Overexpression of the Heme Oxygenase Gene in Renal Cell Carcinoma

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Abstract. **Heme oxygenase (HO) activity has been implicated in the regulation of renal function and cell growth in normal and disease states. Expression of HO genes has been shown to regulate important hemoprotein(s) such as cytochrome P450. In the present study, HO activity was measured in samples of human adenocarcinoma, juxtatumor, and normal renal tissues. The samples were histologically examined to verify the malignant and normal nature. HO activity was 4-fold higher in the adenocarcinoma than in either normal or juxtatumor tissues. We designed a reverse transcriptase–polymerase chain reaction (RT-PCR) method to assess the presence of HO-1 and HO-2 mRNA in biopsy samples of various human renal tissues. Total RNA from renal samples was reverse transcribed and amplified simultaneously by PCR using specific primers for HO-1 and HO-2. Results show that both HO-1 and HO-2 mRNAs were expressed in all renal tissues examined and that HO-1 appeared to be amplified more than HO-2. Northern blot analysis revealed that HO-1 mRNA was elevated by several-fold in adenocarcinoma compared with juxtatumor or normal tissues. In contrast, no differences in HO-2 mRNA levels were observed using either RT-PCR or Northern blot. Cytochrome P450 arachidonic acid epoxygenase and ω-hydroxylase activities were markedly reduced in the tumor tissues, whereas, in the juxtatumor tissue, cytochrome P450 ω-hydroxylase activity was significantly increased. Northern blot analysis using cytochrome P450 cDNA probe 4A2 cDNA for the ω-hydroxylase gene family revealed that mRNA levels for ω-hydroxylase transcripts were significantly decreased in the adenocarcinoma compared with juxtatumor. The decrease in cytochrome P450 4AII mRNA levels correlated with a decrease in the arachidonic acid ω-hydroxylation metabolite, 20-HETE. The production of 20-HETE was significantly higher in juxtatumor in agreement with ω-hydroxylase mRNA. Higher levels of HO-1 may be a contributing factor for the undetectable levels of cytochrome P450 arachidonic acid metabolites, 20-HETE, in the adenocarcinoma. Our results suggest that increased generation of mitogenic activities by ω-hydroxylase and 20-HETE in the juxtatumor may be a contributing factor in the development and growth of neoplastic tissues, and the induction of HO in the tumor tissue may be an attempt to limit oxidative injury caused by the cytochrome P450 metabolites and other oxidative stress.** [P.S.E.B.M. 1997, Vol 214]

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A variety of oxidative stress-inducing agents, such as viral and bacterial toxin, metals, heme, and hemoglobin, have been implicated in the pathogenesis of the inflammatory process. The cellular response to such agents involves the production of a number of soluble mediators, including acute-phase proteins, eicosanoids, and various cytokines.

The rate-limiting enzyme in heme catabolism, heme oxygenase (HO), is a stress protein and its induction has been suggested to represent an important protective response against oxidative stress produced by heme and hemoprotein (1–7). Induction of HO may specifically decrease

cellular heme (prooxidant) and elevate bilirubin (antioxidant) levels (8–10). Elevation of HO in tumor-bearing rats (11) and in partial hepetectomy rats (12) resulted in a decrease in renal and liver hemoproteins such as cytochrome P450. Two HO isozymes, the products of distinct genes, have been described (13, 14). HO-1 is ubiquitously distributed in mammalian tissues and is strongly and rapidly induced by many compounds that elicit cell injury. The natural substrate of HO, heme, is itself a potent inducer of the enzyme (1). HO-2, which is believed to be constitutively expressed, is present in high concentrations in such tissues and in the brain and testis (13). In human skin fibroblasts, both HO-1 and HO-2 genes contribute to the enzymatic activity in stressful states (15).

Endotoxin, interleukin-1, and other stress agents cause a rapid (within 5–10 min) activation of the HO gene and subsequent accumulation of HO mRNA (16–18). This process involves transcriptional activation of several regulatory sites in the human HO promoter region (4, 19). A recent study from this laboratory demonstrated that the proximal promoter region of the human HO gene contains NFκB and AP-2 binding sequences (19). The finding of these binding sites on the HO promoter suggests the importance of HO in the stress response, when these transcriptional factors are known to be activated (19). Substantial evidence indicates that induction of renal HO resulted in a decrease in hemoproteins and thromboxane synthesis (20), and in cytochrome P450 arachidonic acid–dependent metabolites (21, 22). Human renal and liver cytochrome P450 metabolizes arachidonic acid epoxygenase and ω-hydroxylase to novel metabolites which have been shown to influence renal function (23, 24).

