Detection of Human CYP2C8, CYP2C9, and CYP2J2 in Cardiovascular Tissues

Tracy C. DeLozier, Grace E. Kissling, Sherry J. Coulter, Diana Dai, Julie F. Foley, J. Alyce Bradbury, Elizabeth Murphy, Charles Steenbergen, Darryl C. Zeldin, and Joyce A. Goldstein

Human Metabolism Section, Laboratory of Pharmacology and Chemistry (T.C.D., S.J.C., D.D., J.A.G.), Laboratory of Respiratory Biology (J.A.B., D.C.Z.), Laboratory of Experimental Pathology (J.F.F.), Biostatistics Branch (G.E.K.), and Laboratory of Signal Transduction (E.M.), National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; and Department of Pathology, Duke University Medical Center, Durham, North Carolina (C.S.)

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ABSTRACT:
The cytochrome P450 (P450) enzymes CYP2C8, CYP2C9, and CYP2J2 metabolize arachidonic acid to epoxygenesatrienoic acids, which are known to be vital in regulation of vascular tone and cardiovascular homeostasis. Because there is limited information regarding the relative expression of these P450 enzymes in cardiovascular tissues, this study examined the expression of CYP2C8, CYP2C9, and CYP2J2 mRNA and protein in human heart, aorta, and coronary artery samples by real-time polymerase chain reaction, immunoblotting, and immunohistochemistry. CYP2J2 and CYP2C9 mRNA levels were highly variable in human hearts, whereas CYP2C8 mRNA was present in lower abundance. CYP2J2 mRNA was approximately 10² times higher than CYP2C9 or CYP2C8 in human heart. However, CYP2C9 mRNA was more abundant than CYP2J2 or CYP2C8 in one ischemic heart. In human aorta, mean CYP2C9 mRNA levels were ~50 times higher than that of CYP2J2 and 5-fold higher than that of CYP2C8. In human coronary artery, mean values for CYP2C9 mRNA were ~2-fold higher that of CYP2J2 mRNA and 6-fold higher than that of CYP2C8 mRNA. Immunoblotting results show relatively high levels of CYP2J2 and CYP2C8 protein in human hearts, which was confirmed by immunohistochemistry. CYP2C9 protein was also detected at high levels in one ischemic heart by immunoblotting. CYP2C9 was present at higher levels than CYP2J2 in aorta and coronary artery, whereas CYP2C8 protein was below the limits of detection. The expression of CYP2J2 and CYP2C8 in human heart, and CYP2C9 and CYP2J2 in aorta and coronary artery is consistent with a physiological role for these enzymes in these tissues.

Coronary heart disease, including myocardial infarction, is one of the leading causes of morbidity and mortality in North America. It is now known that the endothelium, a single layer of cells lining the vascular wall, plays a significant role in regulation of vascular tone and homeostasis (Busse and Fleming, 2003). Disruption of endothelial cell homeostasis can lead to hypertension, atherosclerosis, and cardiovascular diseases (Cines et al., 1998; Russo et al., 2002; Voetsch et al., 2004). Nitric oxide has been shown to play a significant role in regulation of vascular tone and homeostasis in humans (Fleming, 2001). CYP2J2 has been widely studied in human heart and has been found to be constitutively expressed with a large degree of interindividual variation (Wu et al., 1996). CYP2J2 is also localized in the endothelium of both large blood pressure (Sinal et al., 2000), and hypertension (Imig et al., 2002). CYP2C enzymes (CYP2C8/CYP2C9) have been proposed as aspirin resistance, and EETs are currently being studied as potential treatment targets.

