Reduction of ischemia and reperfusion-induced myocardial damage by cytochrome P450 inhibitors

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Ischemia and reperfusion both contribute to tissue damage after myocardial infarction. Although many drugs have been shown to reduce infarct size when administered before ischemia, few have been shown to be effective when administered at reperfusion. Moreover, although it is generally accepted that a burst of reactive oxygen species (ROS) occurs at the onset of reperfusion and contributes to tissue damage, the source of ROS and the mechanism of injury is unclear. We now report the finding that chloramphenicol administered at reperfusion reduced infarct size by 60% in a Langendorff isolated perfused rat heart model, and that ROS production was also substantially reduced. Chloramphenicol is an inhibitor of mitochondrial protein synthesis and is also an inhibitor of a subset of cytochrome P450 monooxygenases (CYPs). We could not detect any effect on mitochondrial encoded proteins or mitochondrial respiration in chloramphenicol-perfused hearts, and hypothesized that the effect was caused by inhibition of CYPs. We tested additional CYP inhibitors and found that cimetidine and sulfaphenazole, two CYP inhibitors that have no effect on mitochondrial protein synthesis, were also able to reduce creatine kinase release and infarct size in the Langendorff model. We also showed that chloramphenicol reduced infarct size in an open chest rabbit model of regional ischemia. Taken together, these findings implicate CYPs in myocardial ischemia/reperfusion injury.

Current treatment of myocardial infarction is directed at the restoration of blood flow to the ischemic region and reduction of myocardial oxygen demand. However, during reperfusion, the heart undergoes further damage due, in large part, to the generation of reactive oxygen species (ROS) (1). It is clear that permanent ischemia results in necrotic cell death. However, it is unclear whether reperfusion itself induces apoptosis or merely permits the manifestation of cell death processes that were initiated and irreversibly committed to during ischemia. Moreover, the relative contributions to tissue injury by the ischemic phase and by reperfusion have been difficult to evaluate. Resolving this question carries important therapeutic implications, as efforts directed toward treating reperfusion injury would have limited value if most cell death were predestined during ischemia.

Although we initially hypothesized that the protective effect of chloramphenicol was caused by inhibition of mitochondrial protein synthesis, we did not see down-regulation of mitochondrial-encoded proteins after chloramphenicol infusion. The energy-sparing effects of inhibition of cytosolic protein synthesis have been described (2), and our previously reported observation that mitochondrial elongation factor Tu is phosphorylated during ischemia suggests a similar process may take place in the mitochondria (3). However, because chloramphenicol also inhibits some cytochrome P450 monooxygenases (CYPs), it was important to determine whether that inhibitory effect was relevant to cardioprotection. In this report, we show that chloramphenicol and the structurally unrelated CYP inhibitors cimetidine and sulfaphenazole, which do not inhibit mitochondrial protein synthesis, also reduce infarct size and creatine kinase (CK) release. These observations strongly implicate CYP mono-

oxygenases in the heart as being important mediators of myocardial damage after ischemia and reperfusion.

CYPs have not heretofore been given a great deal of attention in the heart, although their importance to hepatic drug metabolism is well recognized. It is becoming increasingly clear that CYP enzymes play a key role in the modulation of vascular homeostasis through the conversion of arachidonic acid to vasoactive eicosanoids (4, 5). There are a large number of CYP enzymes, and these are not well conserved across species. A recent RT-PCR analysis of explanted human heart tissue revealed the presence of cytochromes 1A1, 2B6/7, 2C8–19, 2D6, and 2E1 (6) as well as a CYP with arachidonic acid epoxygenase activity, CYP2J2 (7). CYP2C9 has also been shown to be present in human coronary arteries and to represent a potent source of superoxide (8). An isozyme similar to CYP2C9 has also been demonstrated in rat mesenteric arteries and shares immunoreactivity and sensitivity to the selective inhibitor, sulfaphenazole (9).

