Expression of epoxygenases belonging to CYP2 family in rat myocardial ischemia/reperfusion injury in vivo

Ying Ao, MS, *Ren-Xiu Peng,* MD, *Jing Yang,* PhD, *Bing-Hua Wang,* MS, *John R. Falck,* PhD, *Hong Dan,* MS, *Ying-Hui Liu,* MS.

ABSTRACT

الأهداف: من أجل توضيح مظهر مخلقات الايبوكسي العائدة لعائلة (سي واي بي ٢) والتي تسبب نقص تروية عضلة القلب عند فترات إعادة الإرواء المتغيرة وأثر مثبط مخلقات الايبوكسي على حالة ما بعد التعرض لنقص تروية القلب.

الطريقة: أجريت الدراسة الحالية بقسم الصيدلة بكلية الطب بجامعة ووهان بالصين. في الفترة ما بين سبتمبر ٢٠٠٤م ويونيو ٢٠٠٥م. تم تعريض الجرذان لنقص تروية عضلة القلب لمدة ٤٠ دقيقة عقبها تلقت ١٨٠،٦٠،١٥،٠ دقيقة من إعادة الإرواء. تمت معايرة توليد الأكسيد الفائق بواسطة المجهر، وتم تحديد مظهر الجين سي واي بي ٢، ٢سي٦، ٢إي١، ٢جي٣ بواسطة طريقة تفاعل سلسلة الخمائر الناقلة. تم قياس تركيز حمض ديهايدروكسيكوساترينويك (دي اتش أي تي) بواسطة طريقة إليسا (أي ال اس أيه). كما تم تقييم أثر المثبط الابيوكسي إن- ميثيلسولفونيل-٦-(٢-بروبارجليوكسيفينيل) هيكساناميد (ام اس- بي بي أو اتش) على تلف عضلة القلب وتوليد الأكسيد الفائق المسبب بواسطة إعادة الإرواء عند ٦٠ دقيقة.

النتائج: خلال نقص التروية/ إعادة الإرواء لعضلة القلب (ام أي/ آر) تم رفع تنظيم مظهر سي واي بي ٢ سي ٦ و ٢جي ٣ ام آر ان أيه مع مستوى الذروة عند ١٥ دقيقة من إعادة الإرواء: انخفض مظهر جين سي واي بي ٢ إي ١ في مسألة الوقت ووصل المستوى الأدنى عند ١٨٠ دقيقة لحالة ما بعد الإصابة بنقص التروية لعضلة القلب : بينما لم يتم ملاحظة تغيرات في مظهر جين سي واي بي ٢ بي ١/٢ أثناء فترات إعادة الإرواء المختلفة. إزداد حمض ديهايدروكسيكوساترينويك (دي اتش أي تي) ١٤،١٥ بشكل ملحوظ أثناء إعادة الإرواء في نقص تروية القلب. يخفض (ام اس- بي بي أو اتش) ما قبل المعالجة (١٥ ملجم/كجم) بشكل فعال من تلف عضلة القلب وإنتاج الأكسيد الفائق.

خاتمة: هنالك تغيرات في مظهر الجين للأنزيمات المثلية ورفع نشاط مخلقات الايبوكسي سي واي بي المتعلقة بإصابة إعادة الإرواء لعضلة القلب في الجسم. يلعب مثبط مخلقات الايبوكسي دوراً حامياً في التلف بعد الإصابة بنقص التروية.

Objectives: To elucidate the expression of epoxygenases belonging to cytochrome P-450 mono-oxygenases (CYP2) family in rat ischemic myocardium at varying reperfusion periods, and the effect of epoxygenase inhibition on the post-ischemic heart.

Methods: The current study was conducted in the Department of Pharmacology, Medical College of Wuhan University, China, between September 2004 and June 2005. Rats were subjected to 40 minutes of myocardial ischemia, followed by 0, 15, 60, and 180 minutes of reperfusion. Superoxide generation was assayed by confocal microscopy, CYP2B1/2, 2C6, 2E1, 2J3 gene expressions were determined by reverse transcriptase polymerase chain reaction. Fourteen, 15-dihydroxyeicosatrienoic acid (DHET) concentration was measured by enzyme-linked immunosorbent assay. The effects of the CYP epoxygenase inhibitor N-methylsulphonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH) on myocardial damage and superoxide generation caused by 60 minutes of reperfusion were also evaluated.

Results: During myocardial ischemia/reperfusion, CYP2C6 and 2J3 mRNA expression were up-regulated with the peak level at 15 minutes of reperfusion; CYP2E1 gene expression decreased in a time dependent manner and reached the minimum level at 180 minutes of post-ischemia. Meanwhile, no obvious variations of CYP2B1/2 gene expression were detected during different reperfusion periods. Fourteen, 15-DHET significantly increased during reperfusion in ischemic hearts. The MS-PPOH pretreatment (15 mg/kg) effectively reduced myocardial damage and superoxide production.