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ABBREVIATIONS: ROS, reactive oxygen species; P450, cytochrome P450; EET, epoxyeicosatrienoic acid; EDHF, endothelium-derived hyperpolarizing factor; PCR, polymerase chain reaction.
and small coronary arteries (Node et al., 1999), CYP2C9 and CYP2C8 mRNAs have been reported in human myocardium (Enayetallah et al., 2004) as well as endothelial cells (Lin et al., 1996). More specifically, polymorphisms in CYP2J2, CYP2C8, and CYP2C9 have been implicated in cardiovascular disease (Yasar et al., 2003; Spiecker et al., 2004). One study (Yasar et al., 2003) suggested a link between myocardial infarctions and CYP2C9*2 and CYP2C9*3 polymorphisms in female patients as well as CYP2C8*3 polymorphisms in male and female patients.

It has been hypothesized that during reperfusion, after a long period of ischemia, the heart undergoes damage brought about by the presence of P450 enzymes in this tissue (Gottlieb, 2003). This damage occurs by the activation of cardiac P450 enzymes that lead to the production of ROS (Gottlieb, 2003). ROS is believed to contribute to myocardial infarction as well as vascular dysfunction by damaging DNA, proteins, and lipids. In contrast, CYP2J2 overexpression has been found to be cardioprotective after hypoxia/reoxygenation injury (Yang et al., 2001; Seubert et al., 2004). Inhibitors of P450s have also been shown to decrease postischemic infarct size in rats and rabbits (Granville et al., 2004; Gross et al., 2004; Hunter et al., 2005). More specifically, sulfaphenazole, a specific inhibitor of CYP2C9, has been shown to have a protective effect and has been proposed to be a possible therapeutic agent in patients with ischemic heart disease (Hunter et al., 2005).

Recent reports have provided limited information on the relative levels of the CYP2C enzymes in cardiovascular tissues (Thum and Borlak, 2000; Larsen et al., 2006). Because of their extensive homology, more specific antibodies and oligonucleotide primers are needed to determine the levels of the individual CYP2C8/CYP2C9 proteins and mRNAs. This study is the first, to our knowledge, to use specific quantitative and qualitative techniques to localize CYP2C8, CYP2C9, and CYP2J2 in human cardiovascular tissues. The findings provide evidence of the relative expression of these enzymes at the mRNA and protein level in cardiovascular tissues. Our data are consistent with the hypothesis that these enzymes may play a significant role in the regulation of cardiovascular homeostasis.

**Materials and Methods**

**Heart Tissues.** Paraffin blocks and frozen tissue of two human hearts (G and H) were obtained from the Cooperative Human Tissue Network, Southern Division (University of Alabama at Birmingham, Birmingham, AL). Five hearts (coded A–E) were obtained from the National Disease Research Interchange. One additional heart (F), and five coronary artery and five aorta tissues were obtained from discarded heart transplant tissues at Duke University Hospital. These tissues were considered exempt by the National Institute of Environmental Health Sciences Institutional Review Board for Research on Human Subjects since discarded tissues with no patient identifiers were used. Most heart samples were from the left ventricle; a list of heart samples and diagnoses of condition at the time of retrieval is given in Table 1. Available clinical information on coronary artery and aorta tissues from five patients (numbered 1–5) is shown in Table 2. Due to the availability of appropriate samples, coronary and aorta samples were obtained from the same patients, whereas heart samples originated from different individuals.

**Isolation of Total RNA and Preparation of cDNA.** Total RNA was extracted from human tissues using RNeasy from QIAGEN (Valencia, CA) following the manufacturer’s protocol. RNA (200 ng) was transcribed to cDNA using 200 units of Superscript II reverse transcriptase from Invitrogen (Carlsbad, CA), 100 ng of random hexamers, and 10 mM each, dGTP, dATP, dTTP, and dCTP in 1× buffer in a total volume of 20 μl following the manufacturer’s protocol. The reaction was initially incubated at 25°C for 2 min and then at 25°C for 10 min after addition of Superscript with a final incubation at 42°C for 50 min.