Materials and Methods

Langendorff Heart Perfusions. All procedures were approved by the Animal Care and Use Committee at The Scripps Research Institute and conform to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication no. 85-23, revised 1996). Rat hearts were perfused in Langendorff mode with Krebs–Ringer buffer as described (3). Chloramphenicol (300 μM) (Calbiochem), gentamicin sulfate (50 mg/ml), cimetidine (200–600 μM), or sulfaphenazole (10–300 μM) (Sigma) was added to the perfusion buffer 20 min before ischemia or upon reperfusion. No-flow ischemia was maintained for 30 min, and reperfusion was accomplished by restoring flow for 15 min for CK release determination by using the CK EC 2.7.3.2 UV test kit (Sigma) and dihydroethidium staining, or for 120 min for infarct size determination by triphenyl tetrazolium chloride staining (3). Ongoing production of superoxide in heart slices after the perfusion protocol was quantified by measuring ethidium fluorescence as described (10, 11).

Rabbit Coronary Artery Occlusion. Twelve New Zealand White rabbits (2.8–3.9 kg) were randomized to receive an i.v. bolus of chloramphenicol (20 mg/kg in sterile saline) or no drug 30 min before regional ischemia. Rabbits were mechanically ventilated with room air by means of a tracheostomy, and the heart was exposed by a median sternotomy. The left anterior descending coronary artery was snare-occluded for 30 min and then reperfused for 4 h as described (12). Infarct size as a percentage of the area at risk was determined by an observer blinded to the treatment status of the hearts. Area at risk did not differ between the two groups (data not shown).

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Measurement of CYP Activity in Isolated Microsomes. Immediately after ischemia and 15-min reperfusion in the presence or absence of chloramphenicol (300 μM), rat hearts were homogenized in a postmounted rotor-stator tissue homogenizer in KCl buffer (0.15 M, pH 7.4). For studies of sulfaphenazole inhibition, rat livers (Sprague–Dawley, male, 6–8 weeks) were used as the source of microsomes, which were isolated by differential centrifugation as described by Walles *et al.* (13). Cellular debris, nuclei, and mitochondria were pelleted by centrifugation of the homogenate at $11,000 \times g$ for 30 min at 4°C. A crude membrane fraction was isolated by centrifugation at $170,000 \times g$ for 60 min at 4°C. The resulting pellet was resuspended in KCl buffer, and a pellet enriched with microsomes was isolated by centrifugation at $200,000 \times g$ for 40 min at 4°C. The microsomal fraction was then transferred into Tris-sucrose buffer (0.25 M sucrose/20 mM Tris buffer/5 mM EDTA) and stored at -80°C until further use. Assays were carried out in parallel at 37°C in fluorescence detector microtiter plates (96-well). CYP2D2 supersomes, substrates [5-acetylamino-6-amino-3-methyluracil (AMMC)] and product standards 3-[2-(*N,N*-diethyl-*N*-methylamino)ethyl]-7-hydroxy-4-methylcoumarin (AHMC), and the NADPH generating system were obtained from BD Gentest (Woburn, MA). Reaction components were prewarmed to 37°C. The assay was initiated by addition of the NADPH-regenerating system to substrate concentrations selected to give linear time-course profiles (AMMC, 30 μM) and rat liver microsomal protein (10 mg/ml) that was isolated as described above in phosphate buffer (50 mM, pH 7.4) (final organic cosolvent concentration, 0.1% acetonitrile) in the presence or absence of sulfaphenazole (50 μM AMMC). Reaction progression was followed by continuously monitoring AHMC formation ($\lambda_{ex} = 390$ nm and $\lambda_{em} = 460$ nm) by using a SPECTRAmax GEMINI dual-scanning microplate spectrofluorometer for 1 h at 37°C. The residual absorption caused by NADPH was compensated for throughout the analysis. Each assay was performed in triplicate. Reaction progress was followed for no more than 10% of total reaction during which time the reaction rate was linear ($r^2 > 0.985$). These linear initial changes in residual fluorescence emission were converted to concentration and hence rates of product formation by comparison to standard curves. IC_{50} determination for AHMC was performed by using PRISM software (GraphPad, San Diego). For cardiac microsomes, the inhibition by chloramphenicol was calculated, as a percent, by a comparison of the initial rates of AHMC formation between the cardiac microsomal preparations obtained from the chloramphenicol-treated and -untreated groups.