Conclusion: There are changes in gene expression of individual isozymes and an elevation of CYP epoxygenase activity involved in myocardial reperfusion injury *in vivo*. Epoxygenase inhibition plays a protective role in cardiac post-ischemic damage.

Saudi Med J 2008; Vol. 29 (1): 23-29

From the Departments of Pharmacology (Ao, Peng, Yang, Dan, Liu), and Biochemistry (Wang), Medical College of Wuhan University, Wuhan, China, and the Department of Biochemistry and Pharmacology (Falck), University of Texas Southwestern Medical Center, Dallas, United States of America.

Received 6th June 2007. Accepted 22nd September 2007.

Address correspondence and reprint request to: Prof. Ren-Xiu Peng, Department of Pharmacology, Medical College of Wuhan University, Donghu Road, Wuhan 430071, China. Tel. +86 (27) 68758665. Fax. +86 (27) 87331670. E-mail: aygj85@yahoo.com.cn

Cytochrome P-450 mono-oxygenases (CYPs) catalyze oxidation of a wide range of xenobiotics and are responsible for the metabolism of endogenous compounds, including steroids, cholesterol, fatty acid, and arachidonic acid (AA). A recent reverse transcriptase polymerase chain reaction (RT-PCR) analysis of explanted human heart tissues revealed that the heart contains CYP subtypes,[1] albeit less abundant than in liver. It has recently become apparent that CYPs contribute to the modulation of vascular homeostasis,[2] involved in pathogenesis of various cardiovascular diseases such as hypertension,[3] cardiac hypertrophy,[3] heart failure,[4] and myocardial ischemia/reperfusion (MI/R) injury. The diverse threads of cardiovascular risk factors seem to converge on the CYP enzymes. Among the players responsible for reperfused myocardial damage, much attention has been focused on the CYP pathway of AA metabolism. The AA is metabolized by CYP omega-hydroxylases to hydroxyeicosatetraenoic acid (HETE) and by epoxygenases to epoxyeicosatrienoic acids (EETs), respectively.[5] The CYP omega-hydroxylases have been demonstrated to mediate myocardial reperfusion damage in canine and rodent hearts,[5,6] whereas, studies on the role of CYP epoxygenases in cardiac post-ischemic recovery produced diverse results.[5,7] The EETs are predominantly synthesized by epoxygenases belonging to the CYP2 family, including CYP2B, 2E, 2C and 2J.[2] It has been shown that CYP2B1/2 and 2E1 mRNA are present in adult cardiomyocytes of the rat,[8] CYP2C and 2J3 protein have also been detected in normal rat hearts.[9,10] However, little information is available on CYP2 family expression during MI/R. Thus, it seems reasonable to characterize the expression of CYP2 subtypes in reperfused hearts, and that may help elucidate their roles in cardiac reperfusion damage.

In the present study, we investigated the gene expression of CYP2B1/2, 2C6, 2E1, and 2J3 in rat hearts at varying reperfusion periods, and determined the effect of the selective CYP epoxygenase inhibitor N-methylsulphonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH) on myocardial reperfusion damage.

Methods. *Animals.* Male Sprague-Dawley rats (250-300 g), specific pathogen free (SPF) grade, were supplied by Experimental Animals Center, Medical College of Wuhan University, China. All study protocols followed internationally accepted principles and the Guidelines for the Care and Use of Wuhan University, Wuhan, China. The following study was conducted in the Department of Pharmacology, Medical College of Wuhan University, China, between September 2004 and June 2005. Ethical approval was also obtained prior to the study.

Surgical procedure. Myocardial I/R was induced as described with minor modifications.[11] Briefly, after nembutal anesthesia (40 mg/kg, intraperitoneally), the animals were intubated and mechanical ventilation was achieved with a positive rodent respirator (Crompton Parkinson Ltd, Doncaster, Yorkshire, UK) using atmospheric air at a tidal volume of 15 ml/kg and a rate of 50 strokes/minute. A thoracotomy was performed at the fifth intercostal space and the pericardium was opened to expose the heart. The left arterial descending coronary artery (LAD) was ligated 2 mm from its origin by a 5/0 prolene suture with an atraumatic needle. To minimize the damage to the coronary artery and hence maximize chances for reperfusion, the suture was made slightly deeper in the myocardium, and a 2-3 mm segment of 2/0 suture was placed parallel to the vessel within the ligature. This procedure cushioned the artery during occlusion and prevented major damage to the artery by the ligature. The LAD was occluded by tying the suture with double slipknots. A standard limb lead II electrocardiogram (ECG) was monitored with a cardiograph (ECG-6511, Electro, China). The onset of ischemia was confirmed by development of cyanosis and typical elevation of the ST segment in the ECG. Forty minutes after occlusion, the heart was reperfused by pulling free the ligature. Sham-operated rats were subjected to the same surgical procedures, except that the artery was not ligated.