**Quantitative Real-Time PCR.** Quantitative real-time PCR was performed using the following primer and probe sets from Applied Biosystems (Foster City, CA): CYP2C8 (catalog number Hs00426387_m1), CYP2C9 (catalog number Hs00426397_m1), CYP2J2 (catalog number Hs00356035_m1), and GAPDH (catalog number Hs99999905_m). The TaqMan probe and primer sets used for CYP2C8, CYP2C9, CYP2J2, and GAPDH mRNA quantitation have been reported to have equal efficiencies (100 ± 10%) (http://docs.appliedbiosystems.com/pbi晓得/00113186.pdf). The cDNAs from each reversed transcribed reaction were diluted 4-fold, mixed with TaqMan 2X Universal PCR Master Mix No AmpErase UNG, and amplified using cycling conditions including a denaturation step at 94°C for 10 min, another denaturation step at 95°C for 15 s, and an annealing step at 60°C for 60 s for 45 cycles. Reactions were run in an ABI Prism 7900 Sequence Detector from Applied Biosystems. Relative mRNA expression of CYP2C8, CYP2C9, and CYP2J2 was determined using the ΔΔct method (value obtained by subtracting the Ct value of GAPDH mRNA from the Ct value of the target mRNA). Data were expressed as the ratio (calculated using 2ΔΔct) of target mRNA to GAPDH mRNA (Livak and Schmittgen, 2001). The studies were performed in triplicate at least three times and the data were summarized as mean ± S.E. values of all values.

**Preparation of Heart Microsomes and Aorta and Coronary Tissue Lysates.** Heart tissue was homogenized in 50 mM Tris-HCl, pH 7.4/1 mM EDTA/1.15% KCl/0.5 mM phenylmethylsulfonyl fluoride using an Omni tissue grinder (Omni International, Warrenton, VA) on ice, then centrifuged at 800g for 10 min. The resulting supernatant was centrifuged at 10,000 g for 2 min, two times. The 10,000 g supernatant was then recentrifuged at 100,000 g for 70 min. The pellet was washed in 100 mM sodium pyrophosphate, pH 7.4/0.1 mM EDTA and recentrifuged at 100,000 g for 70 min. All procedures were performed at 4°C. The final microsomal pellet was resuspended in 10 mM KPO4, pH 7.4/1 mM EDTA/20% glycerol and stored at −80°C.

To prepare aorta and coronary artery lysates, frozen tissues (0.01–0.1 ng) were added to 1 ml of lysis buffer [50 mM Tris-HCl, pH 7.4, 1% Triton X-100, 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (0.25 M stock), 0.25% sodium deoxycholate, 1 μg/ml aprotinin, 1 μg/ml pepstatin, 1 μg/ml leupeptin, 1 mM sodium orthovanadate, 1 mM sodium fluoride] and homogenized immediately on wet ice. The samples were transferred to dry ice for 5 min and then thawed on wet ice. After thawing, the samples were vortexed and centrifuged for 5 min at 13,400g at 4°C. The supernatants were sonicated and the resulting cell lysates stored at −80°C.