Western Blot Analysis. Rat heart microsomes were resolved on SDS/PAGE and transferred to poly(vinylidine difluoride) membranes, which were blocked with 5% nonfat milk and 3% BSA in Tris-buffered saline with 0.05% Tween 20 (TBST) overnight before adding anti-2C9 antibody (Research Diagnostics) diluted 1:200 in 5% milk in TBST for 1 h. After three washes, secondary antibody conjugated to horseradish peroxidase was added for 1 h at room temperature, followed by enhanced chemiluminescence detection (Amersham Biosciences).

Statistical Analysis. Statistical analysis was performed between groups by ANOVA by using INSTAT 4.10 software (GraphPad). A P value <0.05 was considered significant.

Results

The effects of chloramphenicol administration on CK release, coronary flow, and infarct size during reperfusion were assessed in isolated rat hearts perfused in Langendorff mode. Treatment with 300 μM chloramphenicol, both before and after global no-flow ischemia, resulted in a significant reduction in infarct size from $43.2 \pm 3.2\%$ (SEM) ($n = 16$) (untreated) to $16.0 \pm$
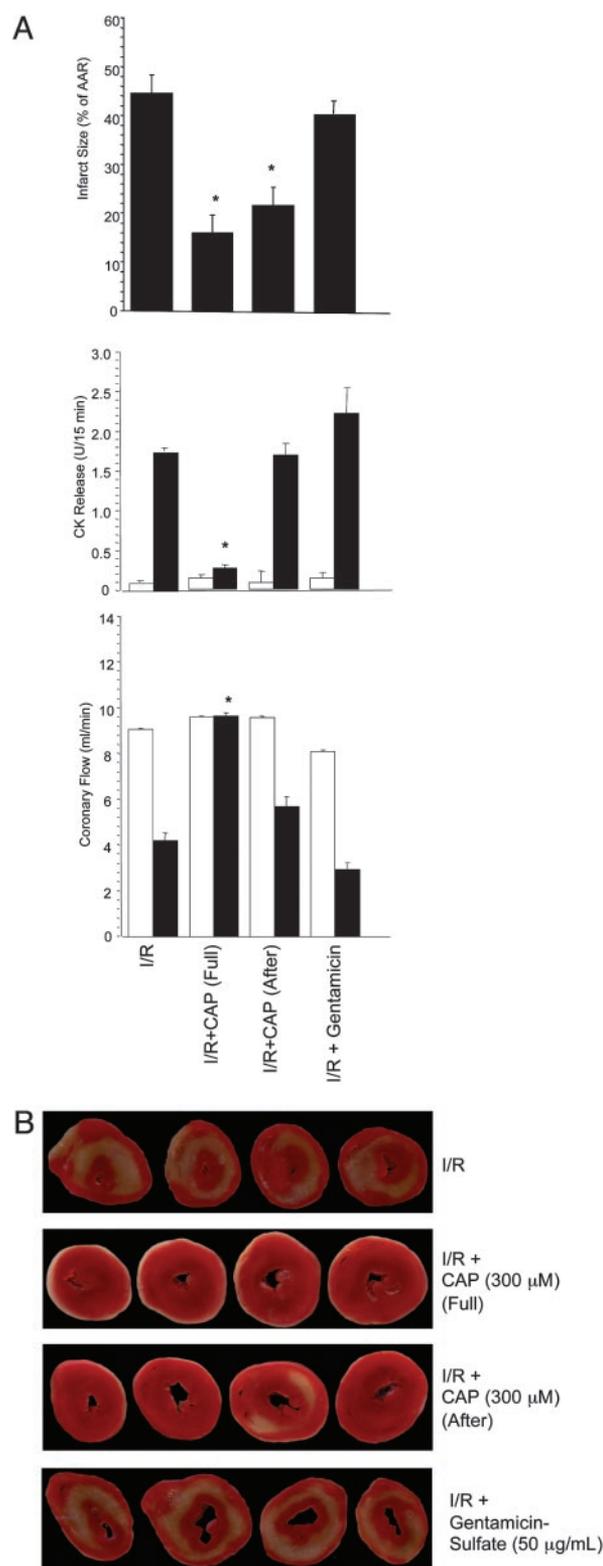