Several studies by our laboratory and others demonstrated that cytochrome P450 epoxygenase and ω-hydroxylase metabolite epoxyeicosatrienoic acid (EETs), and 20-HETE, respectively, possess a wide range of biological functions (20, 23–26). These include stimulation of peptide hormone release, modulation of Na^+, K^+-ATPase, vasodilation, and inhibition of platelet aggregation (for review, see references Refs. 27 and 28).

Our goal in these studies was to examine HO activity in human renal tissues and to determine whether HO-1 and HO-2 could be involved in heme catabolism in normal and pathological states (adenocarcinoma). We used reverse transcriptase–polymerase chain reaction (RT-PCR) method in conjunction with Northern blot analysis to assess the levels of HO-1 and HO-2 mRNA in adenocarcinoma, juxtatumor, and normal tissues; other investigators have also used this method with success in assessing the role of these two genes in oxidative stress (29).

Our data demonstrate that it is possible to assess the basal level of HO-1 and HO-2 in renal biopsies, using RT-PCR, and that Northern blot analysis confirmed these findings. There was overexpression of the HO-1 gene in adenocarcinoma compared with juxtatumor and normal tissues. Our study also demonstrated that elevation of HO-1 mRNA in adenocarcinoma may be responsible for the observed decrease in the hemoprotein and cytochrome P450-dependent arachidonic acid metabolism. These data also directly indicate that heme catabolism in adenocarcinoma is a result of overexpression of HO-1, not HO-2.

Materials and Methods

Handling of Human Kidney Samples. The specimens were obtained immediately after nephrectomy. Each kidney was cut horizontally into two parts. Three samples ($\approx$1 cm^3) were removed and placed in liquid nitrogen. The normal kidney sample was taken from a cortical portion of the kidney not in the vicinity of the tumor. The juxtatumor was excised from apparently normal tissue adjacent to the adenocarcinoma (3 mm). When the tumor sample was removed, areas with a high degree of necrosis were excluded. A portion of each specimen was processed for histological examination by a pathologist.

Preparation of Microsomes. Tissues were placed in ice-cold 0.15 *M* KCl and homogenized (4 ml/g wet wt) in 10 m*M* Tris buffer, pH 7.5, containing 0.25 *M* sucrose. The tissue homogenates were centrifuged at 27,000*g* for 20 min at 4°C. The supernatant was centrifuged at 105,000*g* for 1 hr at 4°C, and the resulting microsomal pellet was resuspended in 0.1 *M* potassium phosphate buffer, pH. 7.6, and used for determination of cytochrome P450 content, cytochrome P450 monooxygenase activities (aryl hydrocarbon hydroxylase and arachidonic acid epoxygenase, ω/ω-1 arachidonate hydroxylases) and HO activity. Protein concentration was determined by the method of Lowry *et al.* (30) with bovine serum albumin (Fraction V) as a standard.

Cytochrome P450–Arachidonic Acid Epoxygenase and ω-Hydroxylase. Arachidonic acid epoxygenase and ω-hydroxylase activities were measured as previously described (23, 24). Briefly, microsomal suspensions (0.3 mg protein) were incubated with [1-^{14}C]-arachidonic acid (0.4 μCi, 7 μ*M*) with or without β-nicotinamide adenine dinucleotide phosphate (NADPH) (1 m*M*), or SKF-525A (100 μ*M*) or both, for 30 min at 37°C. The reaction was terminated by acidification with citric acid to pH 4.5–5.0 and extracted twice with two volumes of ethyl acetate. The extraction efficiency was 70%–75%. The final extracts were evaporated to dryness under N_2 and resuspended in 100 μl of methanol. Arachidonic acid metabolites were separated by reverse-phase HPLC on a C_{18} Bondapack column (Z module; Waters, Milford, MA) with a linear gradient from acetonitrile/water/acetic acid (50:50:1, v/v/v) to acetonitrile: acetic acid (100:1, v/v) at a flow rate of 1 ml/min for 40 min. Radioactivity was monitored by a flow detector (Radiomatic Instruments and Chemicals, Tampa, FL). In addition, fractions of 0.5 ml each were collected and aliquots from each fraction were taken for liquid scintillation counting. The recovery efficiency of the separation procedure was 80%–85%.