**Protein Immunoblotting.** Polyclonal rabbit anti-CYP2C8 and anti-CYP2C9 antibodies were raised in rabbits against recombinant CYP2C8 and CYP2C9 using protocols previously described (Cutler et al., 2004). Monoclonal antibodies against CYP2C9 were a gift from the National Institutes of Health. Oligonucleotide primers for human CYP2C8 and CYP2C9 genes were synthesized (Integrated DNA Technologies, Coralville, IA). PCR reactions were run in an ABI Prism 7900 Sequence Detector with universal PCR master mix (Applied Biosystems), using the following primer and probe sets from Applied Biosystems (Foster City, CA): CYP2C8 (catalog number Hs00426387_m1), CYP2C9 (catalog number Hs00426397_m1), CYP2J2 (catalog number Hs00356035_m1), and GAPDH (catalog number Hs99999905_m). The TaqMan probe and primer sets used for CYP2C8, CYP2C9, CYP2J2, and GAPDH mRNA quantitation have been reported to have equal efficiencies (100 ± 10%) (http://docs.appliedbiosystems.com/pbi晓得/00113186.pdf). The cDNAs from each reversed transcribed reaction were diluted 4-fold, mixed with TaqMan 2X Universal PCR Master Mix No AmpErase UNG, and amplified using cycling conditions including a denaturation step at 94°C for 10 min, another denaturation step at 95°C for 15 s, and an annealing step at 60°C for 60 s for 45 cycles. Reactions were run in an ABI Prism 7900 Sequence Detector from Applied Biosystems. Relative mRNA expression of CYP2C8, CYP2C9, and CYP2J2 was determined using the ΔΔct method (value obtained by subtracting the Ct value of GAPDH mRNA from the Ct value of the target mRNA). Data were expressed as the ratio (calculated using 2ΔΔct) of target mRNA to GAPDH mRNA (Livak and Schmittgen, 2001). The studies were performed in triplicate at least three times and the data were summarized as mean ± S.E. values of all values.
CYP2C9 were raised to bacterially expressed human CYP2C8 or CYP2C9 (Covance, Denver, Pa), respectively, using a standard NIEHS rabbit antibody production protocol. Recombinant CYP2C protein standards were from a yeast cDNA expression system since no N-terminal modification of the cDNA is used for expression of cytochromes P450 in yeast (Romkes et al., 1991). Therefore, the mobility of the yeast-expressed standards is identical to those of the human P450 proteins expressed in vivo. The CYP2J2-specific peptide antibody HMDQFNGRNPTM (anti-CYP2J2pep1) was prepared as described previously (Wu et al., 1996). CYP2J2 recombinant protein expressed in baculovirus was prepared as described previously (Wu et al., 1996).

Aorta and coronary artery lysates, heart microsomes, and recombinant P450 proteins were electrophoresed in SDS-10% (w/v) polyacrylamide gels and transferred to polyvinylidene difluoride membranes. Membranes were immunostained with rabbit anti-CYP2C8, rabbit anti-CYP2C9, and rabbit anti-CYP2J2 and goat anti-rabbit IgG conjugated to horseradish peroxidase from Pierce (Rockford, IL). Polypeptide bands were visualized using SuperSignal West Femto Chemiluminescent Substrate from Pierce and a SynGene GeneGnome chemiluminescence detection system from Synoptics (Cambridge, UK). Polypeptide bands representing authentic recombinant P450 standard proteins were quantitated with GeneTools version 3.06 (Synoptics) using manual band quantification and the quadratic curve-fitting option. Immunoreactive polypeptide bands in lysates and microsomes with electrophoretic mobility identical to those of the recombinant P450 standards were quantified by comparison to the standard curves for the recombinant proteins.

**Real-Time PCR.** The expression of CYP2C8, CYP2C9, and CYP2J2 mRNA in cardiovascular tissues was measured relative to GAPDH using real-time-PCR analysis. CYP2J2 mRNA expression was relatively abundant in all hearts. With the exception of heart H, CYP2J2 mRNA expression was greater than that of CYP2C9 (p < 0.01) or CYP2C8 (p < 0.05) in each of the remaining seven hearts (Fig. 1a). Mean values for expression of each mRNA relative to GAPDH are shown in Table 3. The mean for CYP2C9:GAPDH excluded heart H since the level of CYP2C9 mRNA in this heart was ~800 times greater than the mean for the remaining samples. Mean values for CYP2J2 mRNA were ~900 times higher than that of CYP2C9 (p < 0.0001) in the remaining seven hearts and 1600 times higher than that of CYP2C8 (p < 0.0001). In contrast, CYP2C9 mRNA expression was 200-fold greater than that of CYP2J2 (p < 0.05) and ~1000-fold greater than that of CYP2C8 (p < 0.05) in heart H from an individual who died from ischemic heart disease (Fig. 1a).

