP) Fellow
Brookhaven National Laboratory
Upton, NY (2008 - 2008)

- Radiolabeling and Validation of Precursors used to trace Cannabinoid (CB_1) receptors
- Synthesis and Labeling of SSR149415

Model Institute for Excellence (MIE) Research Fellow
Spelman College
Atlanta, GA (2004-2006)

- Determined optimal conditions for the synthesis of the benzimidazole derivatives
- Microwave synthesis of aniline and benzimidazole derivatives
- Purification of molecules by flash chromatography and recrystallization
- Characterization by NMR and IR
- Assisted group members with various projects
- Maintained chemical stock

Alliance for Graduate Education and Professorate (AGEP)
Research Assistant
University of Iowa
Iowa City, IA (Summer 2005)

- Project Title: "Biotransformation of Indene by *Candida* Strains"
- Frozen stock was used to incubate shaker flask in order to produce a growth curve for various *Candida* strains
- Produced indandiol and indanoxide using strain UI-9773 which was analyzed by NMR and TLC

Multicultural Academic Opportunity Program (MAOP) Research Assistant
Virginia Polytechnic Institute and State University
Virginia Bioinformatics Institute (VBI)
Blacksburg, VA

- Participated in research: "Sequence Data Annotation of Expressed Sequence Tags of *Phytophthora*"
- Manually reviewed and organized singlet data
- Produced database of metabolic pathways for *Phytophthora*
- Created web-based data using HTML

Health Careers Opportunity Program (HCOP) Research Assistant
Albany State University
Albany, GA (Summer 2002)

- Participated in research: "Utilizing Susceptibility Test to Optimize Antibiotic Therapy"
- Employed laboratory research techniques, laboratory safety, literature and database research

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Summer Science Engineering and Mathematical Institute Research Assistant
Clark Atlanta University
Atlanta, GA

- Participated in enrichment coursework
- Introduced to research techniques and laboratory safety

Research and Professional Organizations

- National Organization for the Professional Advancement of Black Chemist and Chemical
      Engineers (NOBCChe)
- American Chemical Society
- Black Biomedical Graduate Student Organization (vice-president 2010- , secretary 2009-2010)

Honors and Awards

- Integrated Programs in Biomedical Sciences Fellow, University of Louisville School of Medicine

- National Science Foundation Greater Philadelphia Alliance for Minority Participation Bridge to the Doctorate Fellowship, Delaware State University

- Graduate Research Internship Program (GRIP) Fellow, Brookhaven National Laboratory

- Model Institute for Excellence (MIE) Fellowship, Spelman College

- Chemistry Department Teaching Assistantship, Spelman College

- Helping Outstanding Pupils Educationally (HOPE) Scholar, Spelman College

Abstracts and Presentations

Carmine S. Leggett, David F. Barker, Mark A. Doll, Lori M. Millner, J.Christopher States, and David W. Hein. Construction and Functional Characterization of Nucleotide Excision Repair – Deficient SV40-Transformed Human Fibroblast Expressing Human CYP1A2 and NAT2 Genetic Variants. Fifth International Workshop on the Arylamine *N*-acetyltransferases **University of Paris Diderot**. Paris, France. 2010

Carmine S. Leggett, Mark A. Doll, John O. Trent, Jason M. Walraven, Lori M. Millner, David W. Hein. Identification and Characterization of Novel Arylamine N-Acetyltransferase Small Molecule Inhibitors.*University of Louisville School of Medicine Research Louisville.* Louisville, KY. 2009

Carmine Leggett, Joanna Fowler, Jacob Hooker, Alexander Makryannis, Alicia Reid, and Alexander Zvonok.: Development and Validation of [^{11}C]SSR149415 and Indole Derivatives as PET agents for Imaging of Cannabinoid(CB_1) and Vasopressin(V_{1b}) Receptors. *Brookhaven National Laboratory Research Symposium*. Upton,NY. 2007

Carmine Leggett, Leyte Winfield. Using ADMET Predictor to Measure Parameters of Heterocyclic Molecules. *Spelman College Research Day*. Atlanta, GA. 2006

Carmine Leggett, Emmanuel Ankudey, Tonya Peeples. Biotransformation of Indene by Candida strains. *Alliance for Graduate Education and Professorate Research Symposium*. University of Iowa. Iowa City, Iowa 2005.

Carmine Leggett, Erika Thomas, Leyte Winfield. Synthesis of Benzimidazole Derivatives. *Model Institute of Excellence Research Day*. Spelman College. Atlanta, GA. 2005.

Carmine Leggett, LaChelle Waller, Brett Tyler. Sequence Data Annotation of Expressed Sequence Tags of Phytophthora. *Virginia Tech Research Symposium*. Blacksburg, VA.2004

Publications

Matthias Schönberger, Carmine Leggett, Sung Won Kim, and Jacob M. Hooker. Synthesis of [^{11}C]SSR149415 and preliminary imaging studies using positron emission tomography. *Bioorganic and Medical Chemistry Letters* 15: 3103-3106, 2010. (Epub April 2, 2010) [PMID: 20400305]

Hein, D.W., Millner, L.M., Leggett, C.S. and Doll, M.A.: Relationship between N-acetyltransferase 2 single nucleotide polymorphisms and phenotype. *Carcinogenesis* 31: 326-327, 2010. (Epub November 23, 2009). [PMCID: 2812573]

Winfield, Leyte; Smith, Dayle M.; Halemano, Kalani; Leggett, Carmine S. A Preliminary Assessment of Structure- Activity Relationship of Benzimidazole-Based Anti-Proliferative Agents. *Letters in Drug Design and Discovery* 5: 369-376, 2008. [ISSN:1570-1808]