Assay of HO Activity. The activity of human kidney microsomal HO in all tissues was assayed as previously

described (31). Briefly, the volume of the assay medium was 1.0 ml and contained 105,000*g* microsomal supernatant fraction (3.0 mg of protein) as a source of biliverdin reductase and 17 μmol of hemin. The reaction was terminated after 15 min by addition of 0.1 ml of 0.01 *M* HCl. The mixture was extracted three times with 1 ml of chloroform to remove bilirubin. The chloroform fraction was then evaporated under N_2-oxygen free, to a final volume of 0.8 ml, and the extract was scanned in an Aminico DW-2C spectrophotometer in the split-beam mode. The concentration of bilirubin was calculated from the difference in absorption between 465 and 530 nm utilizing an absorption coefficient of 40 $nM^{-1} \cdot cm^{-1}$. Differing conditions used for the assay of HO are outlined in the legends to the appropriate figures.

RNA Extraction and Northern Blot Analysis. Total RNA was extracted by the technique of Chomczynsky and Sacchi. Briefly, tissue was homogenized in a solution containing 4 *M* guanidine thiocyanate; 25 m*M* sodium citrate, pH 7.0; 0.5% sarcosyl; and 0.1 *M* 2-mercaptoethanol. RNA was extracted first with phenol-chloroform followed by an additional extraction with chloroform and precipitated with isopropanol. Total RNA was vacuum dried, redissolved in water and treated with diethyl pyrocarbonate. Concentration and purity of RNA ($O.D._{260\ nm}/O.D._{280\ nm} > 1.7$ in all samples) was determined. Total RNA (20 μg) from various tissue samples was denatured, electrophoresed on 1% agarose gels containing 1 *M* formaldehyde, transferred to Hybond N^+ filter (Amersham, Arlington Heights, IL) and hybridized with the cDNA for the human kidney CYP 4AII (*Nde*I-*XbQ*I fragment of pBluescript), which was a generous gift from Dr. Eric Johnson, The Scripps Research Institute, La Jolla, CA. The probe used for HO-1 was the 833 bp *Eco*RI/*Hin*dIII fragment prepared in the pRHOI vector, a plasmid containing full-length cDNA for HO (18). The probe for HO-2 was generated by RT-PCR from human kidney poly(A)$^+$RNA preparations as described in the RT-PCR method and purified by agarose gel electrophoresis. The probe was labeled with ^{32}P using the Nick Translation System (Promega, Madison, WI). The hybridization solution contained 1% BSA, 7% SDS, 1 m*M* EDTA, and 2× SSPE. The temperature was 60°C. The filters were washed in 0.5% BSA, 5% SDS, 1 m*M* EDTA, and 0.2× SSPE at 60°C for 30 min with two changes of solution. X-ray films were exposed to the filters at −80°C with an intensifying screen.

RT-PCR. The method used for RT-PCR has been previously described (29). Briefly, 5 μg of RNA were reverse transcribed using the 1st-Strand cDNA synthesis kit (Clontech, Palo Alto, CA). The PCR was performed on a Perkin Elmer Cetus DNA Thermal Cycler. Primers 5′-CAGGCAGAGAATGCTGAGTTC and 5′-GCTTCACATAGCGCTGCA were used to amplify the 79- to 429-bp region of HO-1 cDNA. Primers 5′-GCAATGTCAGCGGAAGTGGAA and 5′-AATGCACCTGAGGTGGTAGTT were used to amplify the −3- to 1036-bp region of HO-2 cDNA. The reaction mixture (50 μl) contained primers for both cDNAs (0.5 μ*M* each), dNTP (200 μ*M*), [^{32}P]dCTP (5 μCi; 3000 Ci/mmol), 10 m*M* Tris HCl, pH 8.3, 75 m*M* KCl, 3 m*M* $MgCl_2$, and 2.5 U Taq DNA polymerase. After layering with 50 μl of mineral oil to prevent evaporation, 22 cycles were performed. Each cycle consisted of 30 sec at 94°C, 60 sec at 58°C, and 60 sec at 72°C. PCR products (20 μl) were separated on a polyacrylamide gel (4%). The dried gel was exposed to x-ray film.

Statistical Analysis. Results are expressed as mean ± SEM. Statistical significance was determined by analysis of variance (ANOVA) for multiple comparisons, followed by a modified *t* test to determine differences among groups. In all test cases, a critical significance level of 0.05 was assumed for statistical significance.

