Arachidonic Acid-dependent Peroxidative Activation of Carcinogenic Arylamines by Extrahepatic Human Tissue Microsomes

Thomas J. Flammang,1 Yasushi Yamazoe,2 R. Wayne Benson, Dean W. Roberts, David W. Potter,3 David Z. J. Chu, Nicholas F. Lang, and Fred F. Kadlubar

Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079 [T. J. F. Y., Y. R. W. B., D. W. R., D. W. P., F. F. K.], and Department of Surgery, University of Arkansas for Medical Sciences and John L. McClellan Memorial Veterans Administration Medical Center, Little Rock, Arkansas 72205 [D. Z. J. C., N. P. L.]

ABSTRACT

Prostaglandin H synthase (PHS), an arachidonic acid-dependent peroxidase, has been implicated in the peroxidative activation of carcinogenic aromatic amines in extrahepatic carcinogen target tissues of experimental animals. We have examined the arachidonic acid-dependent activation of [3H]benzidine DNA-bound products by microsomal preparations from 75 normal human tissues obtained during surgical procedures. For several samples of urinary bladder epithelium, prostate epithelium, colon mucosa, and peripheral lung tissue, an arachidonic acid-dependent, microsomal-catalyzed activation of benzidine was observed; and the activity could be inhibited appreciably by indomethacin, a known inhibitor of PHS. Little or no arachidonic acid-dependent activity was detected in human placenta, breast, or liver microsomes or the majority of colon microsomes. Substrate specificity was also examined with purified rat PHS and with human bladder and with active colon preparations. Purified PHS catalyzed the activation of benzidine ≫ 2-naphthylamine, 2-amino-6-methylidipyr(1,2-a;3',2'-d)imidazole ≫ 4-aminobiphenyl ≫ 2-amino-3-methylimidazo(4,5-f)quinoline ≫ 3-amino-1-methyl-5-//-pyrido[4,3-b]indole. In comparison, human bladder and colon microsomes catalyzed the activation of benzidine ≫ 4-aminobiphenyl, 2-amino-6-methylidipyr(1,2-a;3',2'-d)imidazole ≫ 2-amino-3-methylimidazo(4,5-f)quinoline, 3-amino-1-methyl-5-//-pyrido[4,3-b]indole. To confirm the occurrence of PHS antigen in human extrahepatic tissues, an avidin/biotin-amplified competitive enzyme-linked immunosorbent assay was developed with purified rat PHS and a commercially available monoclonal antibody known to cross-react with human platelet PHS. The avidin/biotin-amplified competitive enzyme-linked immunosorbent assay, which detected ng quantities of PHS, clearly established the presence of the PHS protein in human bladder, prostate, and lung microsomes. In contrast, PFS antigen was not detected in the liver or placental microsomes. The interindividual and tissue-dependent variability of PHS and its role in aromatic amine carcinogenesis are discussed.

INTRODUCTION

The primary aromatic amines include well-known human carcinogens that have been used in the chemical industry, and that are also found to occur in the environment, such as cigarette smoke, and in other sources (1–3), e.g., benzidine, 2-naphthylamine, and 4-aminobiphenyl. In addition, structurally more complex heterocyclic aromatic amines have been isolated from cooked foods, and are known to be highly mutagenic and moderately: carcinogenic in short-term test systems and in rodent bioassays (4–6), e.g., Glu-P-1,4 IQ, and Trp-P-2. The tumorigenic potential for each of the compounds in the human population may depend on many factors, including the extent to which each is metabolized to an ultimate carcinogen that forms covalent adducts with DNA. In the last few years, studies in experimental animals have suggested that extrahepatic peroxidative enzymes convert aromatic amines to reactive ultimate carcinogens (7, 8).

PHS, a mammalian peroxidase, is known to be present in the microsomal fraction of several tissues (9). PHS is a bifunctional enzyme that initiates prostaglandin biosynthesis through oxidation of arachidonic acid to a 15-hydroperoxy-9,11-endoperoxide which is then enzymatically reduced to its hydroxy endoperoxide (10). Several chemical carcinogens, including aromatic amines, function as reducing cofactors for the peroxidase and thereby undergo peroxidative metabolism (11–16). The resultant oxidized amine can then react with DNA, forming covalently bound carcinogen-DNA adducts that may lead to initiation of the neoplastic process.