Experimental design. Two protocols were performed. To observe the dynamic expression of CYP 2 isozymes, rats were randomly separated into 5 groups, 12 animals for each group. After 40 minutes (min) of myocardial ischemia, the animals were sacrificed at 0, 15, 60, and 180 min of reperfusion, with the sham as the control. The blood was collected for biochemical studies, while left ventricles were obtained for superoxide determination, CYP 2 isozyme mRNA analysis and 14, 15-dihydroxyeicosatrienoic acid (DHET) assessment. For the treatment protocol, rats were randomly assigned to 4 groups, 12 animals for every group: vehicle (45% 2-hydroxypropyl-β-cyclodextrin)-treated sham; vehicle-treated I/R; MS-PPOH-treated I/R, in which the rats were subdivided into 5 and 15 mg/kg groups. The MS-PPOH or vehicle was administered by intravenous infusion 10 min prior to LAD-occlusion. Forty min of ischemia was followed by a 60 min reperfusion period, after which the rats were killed for superoxide determination, 14, 15-DHET assessment and tissue damage analysis.

Measurement of plasma creatine kinase (CPK), lactate dehydrogenase (LDH) activities, and malondialdehyde (MDA) level. Blood samples were collected in heparinized tubes and centrifuged at 2000xg for 15 minutes. The plasma was recovered and aliquots were

used for determination of CPK, LDH activities and MDA level with commercial kits (Nanjing Jiancheng Bio-Tek Corporation, Nanjing, China).

Assessment of myocardial infarct size. On completion of the experimental protocols, the coronary artery was reoccluded, and Evans Blue dye (one ml of 1% w/v) was injected into the left ventricle via the right jugular vein, to distinguish between perfused and nonperfused sections of the heart. The animals were sacrificed with an overdose of anesthetic and the heart excised. The area at risk (AAR) was determined by negative staining with Evans blue, and the infarcted area was identified as the unstained area within the AAR after 1% triphenyl tetrazolium chloride (TTC) staining. Pieces were separated according to staining and weighted to determine the infarct size (IS) as a percentage of the AAR.

Superoxide generation detection. The DHE staining for superoxide was carried out as described previously.[12] Briefly, transmural tissue samples from ischemic myocardium were harvested, embedded in optimal cutting temperature compound (OCT) for cryosectioning. The tissue sections (20 μm) were cut using a cryostat (CM1900, Leica, German) and placed on a glass slide. The dihydroethidium (DHE) (2 μM) was topically applied to each tissue section. Slides were incubated in a light-protected humidified chamber at 37°C for 30 minutes, washed with PBS and coverslipped. Images were obtained with a laser scanning confocal microscope (TCS SP2, Leica, German) (λex: 543 nm, λem: 605 nm). The fluorescence intensity was analyzed with confocal software (Leica, German).

Reverse transcription-polymerase chain reaction. Total ribonucleic acid (RNA) was extracted from ischemic heart tissues using Trizol reagent (Watson Biotechnologies Inc, Shanghai, China) according to the manufacturer's instructions. Two micrograms of total RNA were reverse-transcribed using RevertAid™ First Strand cDNA Synthesis Kit and stored at -20°C. The PCR was carried out in a 25 μL reaction mixture containing 2 μL of cDNA template, 1xPCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of dNTP, 1 unit of Taq DNA polymerase, and 400 nmol/L of each primer as shown in **Table 1**. The PCR amplification cycling conditions were as follows: 94°C for 30 seconds, 56°C for one min, 72°C for one min, 40 cycles for CYP2B1/2, 35 cycles for CYP2E1, and 28 cycles for cyclophilin (CyP); 94°C for 30 seconds, 54°C for one min, 72°C for one min, 35 cycles for CYP2C6 and 2J3. The PCR products were resolved in 1.2% agarose gels stained with ethidium bromide. The relative intensity of CYP subtypes compared with CyP was calculated for each sample by densitometry with image analysis software (VL, France).

Determination of 14, 15-DHET. Half gram of ischemic myocardium was homogenized with triphenyl phosphine. Eicosanoids were extracted thrice with ethyl acetate after acidification with acetic acid. After evaporation, the samples were dissolved in N, N-dimethylformamide (AMRESCO, Solon, OH) and concentrations of the stable EET metabolite 14, 15-DHET were determined by an enzyme-linked immunosorbent assay kit (Detroit R&D, Detroit, MI) according to the manufacturer's instructions.

Statistical analysis. Data were expressed as mean±SD. Statistical analyses were performed by one-way analysis of variants using SPSS Version 11.5. A $p<0.05$ was taken as statistically significant.

Results.

Plasma CPK and LDH activities. The biochemical indicators of cardiac damage were determined in the varying periods of reperfusion. The increase of plasma CPK and LDH activities became evident after 15 minutes of reperfusion and persisted with the prolongation of post-ischemia. They were markedly enhanced by 65% ($p<0.01$) and 123% ($p<0.01$), at 180 minutes reperfusion compared with the sham (**Table 2**).