Materials. β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose-6-phosphate, bovine serum albumin, benzo(a)pyrene and 7-ethoxyresorufin were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose-6-phosphate dehydrogenase was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). All other chemicals, or highest quality, were obtained from either Sigma or Fisher Scientific (Springfield, NJ). [1-^{14}C]-arachidonic acid (56.1 mCi/mmol) was obtained from Amersham Co. (Arlington Height, IL).

Results

HO Activity in Normal Human Renal and in Adenocarcinoma Tissues. Biopsies of tumor and normal tissues were taken from the adenocarcinoma and juxtatumor tissue sites, respectively, of the same kidney just after nephrectomy. Each sample was processed for histological and morphological examination. HO activity was measured in adenocarcinoma and juxtatumor tissue biopsies from six patients undergoing nephrectomy. As seen in Figure 1, in all patients the activity of HO in the adenocarcinoma tissue was 3- to 6-fold higher than the average value in the normal tissue adjacent to the tumor of the same kidney. The adeno-

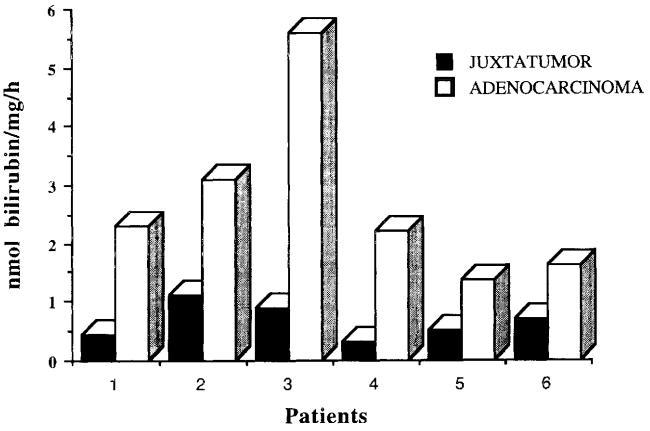

Figure 1. HO activities in tumor and juxtatumor tissues of the hypernephroma kidneys. Microsomes were prepared by different centrifugations and microsomal HO activity was determined by measuring bilirubin formation as described in Materials and Methods. ■, juxtatumor kidney tissue; □, adenocarcinoma.

carcinoma tissue demonstrated an activity that ranged from 1.26 to 5.62 nmol bilirubin/mg/hr (2.66 ± 0.64 [mean ± SEM]; $n = 6$), while in the juxtatumor tissue, HO activities ranged from 0.38 to 1.04 nmol bilirubin/mg/hr (0.66 ± 0.11; $n = 6$). In normal tissues, HO activity was expressed at the same level as that seen in juxtatumor tissues. These results indicated a 4-fold difference ($P < 0.01$) between the carcinoma and juxtatumor tissues. HO activity in the juxtatumor tissues demonstrated a level comparable to that described previously for normal human kidney (23).

HO-1 and HO-2 mRNA Levels in Normal Kidney Tissues, Adenocarcinoma, and Juxtatumor. Expression of the two HO genes has not been evaluated in normal human kidney nor in adenocarcinoma. HO-2 gene expression is elevated in human skin fibroblasts and contributes to the enzyme activities at levels similar to HO-1 (15). We designed an RT-PCR method for evaluation of HO-1 and HO-2 in human kidney tissues and evaluated their basal levels in adenocarcinoma. Two hundred nanograms of total RNA extracted from various renal tissues were reverse transcribed into cDNA as previously described (32) and cDNA was amplified by PCR. Amplified RT-PCR products showed that both HO-1 and HO-2 primers yielded amplification products of the expected size, 350 and 1039, respectively. In addition, the amount of HO-1 amplified from RNA extracted from the adenocarcinoma appeared to be much higher (Fig. 2, Lane 2) than HO-1 amplified from RNA extracted from normal or juxtatumor (Fig. 2, Lanes 1 and 3, respectively).

The RNA from the same tissue amplified using HO-2 primers in the same tube did not show any increase in the RT-PCR products (Fig. 2). Identical results were obtained in six different biopsy donors. When the radioactivity in each RT-PCR signal of HO-1 and HO-2 was assessed in Figure 2 and the ratio of expression of HO-1 to HO-2 was plotted (Fig. 2, lower panel), the results indicated that HO-1 mRNA levels appeared to be increased several-fold higher in adenocarcinoma cells compared with normal or juxtatumor cells.