In this study, we have surveyed the ability of microsomal fractions isolated from several human tissues to catalyze the arachidonic acid-dependent binding of benzidine and other amines to DNA in vitro. We have also utilized a monoclonal antibody that cross-reacts with human platelet PHS, in combination with a competitive A-B ELISA, in order to confirm the presence of PHS antigen in the samples with arylamine peroxidase activity.

MATERIALS AND METHODS

Tissue Preparations. Human tissue samples that were normal in appearance and not required for pathological examination were obtained from specimens removed during routine surgical procedures performed at the John R. McClellan Memorial Veterans Hospital or the University of Arkansas for Medical Sciences Hospital, Little Rock, AR. All samples, except breast and placenta, were obtained from male patients. The samples were immediately placed in cold saline following resection. Within 5–10 min, the specimens were drained, wrapped in several layers of aluminum foil, quickly frozen by immersion in liquid nitrogen, and stored at −80°C until the assays were performed. The bladder epithelium was dissected from the submucosa at surgery and then frozen in the same manner.

The tissue samples were then thawed at 5°C, placed in an ice-cold solution of 50 mM sodium pyrophosphate buffer (pH 7.4) containing 0.1 mM diithiothreitol, mixed with scissors, and washed by decanting one or more times to remove excess blood. For the colon mucosa, which was obtained adjacent to neoplasia, the epithelial cell layer was scraped from the thawed colon as described by Fang and Strobel (17). The washed tissue samples were suspended in 5–6 volumes of an ice-cold 0.25 M sucrose solution containing 20 mM Tris-acetate buffer (pH 7.4), 25 mM phenylmethylsulfonyl fluoride, 20 µM butylated hydroxytoluene, and 0.1 mM EDTA (all from the Sigma Chemical Co., St. Louis, MO). The same methods were also used on tissues obtained fresh and then immediately processed. The tissues were homogenized with a Teflon pestle Potter-Elvehjem homogenizer followed by sonication (2 × 50 W for 5 s) using a Labline Model 9100 instrument. The microsomal fraction was obtained from the homogenate by differential centrifugation at 4°C according to the principles of Hogeboom et al. (18). The microsomal pellet was washed once with the homogenization

Received 7/14/88; revised 12/20/88; accepted 1/19/89.
1 To whom requests for reprints should be addressed, at HFT-110, NCTR, Jefferson, AR 72079.
2 Present address: Department of Pharmacology, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160, Japan.
3 Present address: Department of Toxicology, Rohm & Haas Co., Spring House, PA 19477.
4 The abbreviations used are: Glu-P-1, 2-amino-6-methylidipyr(1,2-a;3',2'-d)imidazole; Q, 2-amino-3-methylimidazo(4,5-f)quinoline; Trp-P-2, 3-amino-1-methyl-5//-pyrido[4,3-b]indole; PHS, prostaglandin H synthase; A-B ELISA, avidin/biotin amplified enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

buffer by resuspension and centrifugation at 105,000 × g for 1 h. The final microsomal pellet was suspended in 0.25 M sucrose solution containing 10 mM Tris-acetate buffer (pH 7.4), 0.1 mM EDTA, and 20% glycerol, and immediately used in the enzyme assays. Purified ram seminal vesicle PHS (No. 85B) was obtained from Oxford Biomedical Research (Oxford, MI). Protein concentrations were determined by the biuret method (19).

Chemicals. [3H]Trp-P-2 (200 mCi/mmol) and [3H]Glu-P-1 (200 mCi/mmol) were a gift from Dr. R. Kato (Keio University, Tokyo, Japan). The remaining radiolabeled substrates, [3H]benzidine (114 mCi/mmol), [3H]2-naphthylamine (33.8 mCi/mmol), [3H]4-aminobiphenyl (37.8 mCi/mmol), and [3H]IQ (118 mCi/mmol), were purchased from Chem-Syn Science Laboratories (Lenexa, KS).