In situ detection of superoxide production in ischemic left ventricles. The DHE reacts with superoxide anions to form ethidium bromide, which in turn intercalates with DNA to provide nuclear fluorescence as a marker of superoxide anion generation.[12] The Eth-DNA fluorescence in the sham group act as the control. A moderate increase by 31% occurred after 40 min of occlusion ($p<0.05$). The fluorescence was drastically

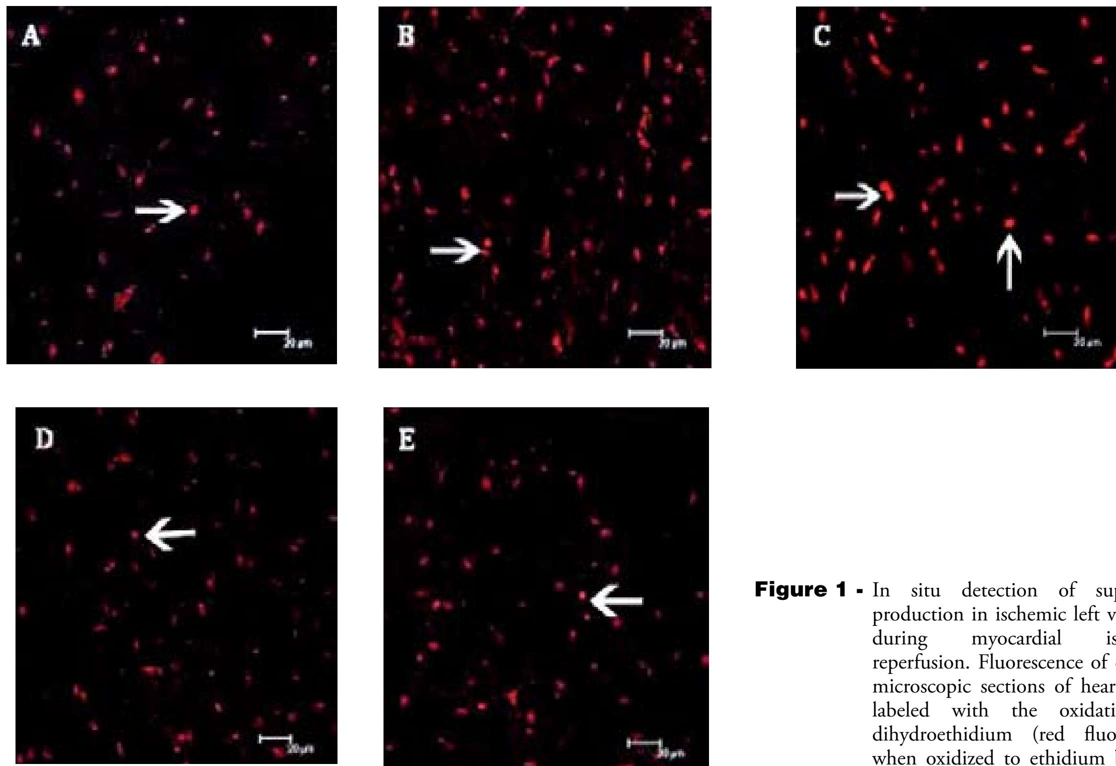
Table 1 - The polymerase chain reaction (PCR) primers used in the reverse transcriptase-PCR.

Accession (n)	Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Product length (bp)
L00316	CYP2B1/2	GAGTTCTTCTCTGGGTTCCTG	ACTGTGGGTCATGGAGAGCTG	549
BC100092	CYP2C6	GGAAAACCAATGGCTCACCCT	TCCCAGCACCAAACAAATCAG	426
M20131	CYP 2E1	CTCCTCGTCATATCCATCTG	GCAGCCAATCAGAAATGTGG	473
U39943	CYP 2J3	GGCATGCCCTTAATCAAAGA	AGCCTCAGCATCTCCTGAAA	364
M19533	Cyclophilin	CTTCGACATCACGGCTGATGG	CAGGACCTGTATGCTTCAGG	265

Table 2 - Plasma creatine kinase (CPK), lactate dehydrogenase (LDH) activities and 14, 15-dihydroxyeicosatrienoic acid (DHET) concentration in rat ischemic left ventricles during myocardial ischemia/reperfusion.

Group	CPK (U/mL)	LDH (U/L)	14,15-DHET (ng/mg protein)
Sham	71 ± 10	440 ± 178	10.1 ± 1.9
40 min of ischemia	81 ± 14	488 ± 139	13.0 ± 2.1
15 min of reperfusion	94 ± 14*	626 ± 148*	14.7 ± 3.6*
60 min of reperfusion	101 ± 24**	640 ± 101*	14.0 ± 2.9*
180 min of reperfusion	117 ± 30**†	981 ± 220**††	16.8 ± 4.7*

For CPK and LDH determination, n = 6~10. For 14, 15-DHET assay, n = 4~5.
*p<0.05, **p<0.01 versus sham group, †p<0.05, ††p<0.01 versus 40 min ischemia group, min - minutes