Northern Blot Analysis of HO-1 Expression in Normal Kidney, Adenocarcinoma, and Juxtatumor. The RNA preparations from the different samples were also subjected to Northern blot analysis and probed for HO-1, HO-2, and G3PDH. As can be seen in Figure 3, Lane 2, the level of HO-1 mRNA in the adenocarcinoma was elevated compared with the transcript levels in normal or juxtatumor (Fig. 3, Lanes 1 and 3, respectively). In contrast, HO-2 transcript levels did not change in any of the three renal tissues. Evaluation of the intensity of the bands by scanning densitometry indicate that adenocarcinoma RNA contains several-fold higher levels of HO-1 mRNA compared with normal tissues. On the other hand, HO-2 mRNA levels did not change in all three tissues (Fig. 3, middle panel). Hybridization of the filters with a radiolabeled G3PDH probe confirmed that a similar amount of total RNA was transferred to the filters in each lane of the paired experiment (Fig. 3, lower panel).

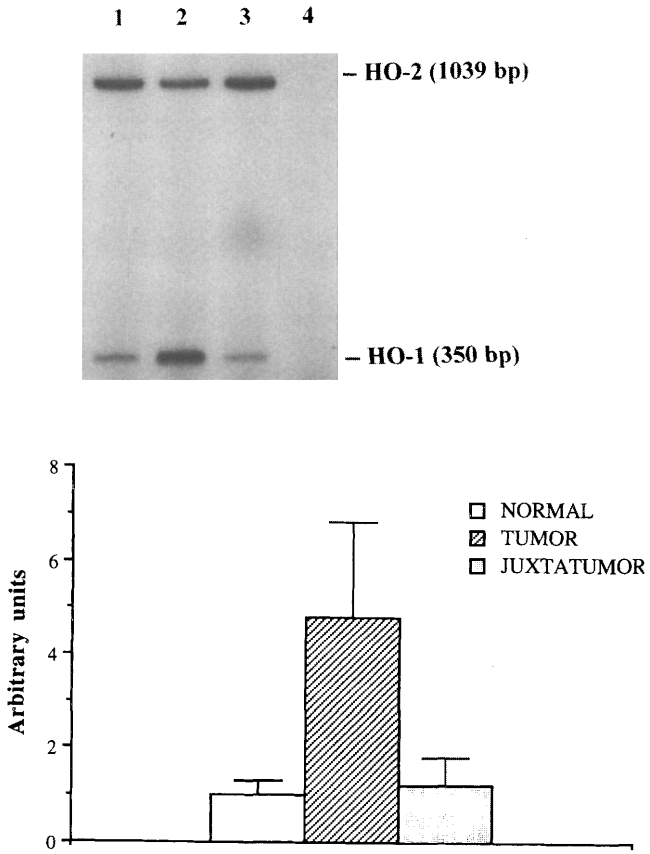

Figure 2. RT-PCR analysis of HO-1 and HO-2 mRNA in human kidney. RT-PCR with primers for HO-1 and HO-2 was performed as described in Materials and Methods. The upper panel indicates that the size of the HO-2 band is 1039 bp and that of the HO-1 band is 350 bp. Lane 1, normal human kidney; Lane 2, renal adenocarcinoma; Lane 3, juxtatumor; Lane 4, negative control—no RNA added. The lower panel indicates the ratio of H-HO-1/H-HO-2 after RT-PCR. The graph represents the mean ± SEM of three different sets of tissues from different patients.

Cytochrome P450–Arachidonic Acid Epoxygenase and ω-Hydroxylase Activity. Arachidonic acid metabolite formation was absolutely dependent on NADPH addition and inhibited by SKF-525A, an inhibitor of cytochrome P450-dependent enzymes. In the absence of NADPH, the conversion of ^{14}C-arachidonic acid was very low (≈3% of total radioactivity) and accounted for mainly by cyclooxygenase metabolites. In the presence of NADPH, arachidonic acid was converted into cytochrome P450 metabolites by all maternal kidney tissue, and to a very low level by the adenocarcinoma tissue samples. A characteristic reverse-phase HPLC chromatogram of cytochrome P450 NADPH-dependent arachidonic acid metabolites formed by microsomes of maternal kidney tissue of Subject B. C. is shown in Figure 4. In the presence of NADPH, two major radioactive peaks at 17 and 20 min were co-eluted with authentic standards of 11,12-DHT and 19/20-HETE, respec-