Enzyme Assays. The peroxidative activation of these arylamines was measured by determining their arachidonic acid-dependent covalent binding to DNA. The assays (1 ml) contained: 50 mM potassium phosphate buffer (pH 7.4), 100 μM arachidonic acid (Nu-Chek Prep, Inc., Elysian, MN), 1 μM hematin (Sigma; with reactions containing purified PHS only), 2.5 mg calf thymus DNA/ml (Type I; Sigma), 20 μM [3H]arylamine, and 50 μg/ml microsomal protein (or 100 units purified PHS); 100 μM linolenate, or linoleate or NADPH (Sigma) was substituted for arachidonate in some reactions as indicated in the results. The reactions were preincubated at 37°C for 2 min and the assay was then started by addition of arachidonic acid. Arachidonic acid stock solutions (10 mM in argon-purged ethanol) were prepared fresh daily. Control reactions without arachidonic acid or without protein were also carried out. When a sufficient quantity of a microsomal sample was available, 0.1 mM indomethacin (Sigma) was included as an inhibitor in an additional incubation mixture. After incubation for 5 min, the reactions were terminated with 5 volumes of cold 95% ethanol containing 1% phenol to precipitate the DNA. The DNA was purified by multiple solvent extractions and precipitations (20) and the levels of [3H]arylamines covalently bound to the DNA were determined by liquid scintillation counting. Binding values were generally single determinations (due to the limited quantities of tissue). Based on arachidonic acid-dependent binding of benzidine to DNA that was catalyzed by purified PHS, the binding values are expected to vary by ±10%. Where indicated the data are expressed as the mean value ±SD, n ≥ 3. Several microsomal preparations were also assayed for arachidonic acid-dependent peroxidative activity by measuring the formation of acetaminophen polymers as described by Potter and Hinson (21). These reaction mixtures (1 ml) contained 0.1 mM potassium phosphate buffer (pH 7.4), 0.2 mM arachidonic acid, 10 mM acetaminophen, and 50 μg microsomal protein (200 μM indomethacin was used as required); and the incubations were carried out at 37°C for 5 min. The assays are single determinations (due to the limited quantities of tissue) and polymer formation is expressed as nmol free radical equivalents per assay (22); the limit of sensitivity is 0.5-nmol equivalents.

Immunossays. The levels of PHS protein in the microsomal preparations were estimated by using a mouse monoclonal antibody to ram seminal vesicle PHS (Oxford No. PG21: anti-PGH synthase, cyo-1), that was known to cross-react with human platelet PHS (23). An A-B ELISA was developed and standardized against the purified ram PHS; 50% inhibition of antibody binding was determined to be 4.52 ± 1.21 ng (SD; n = 4) PHS per well; and antigenicity was destroyed when PHS was heat denatured. Purified PHS in 60 mM sodium carbonate buffer, pH 9.6, was adsorbed (15 ng/well) to 96-well polystyrene plates (Nunc Immuno Plate I; Vangard International, Inc., Neptune, NJ) by incubating in a humidified chamber at 37°C for 1.5 h and then overnight at 4°C. The wells were washed 5 times with 10 mM PBS, pH 7.2, containing 0.05% Tween-20. The remaining binding sites were then blocked by incubating each well with 280 μl PBS containing 0.1% gelatin (Difco, Detroit, MI) and 4% fetal calf serum (Gibco, Grand Island, NY) for 2 h at 37°C. The monoclonal anti-PHS antibody, diluted in assay buffer (PBS containing 0.025% gelatin, 1% fetal calf serum, and 2% Triton X-100), was combined with an equal volume of an appropriate dilution of human tissue microsomes or purified PHS standard diluted in the same buffer such that the final concentration of antibody was 1:1600. The mixture was incubated overnight at 4°C and 100-μl aliquots were then added to duplicate wells and incubated for 1.5 h at 37°C. The wells were washed as before, and 100 μl of biotinylated goat anti-mouse IgG antibody (Enzo Biochemicals, Inc., NY), diluted 1:2000 in assay buffer, was added to each well; and the plates were then incubated in a humidified chamber at 37°C for 1.5 h. Excess biotinylated goat anti-mouse IgG antibody was removed from the wells by washing as before, and 100 μl of a 1:250 dilution of avidin-biotinhorseradish complex (Enzo) was added. After 15 min at 37°C, the wells were again washed and 100 μl of 0.1% (w/v) 5-aminono-2-hydroxybenzoic acid (Sigma; recrystallized) containing 0.005% H2O2 were added to each well. The reaction was terminated after 30 min by the addition of 50 μl of 1 N NaOH. The optical density was measured at 450 nm with an ELISA plate reader (Chromoscan, Bio-tek Instruments, Inc., Burlington, VT) and the data were stored and evaluated on an Apple IIe computer, using routines developed in our laboratory. Percentage of inhibition was determined and the inhibition curves were generated by using a polynomial curve-fitting procedure (24). In each set of immunoassay experiments a sample of purified PHS was used as the positive assay control.