Fig. 1. Effect of chloramphenicol on ischemia/reperfusion in the Langendorff model. Adult rat hearts were perfused in Langendorff mode for 20 min, then subjected to 30 min of no-flow ischemia followed by 2 h of reperfusion (I/R). Chloramphenicol (CAP), or gentamicin was added for the entire procedure. In CAP-After, chloramphenicol was added only during reperfusion. (*A*) After 2 h of reperfusion, hearts were frozen and assessed for infarct size. Coronary effluent was collected for 15 min immediately before and after ischemia and assessed for CK release. Error bars denote SEM. *, $P < 0.01$ (comparison to I/R). (*B*) Representative heart sections stained with triphenyl tetrazolium chloride.
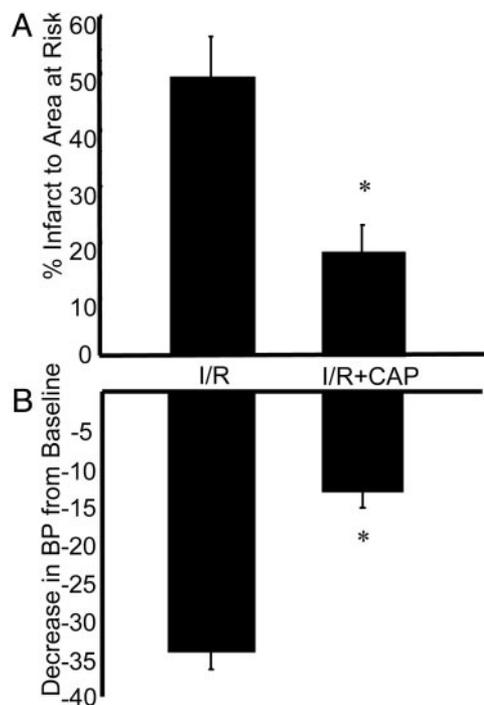
Fig. 2. Effect of chloramphenicol in a surgical model of coronary artery ligation in rabbits. Rabbits were injected with or without chloramphenicol (20 mg/kg) for 30 min, and then the coronary artery was snare-occluded for 30 min followed by 4 h of reperfusion. (*A*) At 4 h, hearts were removed and immediately frozen and assessed for infarct size. (*B*) The decrease in left ventricular systolic pressure from baseline was assessed after ischemia. Error bars represent standard error of the mean (*n* = 6). *, *P* < 0.05.

3.6% ($n = 5$, $P < 0.005$) (chloramphenicol-treated), respectively (Fig. 1). Chloramphenicol administration before ischemia substantially decreased the amount of CK released after ischemia from 1.75 units per 15 min $\pm$ 0.04 (SEM) ($n = 16$) in untreated hearts to 0.27 ± 0.04 ($n = 5$, $P < 0.01$). Effects on coronary flow paralleled cardioprotection. Gentamicin, a structurally unrelated antibiotic, was not cardioprotective in this model, arguing against a possible antimicrobial effect of chloramphenicol. Importantly, administration of chloramphenicol immediately after ischemia also substantially reduced infarct size ($21.7 \pm 3.7\%$, $n = 5$, vs. 43.2%, $P < 0.01$), suggesting that reperfusion injury is a major determinant of tissue damage in this model.