Individual human aortas exhibited varying levels of all three mRNAs. The CYP2C9 mRNA level was considerably higher than those of CYP2J2 (p < 0.05) in all five aortas and CYP2C8 mRNA in four of the aortas (p < 0.05) (Fig. 1b). CYP2C8 mRNA was also more abundant than CYP2J2 in aorta 5 (Fig. 1b). Mean values for CYP2C9 mRNA relative to GAPDH were ~50 times higher than those of CYP2J2 (p < 0.0001) and 5-fold higher than those of CYP2C8 (p < 0.0001) (Table 3). Individual coronary samples also exhibited considerable interindividual variability in expression of the three mRNAs. CYP2C9 mRNA expression was generally greater than CYP2J2 or CYP2C8 (Fig. 1c). Specifically, CYP2C9 mRNA was higher than CYP2J2 mRNA in four of five coronary artery samples (p < 0.02), and CYP2J2 was higher than CYP2C8 in (p < 0.01) in all samples except coronary artery 2. Mean values for CYP2C9 mRNA relative to GAPDH were ~2 times higher those of CYP2J2 (p < 0.0001) and 6-fold higher than those of CYP2C8 (p < 0.0001) (Table 3).

**Western Blotting.** Microsomal fractions of human liver and heart, and lysates of coronary artery and aorta were separated by electrophoresis and subjected to immunoblotting using antibodies to CYP2C8, CYP2C9, and CYP2J2. Recombinant human CYP2C8, CYP2C9, CYP2J2, CYP2C18, and CYP2C19 proteins were used as standards. A polyclonal antibody to CYP2C8 detected recombinant CYP2C8 as an immunoreactive band at 55 kDa, as shown by the arrow (Fig. 2a). The CYP2C8 antibody did not cross-react with recombinant CYP2C18, CYP2C19, or CYP2J2 proteins. Although the CYP2C8 antibody cross-reacts slightly with recombinant CYP2C9, the two proteins can be distinguished by their electrophoretic mobilities. An immunoreactive polypeptide band with molecular mobility identical to that of recombinant CYP2C8 protein was detected at comparable levels in all of the human heart microsomal samples except for heart H, where limited amounts of heart microsomes probably prevented detection of this protein. The amounts of CYP2C8 protein in the heart microsomes were estimated to be 0.20 ± 0.02 pmol/mg tissue by comparison with recombinant standards. The CYP2C9 antibody did not cross-react with recombinant CYP2C8 or CYP2J2 protein. Although the CYP2C9 antibody cross-reacted slightly with recombinant CYP1C9, the two proteins can be distinguished by their mobilities. CYP2C9 protein was below the level of detection in immunoblots of most heart microsomes with the exception of heart H. Despite the limited amount of this heart sample, CYP2C9 was clearly detected at relatively high levels (~5.5 pmol/mg). Notably, heart H also contained high amounts of CYP2C9 mRNA (Fig. 2a). The CYP2J2 antibody did not cross-react with

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<th>Clinical information for human coronary and aorta tissues</th>
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<td><strong>Coronary Tissues</strong></td>
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| **Aorta Tissues** | **Diagnosis** |
| Aorta 1 | No atherosclerosis |
| Aorta 2 | Minimal atherosclerosis |
| Aorta 3 | No atherosclerosis |
| Aorta 4 | No atherosclerosis |
| Aorta 5 | No atherosclerosis |
recombinant CYP2C8, CYP2C9, or CYP2C18 proteins. CYP2J2 protein was detected at relatively high levels in individual heart microsomes, but showed some interindividual variability (0.05–0.4 pmol/mg; mean = 0.17 ± 0.05 pmol/mg) (Fig. 2a).

In lysates from human aorta, CYP2C9 protein was detected at relatively high levels in tissues from all five individuals (1.3 ± 0.3 pmol/mg) (Fig. 2b). CYP2J2 protein was also detected on the immunoblots of the aorta lysates, albeit at significantly lower levels (0.055 ± 0.004 pmol/mg) than CYP2C9 protein (p < 0.01). CYP2C8 protein was not detected in human aorta by immunoblotting (<0.03 pmol/mg). The faint band detected with the CYP2C8 antibody has a slightly lower electrophoretic mobility (higher molecular weight) than CYP2C8 and probably represents cross-reactivity with CYP2C9 protein.