RESULTS

The arachidonic acid-dependent metabolic activation of [3H] benzidine to DNA-bound products was catalyzed by microsomes that were isolated from human bladder, colon, prostate, and lung, but not from liver or breast samples; low levels of binding were also detected in two of twelve incubations fortified with placental microsomes (Fig. 1). The range of arachidonic acid-dependent activity was found to vary widely between the different tissue types as well as with the same type of tissue from different individuals (Fig. 1; Table 1). The highest activities were consistently detected in bladder epithelial microsomes, even though the binding levels varied over a 200-fold range. The next most active tissue appeared to be the prostatic epithelium where one-half of the microsomal samples catalyzed appreciable levels of arachidonic acid-dependent binding of [3H] benzidine to DNA. In contrast, the pattern of arachidonic acid-dependent metabolic activation of benzidine was markedly different in the colon samples. While significant levels of benzidine-DNA-binding activity were found in 9 of the microsomal preparations from colon mucosa, little or no activity was detected in the remaining 21 colon samples examined.

The occurrence of microsomal peroxidative activity in different tissue types was also detected by a rapid high pressure liquid chromatography assay that measures the formation of acetaminophen polymers (21, 22). In all cases where significant benzidine-DNA-binding activity was detected, acetaminophen...
Amines can be activated by arachidonic acid-dependent enzymes that catalyze the binding of benzidine to DNA (Table 1). In addition, both the arachidonic acid-dependent binding of benzidine and the peroxidative activation of carcinogenic arylamines by purified human placental phospholipase A2 (PLA2) and bladder Sample 1 were reduced 4-fold after freezing (Table 1), indicating that the enzyme is easily denatured. Likewise, both the arachidonic acid-dependent acetaminophen polymer formation was inhibited by indomethacin in only one of the four samples tested.

The results shown in Table 3 indicate that lipoxygenase and NADPH oxidase will support the metabolism of benzidine to bind DNA. However, indomethacin did not inhibit the arachidonic acid-dependent activity in the 2 of 12 microsome samples from placenta that catalyzed low levels of benzidine binding. The highest inhibition of benzidine binding (range, 60-100%) was observed in the bladder and prostate samples tested (Tables 1 and 2). Furthermore, indomethacin also blocked arachidonic acid-dependent acetaminophen polymer formation in cross-tested bladder and prostate samples but at reduced efficiency (range, 33-60%) as compared to the inhibition of benzidine-DNA binding (Table 1). A more varied result was observed in inhibition experiments with colon samples where inhibition of benzidine binding varied from 9 to 100% (Tables 1 and 2) and acetaminophen polymer formation was inhibited by indomethacin in only one of the four samples tested.

The presence of PHS in the tissues examined was verified by a competitive A-B ELISA for PHS antigen in the microsomal samples. The sensitivity of the assay (50% inhibition of antibody binding) to human tissue microsomes varied widely, depending on the tissue type (Tables 1 and 2; Fig. 1). In general, indomethacin inhibited the arachidonic acid-dependent activities in the bladder, prostate, and colon samples that were tested. In contrast, indomethacin did not inhibit the arachidonic acid-dependent activity in the 2 of 12 microsome samples from placenta that catalyzed low levels of benzidine binding. The highest inhibition of benzidine binding (range, 60-100%) was observed in the bladder and prostate samples tested (Tables 1 and 2).

Furthermore, indomethacin also blocked arachidonic acid-dependent acetaminophen polymer formation in cross-tested bladder and prostate samples but at reduced efficiency (range, 33-60%) as compared to the inhibition of benzidine-DNA binding (Table 1). A more varied result was observed in inhibition experiments with colon samples where inhibition of benzidine binding varied from 9 to 100% (Tables 1 and 2) and acetaminophen polymer formation was inhibited by indomethacin in only one of the four samples tested.