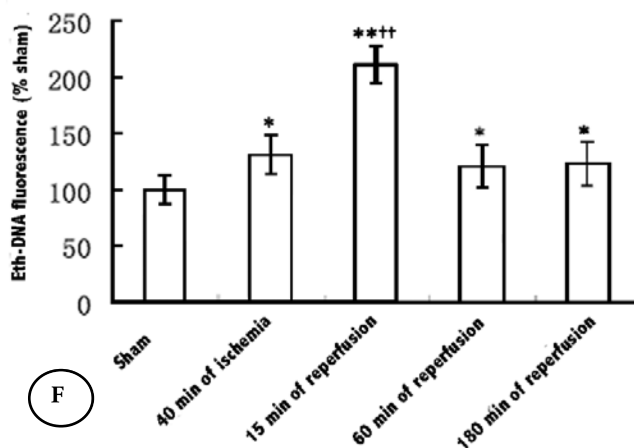


Figure 1 - In situ detection of superoxide production in ischemic left ventricles during myocardial ischemia/reperfusion. Fluorescence of confocal microscopic sections of heart tissues labeled with the oxidative dye dihydroethidium (red fluorescence when oxidized to ethidium bromide by superoxide). **a**) Sham-treated left ventricle; **b**) 40 min of ischemia-treated left ventricle; **c**) 15 min of reperfusion-treated left ventricle; **d**) 60 min of reperfusion-treated left ventricle; **e**) 180 min of reperfusion-treated left ventricle. Bar = 20 µm. **f**) Average fluorescent intensity of each image was quantified using imaging software. *P<0.05, **P<0.01 versus sham group; ††P<0.01 versus 40 min ischemia group.

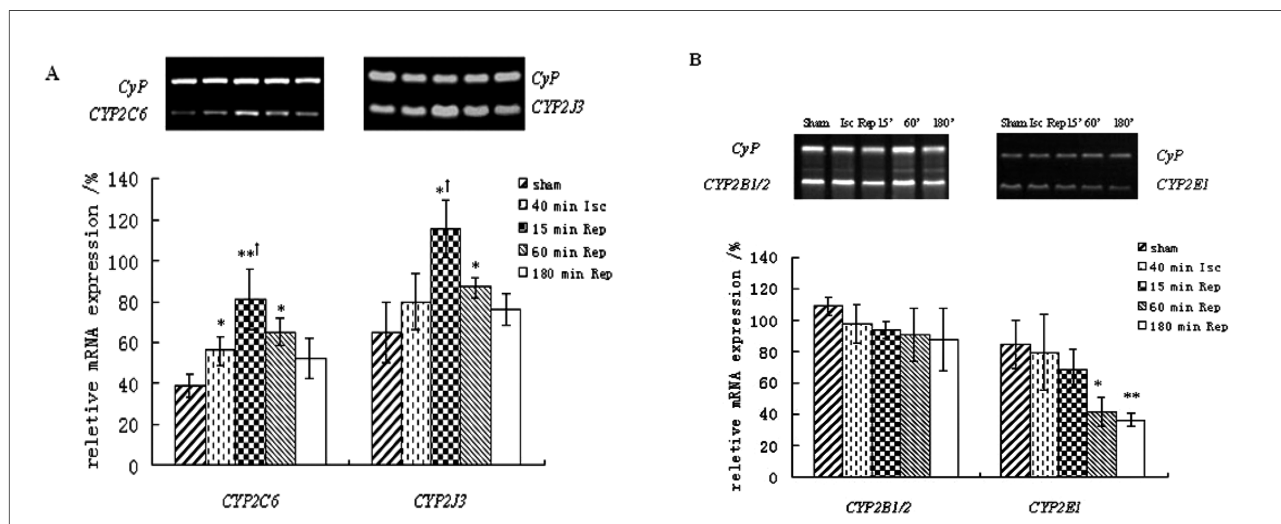


Figure 2 - The mRNA expressions of CYP2 family isozymes in ischemic left ventricles during MI/R showing **a)** CYP2C6 and CYP2J3 mRNA expressions were analyzed by reverse transcription-polymerase chain reaction (RT-PCR), **b)** CYP2B1/2 and CYP2E1 mRNA expressions were analyzed by RT-PCR, n =3, *p<0.05, **p<0.01 versus sham group, †p<0.05 versus 40 minutes ischemia group, isc - ischemia, rep - reperfusion.

Table 3 - Effects of N-methylsulphonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH) on 14, 15-dihydroxyeicosatrienoic acid (DHET) concentrations, myocardial damage and reactive oxygen species production in myocardial ischemia/reperfusion (MI/R) rats.