In lysates from human coronary arteries, CYP2C9 protein was detected in all five samples (1.7 ± 0.2 pmol/mg) (Fig. 2c). Significantly smaller amounts of CYP2J2 (0.06 ± 0.01 pmol/mg) (p = 0.001) were also detected in all coronary artery samples, whereas CYP2C8 protein was not detected in lysates of any of the coronary samples tested (<0.03 pmol/mg). As with aorta, a slightly higher molecular weight immunoreactive band was detected in the coronary artery samples with the antibody to CYP2C8, probably representing cross-reactivity to CYP2C9 protein.

**Immunohistochemistry.** Immunohistochemical staining with the CYP2J2 antibody revealed that CYP2J2 protein was expressed in the cytoplasm of the cardiomyocytes of heart G, as well as in the endothelium of the blood vessels at a 3+ intensity level (out of a 0 to 4+ scale). Immunohistochemical staining with the CYP2C8 antibody within heart G revealed more prominent expression of the CYP2C8 protein in the subendocardial zone of the myocardium (2+ intensity) than in the central portion of the myocardium (1+ intensity). Some, but not all, of the blood vessels in the myocardium also exhibited weak (1+ intensity) staining of the endothelium with the CYP2C8 antibody (Fig. 3). CYP2C9 protein could not be detected in heart G or heart H by immunohistochemistry with our antibody to CYP2C9.

**Discussion**

There have been numerous studies in recent years that have shed light on the potential importance of the cytochrome P450 enzyme superfamily in arachidonic acid metabolism and their physiological role in cardiovascular tissues (Roman, 2002; Gottlieb, 2003; Spiecker and Liao, 2005; Elbekai and El-Kadi, 2006). EET metabolites of P450 enzymes have a number of roles in the vasculature including their involvement in the relaxation of smooth muscle via activation of Ca2+-sensitive K+ channels and vasodilation. Despite an increasing amount of information supporting a physiological role of these enzymes in the cardiovascular tissue, studies identifying the individual P450 isoforms present in these tissues have been limited (Thum and Borlak, 2000; Larsen et al., 2006). It is widely accepted that CYP2J2 is the principal cytochrome P450 enzyme expressed in human heart at the mRNA and protein level (Wu et al., 1996; Node et al., 1999). The present study provides evidence for the relative expression of CYP2J2, CYP2C9, and CYP2C8 in cardiovascular tissues using specific PCR primers and antibodies to these isoforms.
In the present study, considerable interindividual variability was observed in the levels of CYP2J2, CYP2C9, and CYP2C8 mRNA and protein in human heart, coronary artery, and aorta tissues by real-time PCR, immunoblotting, and immunohistochemistry. The majority of the human hearts used in this study were nondiseased hearts. This study shows that CYP2J2 and CYP2C8 are the principal isoforms expressed in nondiseased human hearts. CYP2J2 mRNA was detected at considerably higher levels than either those of CYP2C8 or CYP2C9 mRNA in the majority of nondiseased human hearts. Despite the low levels of CYP2C8 mRNA, CYP2C8 protein was detected in all human hearts. Previous studies have noted discrepancies in expression of mRNA and proteins for P450 enzymes including CYP2J2, which could not be totally explained by genotype (Forrester et al., 1992; Gaedigk et al., 2006). Reasons for discrepancies between mRNA and protein could include the condition of the tissue and integrity of the RNA, differences in pretranslational regulation, and stability of the proteins or mRNA, as well as polymorphisms affecting protein expression. Surprisingly, CYP2C9 protein and mRNA were more abundant than those of either CYP2J2 or CYP2C8 in one heart that was from an individual with ischemia. It is interesting to note that hypoxia has recently been reported to increase CYP2C8 and/or CYP9 mRNA and protein expression in human umbilical vein endothelial cells in culture (Michaelis et al., 2005) and possibly increase transcriptional activity of a transfected CYP2C9 promoter. Our study was originally designed to examine CYP2J2 and CYP2C expression in nonfailing hearts and in aorta and coronary tissues, and thus contained only one diseased heart. Therefore, it is impossible to know whether the disease state contributed to the high CYP2C9 expression. Additional studies with sufficient numbers of nonfailing and failing hearts will be required to address whether there are differences in the expression of the CYP2C and CYP2J2 genes in heart failure from various etiologies.