The varied results with indomethacin inhibition indicated that the arachidonic acid-dependent DNA-binding activity catalyzed by the tissue microsomes and, in particular, those in the colon, may be catalyzed by several enzymes, including the indomethacin-sensitive PHS. To test this hypothesis, incubation mixtures, alternately containing microsomes from bladder, prostate, or colon, were fortified with known cofactors for other peroxidative enzymes such as lipoxygenase and NAPDH oxidase (27-31). The results shown in Table 3 indicate that lino- lenate will support the metabolism of benzidine to bind DNA with each of the tissue types. In contrast, only the colon microsome samples catalyzed the NAPDH-dependent activation of benzidine to bind to the DNA.

The presence of PHS in the tissues examined was verified by a competitive A-B ELISA for PHS antigen in the microsomal samples. The sensitivity of the assay (50% inhibition of antibody polymers were formed (Table 1). On this basis, it was determined that seven samples of colon microsomes would not catalyze the binding of benzidine to DNA (Table 1). In addition, the binding activity of the most active bladder microsomal sample was reduced 4-fold after freezing (Table 1), indicating that the enzyme is easily denatured. Likewise, both the arachidonic acid-dependent DNA binding of benzidine and the peroxidase-mediated acetaminophen polymer formation of colon microsomal preparations was lost after freezing (Table 1). However, the incidence and level of the colon peroxidase activity appears to be the same if measured in microsomes prepared from fresh or from frozen tissue. Thus, microsomes were assayed immediately after they were prepared from fresh or frozen tissue.

Larger samples of bladder and colon tissue afforded microsomal preparations of sufficient quantity to examine the arachidonic acid-dependent metabolic activation of several different aromatic amine substrates. The results in Table 2 indicate that a variety of carcinogenic arylamines and heterocyclic amines can be activated by arachidonic acid-dependent enzymes in human tissue to form DNA-bound products. For comparison, binding levels catalyzed by purified ram PHS are also shown. Benzidine was clearly the most active substrate for DNA binding catalyzed either by the purified PHS or by the bladder and colon microsomal preparations. 2-Naphthylamine, Glu-P-1, and 4-aminobiphenyl were also subject to peroxidative activation by selected microsomal preparations and were generally bound at higher levels than IQ or Trp-P-2.

Two alternative methods were utilized to characterize the nature of the peroxidative activity observed in the larger microsomal samples: (a) inhibition of benzidine-DNA binding or acetaminophen oxidation by indomethacin, a specific inhibitor of PHS cyclooxygenase activity (25, 26); and (b) immunochemical detection of Triton X-100-solubilized microsomal PHS protein antigenicity with a monoclonal anti-PHS antibody. The extent of indomethacin inhibition of benzidine activation mediated by human tissue microsomes varied widely, depending on the tissue type (Tables 1 and 2; Fig. 1). In general, indomethacin inhibited the arachidonic acid-dependent activities in the bladder, prostate, and colon samples that were tested. In contrast, indomethacin did not inhibit the arachidonic acid-dependent activity in the 2 of 12 microsome samples from placenta that catalyzed low levels of benzidine binding. The highest inhibition of benzidine binding (range, 60-100%) was observed in the bladder and prostate samples tested (Tables 1 and 2). Furthermore, indomethacin also blocked arachidonic acid-dependent acetaminophen polymer formation in cross-tested bladder and prostate samples but at reduced efficiency (range, 33-60%) as compared to the inhibition of benzidine-DNA binding (Table 1).

The results shown in Table 3 indicate that lino- lenate will support the metabolism of benzidine to bind DNA with each of the tissue types. In contrast, only the colon microsome samples catalyzed the NAPDH-dependent activation of benzidine to bind to the DNA.

The presence of PHS in the tissues examined was verified by a competitive A-B ELISA for PHS antigen in the microsomal samples. The sensitivity of the assay (50% inhibition of antibody binding) to human tissue microsomes varied widely, depending on the tissue type (Tables 1 and 2; Fig. 1). In general, indomethacin inhibited the arachidonic acid-dependent activities in the bladder, prostate, and colon samples that were tested. In contrast, indomethacin did not inhibit the arachidonic acid-dependent activity in the 2 of 12 microsome samples from placenta that catalyzed low levels of benzidine binding. The highest inhibition of benzidine binding (range, 60-100%) was observed in the bladder and prostate samples tested (Tables 1 and 2). Furthermore, indomethacin also blocked arachidonic acid-dependent acetaminophen polymer formation in cross-tested bladder and prostate samples but at reduced efficiency (range, 33-60%) as compared to the inhibition of benzidine-DNA binding (Table 1). A more varied result was observed in inhibition experiments with colon samples where inhibition of benzidine binding varied from 9 to 100% (Tables 1 and 2) and acetaminophen polymer formation was inhibited by indomethacin in only one of the four samples tested.