Group	Dose (mg/kg)	14,15-DHET (ng/mg protein)	IS (%AAR)	CPK (U/mL)	LDH (U/L)	Eth-DNA fluorescence (% sham)	MDA (nmol/mL)
Sham	--	9.3 ± 1.4	--	92 ± 23	408 ± 103	100 ± 9	4.0 ± 0.6
Vehicle	--	14.5 ± 5.9 *	38 ± 9	121 ± 24*	637 ± 133*	128 ± 14*	5.1 ± 0.8 *
MS-PPOH	5	5.4 ± 1.3 †	29 ± 10	115 ± 28	534 ± 191	125 ± 20	4.2 ± 0.8
	15	2.1 ± 0.6 ††	24 ± 8 †	97 ± 22†	480 ± 102†	111 ± 12†	3.9 ± 0.9 †

Mean ± SD, n = 5~10,*p<0.05 versus sham group,† p<0.05, ††p<0.01 versus vehicle group, MI/R: 40 minutes ischemia/60 minutes reperfusion, vehicle: MI/R + 45% 2-hydroxypropyl-β-cyclodextrin, MS-PPOH: MI/R + MS-PPOH 5 and 15 mg/kg

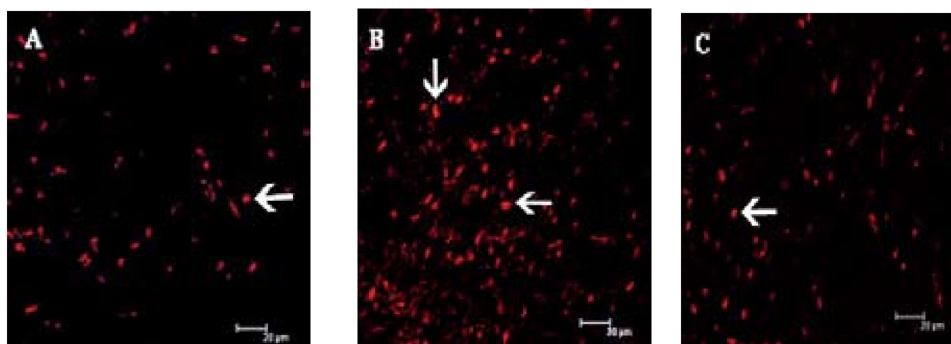


Figure 3 - Decreases in dihydroethidium fluorescence in left ventricles treated with N-methylsulphonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH) before myocardial ischemia/reperfusion (MI/R). Fluorescence of confocal microscopic sections of heart tissues labeled with the oxidative dye dihydroethidium (red fluorescence when oxidized to ethidium bromide by superoxide) showing **a)** sham-treated left ventricle (TLV) in group, **b)** MI/R-TLV, **c)** MI/R + MS-PPOH 15 mg/kg-TLV, bar = 20μm, MI/R: 40 minutes ischemia/60 minutes reperfusion, vehicle: MI/R + 45% 2-hydroxypropyl-β-cyclodextrin.

elevated by 111% ($p<0.01$) at 15 min of reperfusion, although declined in 60 and 180 min of reperfusion, it remained 16% ($p<0.05$) and 21% ($p<0.05$) higher than that of the sham (**Figure 1**).

mRNA expressions of CYP isozymes. The elevation of CYP2C6 gene expression became evident after 40 min of ischemia ($p<0.05$), and significantly up regulated by 108% ($p<0.01$) at 15 min of reperfusion compared with the sham. After 60 and 180 min of post-ischemia, the mRNA expression declined, but was still 67% ($p<0.05$) and 44% higher than the sham. The marked up-regulation of CYP2J3 gene occurred at 15 min of post-ischemia, which was 1.8-fold of the sham ($p<0.05$), then reduced, remaining 45% ($p<0.05$) and 17% higher than the sham after 60 and 180 minutes of reperfusion (**Figure 2a**). In contrast, CYP2E1 mRNA expression decreased in a time dependent manner, it was down regulated by 50.5% ($p<0.05$) after 60 minutes and 56.9% ($p<0.01$) at 180 minutes of reperfusion versus the sham. However, CYP2B1/2 gene level had no significant difference at any varying periods of reperfusion (**Figure 2b**).

The 14, 15-DHET concentrations in rat ischemic left ventricles. The concentrations were slightly increased at 40 minutes of ischemia and significantly elevated during reperfusion. Relative to the sham group, 14, 15-DHET levels were higher of 45.6% ($p<0.05$) at 15 minutes, 39.0% ($p<0.05$) at 60 minutes, and 66.7% ($p<0.05$) at 180 minutes of reperfusion (**Table 2**).

Effect of MS-PPOH on 14, 15-DHET concentrations. The increase of DHET upon 60 minutes of reperfusion was significantly inhibited by MS-PPOH. Compared with vehicle-treated group, the concentrations of 14, 15- DHET were reduced by 62.8% ($p<0.05$) and 85.5% ($p<0.01$), respectively, in 5 and 15 mg/kg of MS-PPOH pre-treated groups (**Table 3**).

Effects of MS-PPOH on myocardial damage. The mean values of the area at risk, expressed as a percentage of left ventricular walls, was similar in all groups (data not shown). In vehicle-treated rats, LAD-occlusion for 40 min followed by 60 min reperfusion resulted in an infarct size of 38±9% of the area at risk. Intravenous administration of MS-PPOH at the dose of 15 mg/kg reduced the infarct size by 36.8% ($p<0.05$). Similarly, plasma CPK and LDH activities in MS-PPOH 15 mg/kg group were decreased by 19.8% ($p<0.05$) and 24.6% ($p<0.05$), relative to the vehicle group (**Table 3**).