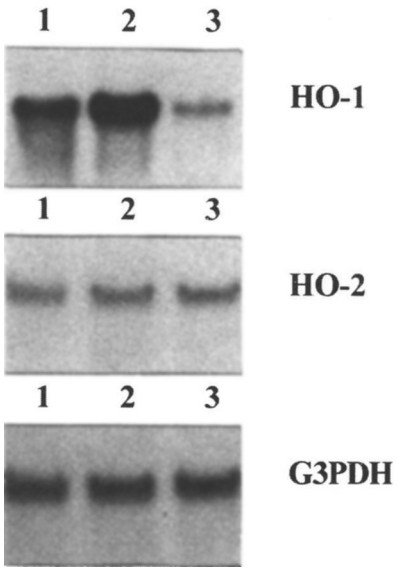

Figure 3. Northern blot analysis of RNA from human kidney. Total RNA (10 μg/lane) was separated on a 1% agarose gel containing 1 *M* formaldehyde. After blotting to Hybond N^+ membranes, the blot was probed with a ^{32}P-labeled cDNA for human HO-1, as described in Materials and Methods. Lane 1, normal kidney; Lane 2, renal adenocarcinoma; Lane 3, juxtatumor.

tively (Fig. 4A). The formation of these radioactive metabolites was inhibited by SKF-525A and was abolished in the absence of NADPH (Fig. 4, B and C, respectively). The activity of ω/ω-1 hydroxylase was determined as the amount of 19/20-HETEs formed per milligram protein in 30 min, whereas the activity of arachidonic acid epoxygenase was considered the sum of DHT and EET formed. In some incubates, the amount of EET (eluting between 24 and 26 min) was very low. This may be due to an active epoxide hydrolase or to nonenzymatic hydrolysis of EETs during acid-lipid extraction.

Similar HPLC chromatograms were obtained for all tissues. We compared cytochrome P450 epoxygenase and ω/ω-1 hydroxylase in renal adenocarcinoma and juxtatumor tissues. As previously described (23), interindividual variations in the activities of the cytochrome P450 enzymes were seen which ranged in juxtatumor tissues between 6.6 and 284.8 pmol/mg/30 min for epoxygenase and 66.0 and 679.8 pmol/mg/30 min for ω/ω-1 hydroxylases, similar to those that have been described for normal human kidneys (23). A dramatic reduction down to undetectable levels in epoxygenase and ω/ω-1 hydroxylase activities in the carcinoma