The varied results with indomethacin inhibition indicated that the arachidonic acid-dependent DNA-binding activity catalyzed by the tissue microsomes and, in particular, those in the colon, may be catalyzed by several enzymes, including the indomethacin-sensitive PHS. To test this hypothesis, incubation mixtures, alternately containing microsomes from bladder, prostate, or colon, were fortified with known cofactors for other peroxidative enzymes such as lipoxygenase and NAPDH oxidase (27-31). The results shown in Table 3 indicate that lino- lenate will support the metabolism of benzidine to bind DNA with each of the tissue types. In contrast, only the colon microsome samples catalyzed the NAPDH-dependent activation of benzidine to bind to the DNA.

The presence of PHS in the tissues examined was verified by a competitive A-B ELISA for PHS antigen in the microsomal samples. The sensitivity of the assay (50% inhibition of antibody binding) to human tissue microsomes varied widely, depending on the tissue type (Tables 1 and 2; Fig. 1). In general, indomethacin inhibited the arachidonic acid-dependent activities in the bladder, prostate, and colon samples that were tested. In contrast, indomethacin did not inhibit the arachidonic acid-dependent activity in the 2 of 12 microsome samples from placenta that catalyzed low levels of benzidine binding. The highest inhibition of benzidine binding (range, 60-100%) was observed in the bladder and prostate samples tested (Tables 1 and 2). Furthermore, indomethacin also blocked arachidonic acid-dependent acetaminophen polymer formation in cross-tested bladder and prostate samples but at reduced efficiency (range, 33-60%) as compared to the inhibition of benzidine-DNA binding (Table 1). A more varied result was observed in inhibition experiments with colon samples where inhibition of benzidine binding varied from 9 to 100% (Tables 1 and 2) and acetaminophen polymer formation was inhibited by indomethacin in only one of the four samples tested.

The varied results with indomethacin inhibition indicated that the arachidonic acid-dependent DNA-binding activity catalyzed by the tissue microsomes and, in particular, those in the colon, may be catalyzed by several enzymes, including the indomethacin-sensitive PHS. To test this hypothesis, incubation mixtures, alternately containing microsomes from bladder, prostate, or colon, were fortified with known cofactors for other peroxidative enzymes such as lipoxygenase and NAPDH oxidase (27-31). The results shown in Table 3 indicate that linolenate will support the metabolism of benzidine to bind DNA with each of the tissue types. In contrast, only the colon microsome samples catalyzed the NAPDH-dependent activation of benzidine to bind to the DNA.
binding) was 4.52 ± 1.21 ng purified PHS/well (Fig. 2). Representative inhibition curves, shown in Fig. 2, clearly demonstrate the presence of PHS antigen in samples of bladder, prostate, and lung microsomes. Furthermore, the reproducible nature of the A-B ELISA was demonstrated in the duplicate determinations with the bladder and prostate microsomes (Fig. 2). PHS antigen was not detected in two samples each of liver microsomal or placental microsomal protein using serial dilutions from 100 to 0.4 μg of protein/assay.

In contrast, atypical inhibition patterns were obtained for the five colon samples tested (data not shown). However, the general shape of the curve with a few colons suggested the presence of PHS antigen, and that a component of colon microsomes interfered with the ELISA. This problem was confirmed as aliquots of the colon microsomal protein added to the PHS standard, also interfered with quantitation of the PHS standard in some undetermined manner; while additions of BSA had no effect on the PHS standard curve. Therefore, the A-B ELISA could not be used to identify unequivocally, PHS antigen in the colon samples.

DISCUSSION

In these experiments, we have demonstrated that arachidonic acid-fortified microsomes obtained from extrapulmonary human tissues, including urinary bladder, colon, prostate, lung, and placenta, but not the breast or liver, support the metabolic activation of several aromatic amine carcinogens, presumably through peroxidation, to reactive intermediates that bind covalently to DNA. Furthermore, arachidonic acid-fortified microsomes from bladder, prostate, and colon also catalyzed the polymerization of acetaldehyde which is thought to occur through free radical intermediates (21, 22). These results are consistent with the peroxidative activities previously reported for human lung (32) and kidney (33), and for prostaglandin biosynthetic activity associated with cultured human bladder (34).