Effects of MS-PPOH on superoxide production and plasma MDA level. Treatment with 15 mg/kg of MS-PPOH decreased superoxide and plasma MDA level by 13.3% ($p<0.05$) and 23.5% ($p<0.05$), compared with the vehicle group (**Table 3, Figure 3**).

Discussion. In the present study, the alteration of epoxygenases belonging to the CYP2 family in rat hearts was investigated during varying periods of myocardial reperfusion and the differing characteristics of the individual epoxygenase isoforms were demonstrated. The AA is metabolized by CYP epoxygenases to force-epoxyeicosatrienoic acids (5, 6-, 8, 9-, 11, 12-, 14, 15-EET) and converted to their corresponding stable metabolites *vic*-dihydroxyeicosatrienoic acids (DHETs).[13] The 14, 15-DHET is one of the major DHET regioisomers and reflects the level of 14, 15-EET,[14] which is the predominant EET catalyzed by epoxygenases in the hearts.[10] In this study, an increase of 14, 15-DHET was observed in post-ischemic heart tissues, consistent with those findings in reperfused dog coronary venous plasma.[14] These results indicate an elevated production of EETs and an enhancement of the activities CYP epoxygenases during MI/R. Tissue damage upon reperfusion is due, in large part, to the generation of reactive oxygen species (ROS). The CYP2C is known to generate ROS (such as superoxide anions) during the CYP reaction cycle[2] and has been proved to be a functionally significant source of ROS in porcine coronary endothelial cells.[15] In our study, consistent with the alteration of superoxide, CYP2C6 mRNA was up-regulated during MI/R with the peak level at 15 minutes of reperfusion. Previously it has been shown that CYP2C inhibition reduces ROS generation and tissue injury during myocardial reperfusion,[9] our results support the suggestion that CYP2C may contribute to ROS production in rat hearts during MI/R. The CYP2J3, homologous to human CYP2J2, is unique in that it is highly expressed in rat hearts, predominantly located in ventricular cardiac myocytes, and active in the epoxidation of AA to EETs.[10] The CYP2J has been reported not to be a significant source of ROS.[2,16] One possible reason for this is that CYP2J isoforms are more tightly coupled and therefore have a reduced ability in generating free radicals.[16] It has been shown that CYP2J2 protected hearts from reperfused insult,[7] and a recent study proved that these cardioprotective effects were the result of an initial burst of ROS.[17] In our study, following the enhanced generation of superoxide during myocardial ischemia, CYP2J3 mRNA expression was up-regulated after reperfusion, which could be a feedback protection triggered by ROS. However, further studies, in which an antioxidant is given prior to the prolonged ischemic time, are needed to prove this hypothesis. The CYP2E1 plays a key role in ROS generation especially in foreign compounds-induced hepatotoxicity.[18] However, to our knowledge, few studies have addressed the alteration of CYP2E1 in myocardial reperfusion damage. In the current study, a decrease in CYP2E1 mRNA expression was observed in rat hearts during MI/R, in accordance with the finding in rat liver microsomes after hepatic I/R-induced oxidative stress.[19] The ROS have been implicated in the transcriptional

regulation of CYPs, particularly CYP2E1.[20] Being part of a biological control mechanism to limit the CYP-mediated toxicity, the CYP2E1 gene promoter has been shown to be repressed by exogenous ROS addition or intracellular ROS production.[21] Therefore, in our study, the reduction of CYP2E1 mRNA in MI/R might be mediated by ROS as a negative limitation mechanism.

In order to clarify the role of CYP epoxygenases, we investigated the effects of the specific epoxygenase inhibitor MS-PPOH on myocardial damage caused by 60 minutes of reperfusion *in vivo*. In the present study, MS-PPOH resulted in cardioprotection by reducing infarct size, decreasing CPK and LDH activities and repressing superoxide generation. These results suggest that epoxygenase inhibition could attenuate the tissue damage through, at least in part, suppressing ROS production during MI/R. There are several signaling pathways[22] involved in the heart post-ischemic injury, such as the K_{ATP} channel, mitogen activated protein kinase (MAPK), the epidermal growth factor receptor (EGFR), and so on. However, in this study, the effect of MS-PPOH to these pathways is still unclear. Thus, we need to further study the mechanism of MS-PPOH to MI/R.

We conclude that an elevation of CYP epoxygenase activity and a diverse gene alteration profile of the individual isozymes after myocardial reperfusion injury in vivo. Although the cardio detrimental and cardioprotective mechanisms seemed to be both activated by different CYP isozymes during MI/R, epoxygenase inhibition may play a protective role in cardiac post-ischemic damage. These results further advance our knowledge of CYP function in MI/R and may have implications in the treatment of MI/R injury.