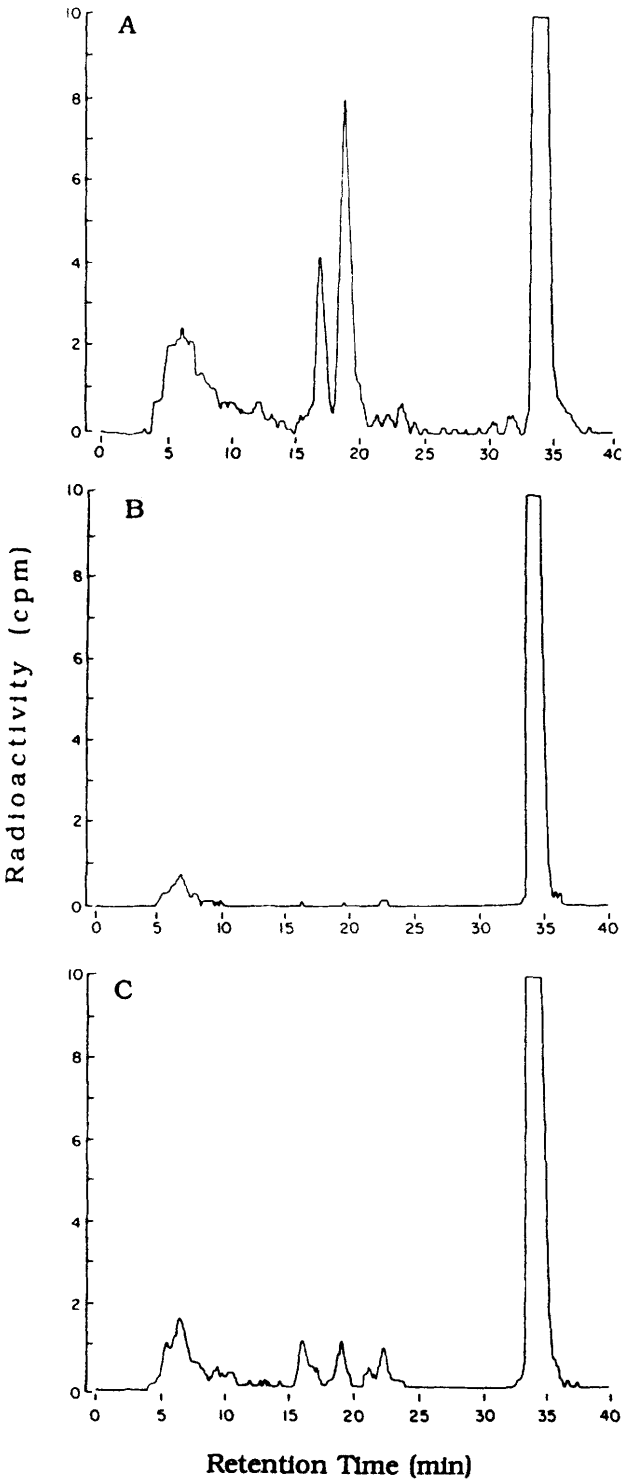

Figure 4. A representative reverse-phase HPLC chromatogram of arachidonic acid metabolites formed by hypernephroma microsomes of Subject B. C. Microsomes (0.3 mg protein), prepared by differential centrifugation, were incubated with ^{14}C–arachidonic acid (0.4 μCi, 7 μ*M*) in the presence and absence of NADPH (1 m*M*) and SKF-525A (100 μ*M*) for 30 min at 37°C. Arachidonic acid metabolites were extracted and separated by reverse-phase HPLC as described in Materials and Methods. I, DHT; II, 19- and 20-HETE; AA, arachidonic acid. (A) In the presence of NADPH. (B) In the absence of NADPH. (C) IN the presence of NADPH and SKF-525A.

tissues of all patients was observed. Juxtatumor tissue demonstrated averages of 304.7 ± 95.6 pmol/mg/30 min for ω/ω-1 hydroxylase and 161.86 ± 44.49 pmol/mg/30 min for epoxygenase, which decreased in the carcinoma tissue to 12.15 ± 7.49 and 14.68 ± 9.49 pmol/mg/30 min, respectively. This represents a significant difference of $P < 0.03$ by paired t test.

Northern Blot Analysis of CYP 4AII Expression. Since the formation of HETEs was significantly different between normal and malignant tissue, we examined the tissue distribution of ω-hydroxylase expression. We used the cDNA for CYP 4AII as a probe, which was recently cloned from human kidney and shown to be an ω-hydroxylase. As can be seen in Figure 5, the expression of CYP 4AII mRNA was much higher in normal kidney and juxtatumor than in the adenocarcinoma tissue. In some patients, CYP 4AII was not detectable in adenocarcinoma tissues. Thus, the very low or undetectable formation of HETEs in the tumor coincides with the low expression of ω-hydroxylase in the same samples. These differences were found in cytochrome P450 AII, but not with other cytochrome P450 enzyme activities such as benzo(a)pyrene hydroxylation (data not shown).

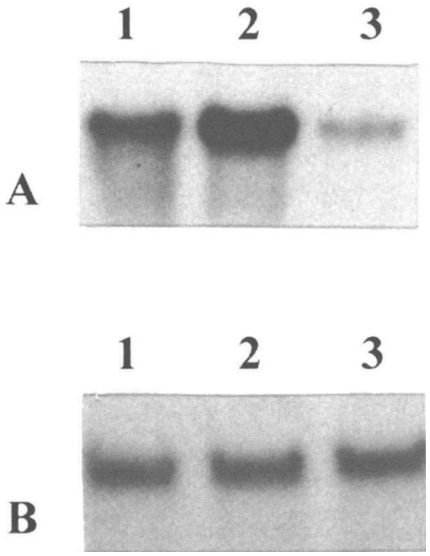

Figure 5. Northern blot analysis of total RNA from human kidney. Total RNA (20 μg/lane) was separated on a 1% agarose gel containing 1 *M* formaldehyde, blotted to Hybond N^+ membranes and probed with a cDNA for CYP 4AII, as described in Materials and Methods. Lane 1, normal kidney; Lane 2, juxtatumor; Lane 3, renal adenocarcinoma.