EETs have been identified as likely candidates for EDHFs (Campbell et al., 1996; Li and Campbell, 1997). The importance of these EET metabolites generated by the P450 enzymes has been reported previously in cardiovascular tissues (Wu et al., 1996; Node et al., 1999; Roman, 2002; Speicker and Liao, 2005) and also have been highlighted in reviews (Campbell and Harder, 1999; McGuire et al., 2001). The vital importance of EETs in homeostasis has been seen in a transgenic mouse model, where overexpression of CYP2J2 resulted in increased EET biosynthesis and improved recovery of left ventricular function after cardiac ischemia and reperfusion (Seubert et al., 2004). These mice also exhibited enhancement of cardiomyocyte L-type Ca\(^{2+}\) currents (Xiao et al., 2004). EETs have also been shown to regulate cardiac electrophysiology and vascular tone via their action on K\(_{ATP}\) channels (Lu et al., 2006). Knowledge of the specific P450 enzymes that generate these EET metabolites has been lacking and selective inhibitors for the various P450 enzymes are limited.

The present study showed that CYP2C9 is the principal cytochrome P450 epoxygenase in aorta and coronary arteries. CYP2J2 protein was also detected at considerably lower levels in aorta, whereas CYP2C8 protein was not detectable by immunoblotting in either coronary artery or aorta samples. CYP2J2 has been previously reported to be localized in the endothelium of both large and small canine and porcine coronary arteries (Zhang et al., 2001) and in our study was found to be localized in cardiac myocytes and in the endothelial cell lining of blood vessels by immunohistochemical staining. Previous reports have shown that overexpression of CYP2C9 in both cultured and native porcine coronary endothelial cells increased both 11,12- and 8,9-EETs (Fleming et al., 2001). The presence of CYP2C9 as well as CYP2J2 in aorta and coronary arteries is consistent with their metabolites acting as potential candidates for EDHF; moreover, 11,12-EET, which is produced by CYP2C8, CYP2C9, and CYP2J2, is also known to improve survival of coronary artery endothelial cells in culture (Dhanasekaran et al., 2006). The presence of CYP2C9 as well as CYP2J2 is also consistent with studies showing that EET metabolites are potent coronary artery vasodilators (Campbell et al., 1996).
Knowledge of the particular cytochrome P450 isoforms that are present in both the coronary artery and aorta is thus particularly physiologically relevant. For example, polymorphisms resulting in variation in CYP2C9 or CYP2J2 activity or expression in coronary arteries would be predicted to alter the production of EET metabolites. Some redundancy in expression of various P450 isoforms such as CYP2C9 and CYP2CJ2, which produce these EETs, could be a protective mechanism.

Our study provides qualitative evidence, using specific primers and immunochemical methods to detect individual P450 isoforms, that CYP2C8, CYP2C9, and CYP2J2 mRNA and protein are present in various amounts in the human heart, coronary artery, and aorta tissues. CYP2J2 and CYP2C8 protein appear to predominate in human heart. In contrast, CYP2C9 expression appears to predominate in aorta and coronary arteries, although CYP2J2 is also expressed in these blood vessels. The expression of these P450s in these tissues is consistent with their proposed role in cardiovascular homeostasis or possibly in cardiovascular pathology. Further investigation into the expression of these enzymes in cardiovascular disease may provide useful information regarding the etiology and treatment of cardiovascular disease, as well as a better understanding of the role of the different P450 enzymes in vascular tone and homeostasis.

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References


Endo- 


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Address correspondence to: Dr. Joyce A. Goldstein, National Institute of Environmental Health Sciences, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709. E-mail: goldste1@niehs.nih.gov