Acknowledgments. *The program of John R. Falck was supported in part by the USPHS NIH (GM31278) and the Robert A. Welch Foundation.*

References

1. Thum T, Borlak J. Gene expression in distinct regions of the heart. ***Lancet*** 2000; 355: 979-983.
2. Fleming I. Cytochrome P450 and vascular homeostasis. ***Circ Res*** 2001; 89: 753-762.
3. Thum T, Borlak J. Testosterone, cytochrome P450, and cardiac hypertrophy. ***FASEB J*** 2002; 16: 1537-1549.
4. Tan FL, Moravec CS, Li J, Apperson-Hansen C, McCarthy PM, Young JB, et al. The gene expression fingerprint of human heart failure. ***Proc Natl Acad Sci USA*** 2002; 99: 11387-11392.
5. Gross ER, Nithipatikom K, Hsu AK, Peart JN, Falck JR, Campbell WB, et al. Cytochrome P450 omega-hydroxylase inhibition reduces infarct size during reperfusion via the sarcolemmal K_{ATP} channel. ***J Mol Cell Cardiol*** 2004; 37: 1245-1249.
6. Nithipatikom K, Gross ER, Endsley MP, Moore JM, Isbell MA, Falck JR, et al. Inhibition of cytochrome P450 omega-hydroxylase: a novel endogenous cardioprotective pathway. ***Circ Res*** 2004; 95: e65-e71.
7. Seubert J, Yang B, Bradbury JA, Graves J, Degraff LM, Gabel S, et al. Enhanced postischemic functional recovery in CYP2J2 transgenic hearts involves mitochondrial ATP-sensitive K^+ channels and p42/p44 MAPK pathway. ***Circ Res*** 2004; 95: 506-514.
8. Thum T, Borlak J. Cytochrome P450 mono-oxygenase gene expression and protein activity in cultures of adult cardiomyocytes of the rat. ***Br J Pharmacol*** 2000; 130: 1745-1752.
9. Grancille DJ, Tashakkor B, Takeuchi C, Gustafsson AB, Huang CQ, Sayer MR, et al. Reduction of ischemia and reperfusion-induced myocardial damage by cytochrome P450 inhibitors. ***Proc Natl Acad Sci USA*** 2004; 101: 1321-1326.
10. Wu S, Chen W, Murphy E, Gabel S, Tomer KB, Foley J, et al. Molecular cloning, expression, and functional significance of a cytochrome P450 highly expressed in rat heart myocytes. ***J Biol Chem*** 1997; 272: 12551-12559.
11. Liu P, Xu B, Cavalieri TA, Hock CE. Age-related difference in myocardial function and inflammation in a rat model of myocardial ischemia-reperfusion. ***Cardiovasc Res*** 2002; 56: 443-453.
12. Miller FJ, Gutterman DD, Rios CD, Heistad DD, Davidson BL. Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. ***Circ Res*** 1998; 82: 1298-1305.
13. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. ***J Biol Chem*** 2001; 276: 36059-36062.
14. Nithipatikom K, DiCamelli RF, Kohler S, Gumina RJ, Falck JR, Campbell WB, et al. Determination of cytochrome P450 metabolites of arachidonic acid in coronary venous plasma during ischemia and reperfusion in dogs. ***Anal Biochem*** 2001; 292: 115-124.
15. Fleming I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, et al. Endothelium-derived hyperpolarizing factor synthase (cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. ***Circ Res*** 2001; 88: 44-51.
16. Yang B, Graham L, Dikalov S, Mason RP, Falck JR, Liao JK, et al. Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. ***Mol Pharmacol*** 2001; 60: 310-320.
17. Gross GJ, Hsu A, Falck JR, Nithipatikom K. Mechanisms by which epoxyeicosatrienoic acids (EETs) elicit cardioprotection in rat hearts. ***J Mol Cell Cardiol*** 2007; 42: 687-691.
18. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. ***Toxicol Sci*** 2002; 65: 166-176.
19. Pahan K, Smith BT, Singh AK, Singh I. Cytochrome P-450 2E1 in rat liver peroxisomes: downregulation by ischemia/reperfusion-induced oxidative stress. ***Free Radic Biol Med*** 1997; 23: 963-971.
20. Zangar RC, Davydov DR, Verma S. Mechanisms that regulate production of reactive oxygen species by cytochrome P450. ***Toxicol Appl Pharmacol*** 2004; 199: 316-331.
21. Morel Y, de Waziers I, Barouki R. A repressive cross-regulation between catalytic and promoter activities of the CYP1A1 and CYP2E1 genes: role of H_2O_2. ***Mol Pharmacol*** 2000; 57: 1158-1164.
22. Gross GJ, Falck JR, Gross ER, Isbell M, Moore J, Nithipatikom K. Cytochrome P450 and arachidonic acid metabolites: role in myocardial ischemia/reperfusion injury revisited. ***Cardiovasc Res*** 2005; 68: 18-25.