Discussion

In the present report, we demonstrated that HO activity in normal human kidney is a result of the activity of two genes. RT-PCR and Northern blot analysis demonstrated that both the HO-1 and HO-2 genes are operative, however, HO-2 mRNA appeared not to be increased in the adenocarcinoma. Northern blot analysis confirmed the RT-PCR results, that HO-1 mRNA is elevated several-fold higher in stress tissues, adenocarcinoma, compared with juxtatumor tissue. Therefore, elevated HO activity in the adenocarcinoma may be attributed solely to HO-1 gene expression. However, renal adenocarcinoma is not the only malignancy in which an alteration of HO activity has been observed. It has also been reported in lymphosarcoma bearing rats (11). Therefore, a relation between malignant behavior and an alteration of HO may exist. This alteration of HO may be the result of local or circulating factors released from malignant cells. Also, hypoxia and oxidative stress occurs through uncontrolled and uncoordinated cell growth, as well as insufficient blood supply in cancerous states. Thus, the elevation of HO-1 in renal adenocarcinoma is also in agreement with the reported finding that induction of HO-1 is a general response to oxidative stress (1–4).

Elevated HO levels in renal adenocarcinoma may be an attempt to provide a protective mechanism against oxidative stress by promoting bilirubin formation and causing a depression of heme and hemoprotein among the cytochrome P450 proteins. The latter may be responsible for the generation of free radicals and may lead to membrane and cell damage, as well as to the formation of endogenous substances that promote tumor growth. On the other hand, cytochrome P450 activity may be important for detoxification of carcinogens, activation of chemotherapeutic drugs and generation of endogenous substances that may attenuate the growth and development of the tumor. If this be the case, inhibitors of HO activity may have potential clinical significance for therapy.

HO-1 is a heat shock protein (33) and has been shown to be an acute phase protein (33, 34) and to be induced under conditions of oxidative stress. Neuzil *et al.* suggest that induction of HO in stress situations enables the rapid metabolism of heme and the generation of bilirubin, a metabolite with potent antioxidant properties (10). Others have shown that increased HO activity, probably by releasing iron from heme, engenders increased ferritin content (9). Thus, an increase in ferritin, the dominant intracellular repository of iron, allows the safe sequestration of the liberated iron and reduces free radical formation. Therefore, enhanced HO activity in situations of stress may be an adaptive, protective mechanism, including the degradation of released heme, the generation of bilirubin, and the procurement of ferritin for iron storage.

Induced expression of HO in proliferating tumor cells may result in the low content of several hemoproteins such as cytochrome P450 isozymes. In fact, cytochrome P450,

arachidonic acid epoxygenase and arachidonic acid ω-hydroxylase activities were markedly decreased in tumor cells. Since a great variety of drugs are metabolized *via* cytochrome P450, we cannot rule out the possibility that increased activity of cytochrome P450 in the juxtatumor tissue, and/or much lower cytochrome P450 content in hypernephroma tissue itself, may be associated with resistance of renal cell carcinoma to cytotoxic therapy (35). Similar to our results, Roy and Liehr (36) found in estrogen-induced kidney tumors in male Syrian hamster cytochrome P450 below detectable levels, while in kidney tissue surrounding estrogen-induced tumors, cytochrome P450 was present, but 50%–60% less than those in untreated control kidney.

Cytochrome P450 epoxygenase and ω-hydroxylase activity was 10 and 30 times higher in juxtatumor tissue than in the adenocarcinoma. We further showed by Northern blot analysis that the RNA level for the CYP 4A2, a P450 fatty acid ω-hydroxylase, is markedly reduced in the carcinoma tissues, in some patients, below detectable levels. In contrast, CYP 4A2 mRNA was elevated in the juxtatumor samples. Recently, we have demonstrated that 20-HETE, the arachidonic acid ω-hydroxylation product, is a potent mitogen to proximal tubular epithelial cell (37). It has also recently been shown that 14,15-EET, a cytochrome P450 epoxygenase metabolite, increases mesangial cell growth (38). Sellmayer *et al.* (39) demonstrated that EGF-induced mesangial cell proliferation is mediated by cytochrome P450–arachidonic acid metabolite(s). Therefore, the possibility exists that cytochrome P450 metabolites promote tumor growth.

In summary, the reciprocal relationship between HO-1 and cytochrome P450 may dictate the state of tumor growth and development, as well as tumor resistance to treatment and the ability of the surrounding, normal tissue to metabolize endogenous substrates such as arachidonic acid, the products of which may possess the ability to modulate growth of the tumor. Although these speculations are not proven in the current results, they suggest the need to examine further the role of HO-1 levels and cytochrome P450–arachidonic acid metabolites in tumor growth and development.

Note. While this manuscript was in preparation, a paper appeared in J Urology **47:**727–733, 1996, describing increased HO-1 expression in BPH and malignant prostate tissue. These results confirm the findings reported here that HO-1 gene expression may be a stress response to abnormal tissue growth.

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