Indomethacin is a known specific inhibitor of PHS (25, 26). Inasmuch as indomethacin was a potent inhibitor of the arachidonic acid-dependent binding of benzidine catalyzed by bladder, prostate, or lung microsomes, as was the polymerization of acetaldehyde in reactions containing bladder and prostate microsomes, it is implied that these reactions are catalyzed mainly by PHS. Similarly, the failure of indomethacin to inhibit the arachidonic acid-dependent binding of benzidine in assays containing placental microsomes implies that reaction was not mediated by PHS. These results are supported by the A-B ELISA experiments which detected the presence of PHS antigen in bladder, prostate, and lung, but not placental microsomes. Also, benzidine was not activated by, and PHS antigen was not detected in, human liver microsomes. These results were consistent with previous findings that PHS-mediated peroxidation can result in the metabolic conversion of different classes of chemical carcinogens to reactive metabolites in extrahepatic mammalian tissues (32-39), but not in rat, rabbit, or dog liver (32, 35, 37).

In contrast, when the same criteria are applied to colon microsomal samples, it appears that the arachidonic acid-dependent metabolic activation of aromatic amines and polymerization of acetaldehyde is due to at least two types of peroxidative metabolism involving both indomethacin-sensitive and -insensitive pathways. Based on indomethacin inhibition studies of benzidine binding, the PHS content of colon appeared to vary over a 10-fold range (i.e., 9-100% inhibition). However, we were not able to unequivocally identify the presence of PHS antigen in colon microsomes by the A-B ELISA. Furthermore, certain samples of colon- and lung-microsomal preparations also catalyzed endogenous indomethacin-insensitive, arachidonic acid-independent, activation of benzidine to form DNA-bound products. Colon microsomes also catalyzed the DNA binding of benzidine in the linolenate- and NADPH-fortified reactions. Together, these observations and the fact that indomethacin effectively inhibits the PHS-cyclooxygenase action, but not the PHS-hydroperoxidase activity (33), suggests that there is more than one peroxidative activity in the colon; but we cannot exclude the presence of PHS. A similar pattern of peroxidative activities and conclusions have been described in experiments with rat colon microsomes (28, 34, 35).

The binding of benzidine to DNA in the presence of NADPH-fortified colon microsomes is interesting since benzidine is not activated by similarly fortified human bladder, prostate, or liver microsomes (Table 2), or by dog bladder, kidney, or liver microsomal preparations (37). Considering these studies and those of Craven et al. (28, 29, 39), it is apparent that the benzidine binding is not catalyzed by cytochrome P-450 and other monooxygenases in the colon. It seems more likely that NADPH-dependent oxidases would mediate this activation through production of endogenous hydroperoxides. These hydroperoxides could then function as oxidants for lipooxygenase (27, 30) or other peroxidases (31, 40, 41), including PHS (42, 43), resulting in benzidine activation through cooxidation.

The reasons underlying the variability of both the levels and incidence of peroxidative activity in the different tissues, in particular the colon, is not known. The identification of the peroxidase activity(ies) in colon also requires additional investigation. A component of colon microsomes, but not BSA, interfered with the A-B ELISA for purified PHS (see "Results"). BSA and colon microsomal protein also reduced the extent of benzidine binding to DNA (10 and 25%, respectively) in reactions catalyzed by purified PHS but not in a manner sufficient to explain failure to detect activity in two-thirds of the colon microsomal preparations. In rodent colon, the extent of PHS activity varies between the cell types (34); however, the small size of human samples precluded a similar analysis. Different physiological states, including the inflammatory response, can alter the levels of PHS, thus contributing to heterogeneity (44). Infiltration of inflamed tissue by eosinophils, neutrophils, macrophages, or platelets, all of which are rich

Fig. 2. Representative competitive avidin-biotin ELISA inhibition curves for PHS antigen in human tissue microsomes. X, purified ram seminal vesicle PHS; O,®, separate determinations with bladder microsomes; Δ, lung microsomes; ©, ¶, separate determinations with prostate microsomes.

5 Unpublished data.
sources of l-oxyperoxidase activity or PHS (45–49), could contribute to a variable response. Endogenous, indomethacin-resistant activity could also arise with inflammation, as the colonic mucosa of patients with chronic bowel diseases also secretes higher levels of the leukotriene B<sub>4</sub> (5-lipoxygenase-derived metabolite of arachidonate) than normal controls (50–52).

Drug treatments prior to surgery could also contribute to the variable results in the tissue samples as nonsteroidal antiinflammatory drugs inhibit the cyclooxygenase action of PHS (25, 26). Although patient anonymity precluded evaluation of this parameter for the individual samples, in general we know that tissue donors did not receive aspirin or other antiinflammatory agents for at least 24 h prior to surgery as a precaution against bleeding.

Bladder microsomes contain higher concentrations of PHS than do the other tissues, as judged by both the levels and indomethacin sensitivity of benzidine binding and on the capacity of the bladder microsomes to inhibit in the A-B ELISA for PHS antigen. Our results in this actuation pathway.

intermediates that bind to DNA and lead to the initiation of methacin-sensitive, arachidonic acid-dependent binding to and prostate epithelium and in peripheral lung tissue. Several acid-dependent binding of aromatic amines to DNA (53).

selected samples of the bladder and colon microsomes also catalyzed the arachidonic-dependent binding of the human bladder carcinogens, 2-naphthylamine and 4-aminobiphenyl to DNA at levels comparable to purified PHS (Table 3). 2-Naphthylamine has been previously shown to serve as a substrate for ram PHS (15, 53) and about 20% of the DNA adducts formed in the urothelium of 2-naphthylamine-treated dogs are estimated to be of peroxidative origin (53). Moreover, peroxidative pathways not involving PHS have been suggested (16). The significance of these observations in relation to arylamine-induced bladder cancer in humans is unknown; however, bladder microsomes obtained from the dog, a species also susceptible to arylamine-induced bladder cancer, contain both high levels of PHS and a high capacity to mediate the arachidonic acid-dependent binding of aromatic amines to DNA (53).

In conclusion, three separate criteria, (a) arachidonic acid-dependent, indomethacin-sensitive, formation of benzidine-DNA adducts; (b) acetaminophen polymer formation; and (c) immunochromatography detection of PHS antigen produced concordant evidence for the presence of PHS in human urinary bladder and prostate epithelium and in peripheral lung tissue. Several samples of colon microsomes supported highly variable indomethacin-sensitive, arachidonic acid-dependent binding to DNA and acetaminophen polymerization, and produced equivocal results in the A-B ELISA for PHS antigen. Our results also indicate that the peroxidation of aromatic amines in human extrahepatic: tissues may serve as a metabolic activation pathway by which these carcinogens can be converted to reactive intermediates that bind to DNA and lead to the initiation of the carcinogenic process. PHS may assume an important role in this activation pathway.

REFERENCES

6. Sugimura, T., Sato, S., Ohgaki, H., Takayama, S., Nagao, M., and Waka-
PEROXIDATIVE METABOLIC ACTIVATION IN HUMAN TISSUE


40. Rahimtula, A. D., and O’Brien, P. J. Hydroperoxide catalyzed liver micro

41. Nordblom, G. D., White, R. E., and Coon, M. J. Studies on hydroperoxide


45. Olsen, R. L., and Little, C. Purification and some properties of myeloper


49. Ho, P. P. K., Towner, R. D., and Esterman, M. A. Purification and charac


51. Sharon, P., and Stenson, W. Enhanced synthesis of leukotriene B4 by colonic muco


Arachidonic Acid-dependent Peroxidative Activation of Carcinogenic Arylamines by Extrahepatic Human Tissue Microsomes

Thomas J. Flammang, Yasushi Yamazoe, R. Wayne Benson, et al.


| Updated version | Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/49/8/1977 |

<table>
<thead>
<tr>
<th>E-mail alerts</th>
<th>Sign up to receive free email-alerts related to this article or journal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reprints and Subscriptions</td>
<td>To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a>.</td>
</tr>
<tr>
<td>Permissions</td>
<td>To request permission to re-use all or part of this article, contact the AACR Publications Department at <a href="mailto:permissions@aacr.org">permissions@aacr.org</a>.</td>
</tr>
</tbody>
</table>