The observed cardioprotective effect of chloramphenicol is not model-specific. In addition to the Langendorff model, we studied the protective effect of chloramphenicol in a rabbit model of coronary artery occlusion. Rabbits were administered either chloramphenicol (20 mg/kg i.v. in saline) or no drug 30 min before snare-occlusion of the coronary artery for 30 min, followed by 4 h of reperfusion. The volume of the area at risk did not differ between the two groups (not shown). However, similar to the Langendorff model, chloramphenicol administration significantly reduced infarct size: 49.3% of the area at risk $\pm$ 17.7% (untreated) vs. $18.3 \pm 12.1\%$ (treated) ($n = 6$, $P < 0.05$) (Fig. 2). Postischemic hypotension was also diminished in the drug-treated rabbits (-34.2 mmHg $\pm$ 2.3 vs. -13.3 ± 2.2, $n = 6$, $P < 0.05$; 1 mmHg = 133 Pa) (Fig. 2).

We previously ruled out the possibility that chloramphenicol artifactually improved triphenyl tetrazolium chloride staining by perfusing chloramphenicol during the last 15 min of the 2-h reperfusion, at which time injury was well established; at that late time point, chloramphenicol did not reduce infarct size as measured by tetrazolium staining (3). Neither compound inter-
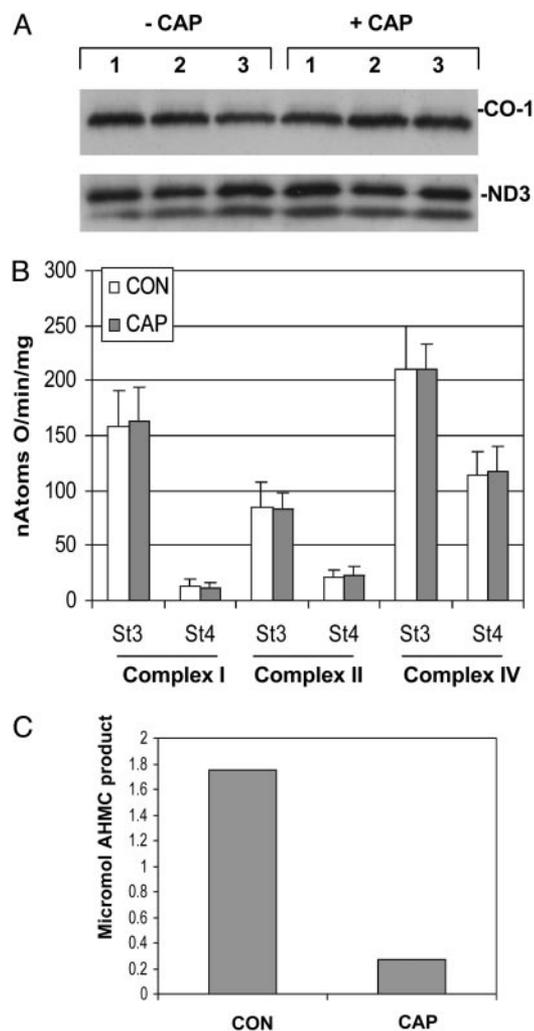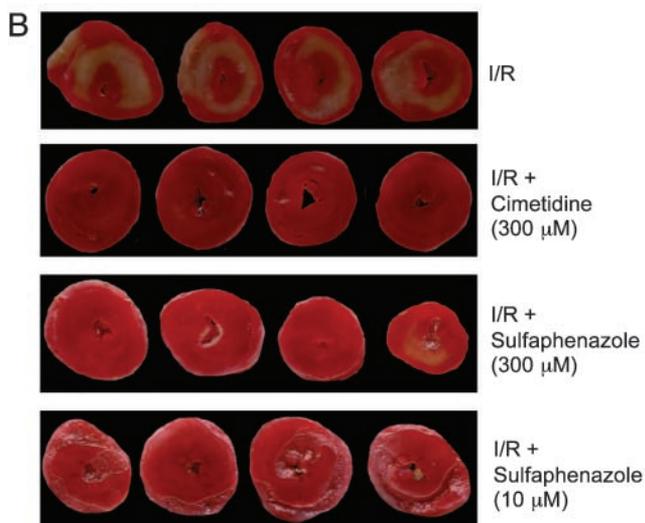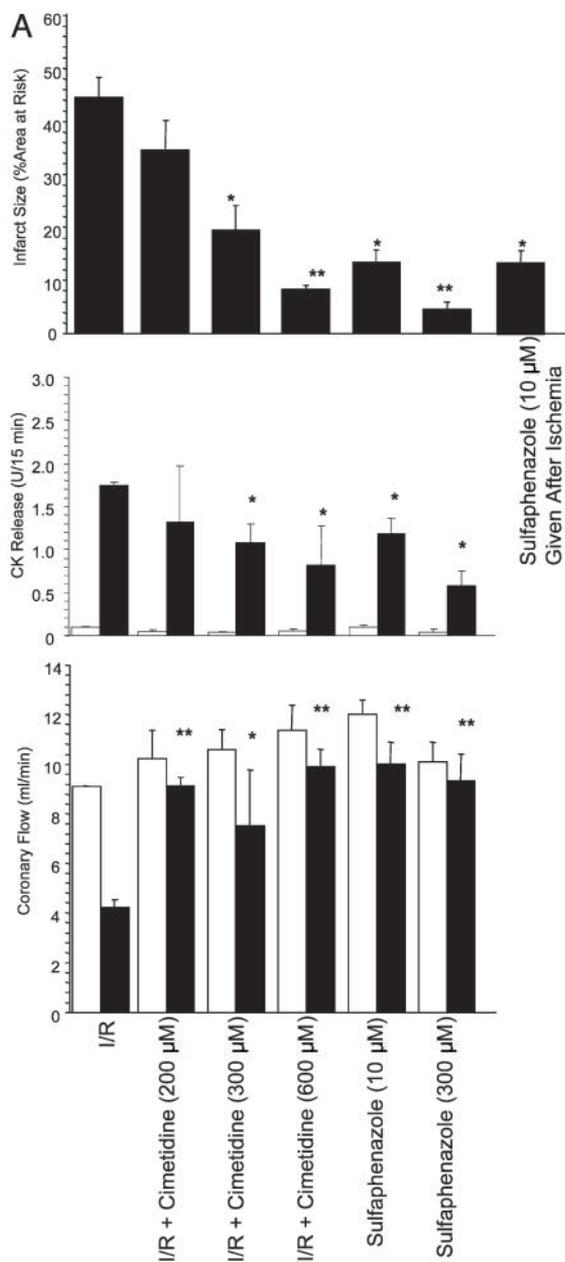
Fig. 3. Effect of chloramphenicol infusion on mitochondria and CYP activity. (*A*) Mitochondria isolated from hearts perfused with or without chloramphenicol were assessed for expression levels of mitochondrial genome-encoded proteins cytochrome oxidase subunit I (CO-I) and complex I subunit 3 (ND3). (*B*) Mitochondrial respiration was measured by polarography. State 3 and state 4 respiration are shown for palmitoylcarnitine (complex I substrate), succinate (complex II substrate), and TMPD/ascorbate (complex IV substrate). Error bars represent standard deviation. (*C*) Cardiac microsomes were prepared from hearts perfused with or without chloramphenicol, and CYP activity was measured as the NADPH-dependent demethylation reaction of AMMC.

feres with dihydroethidium conversion if added to heart sections during staining (data not shown), thereby eliminating the possibility that this is a staining artifact. That this protective intervention is associated with physiologic benefit is suggested by the improved blood pressure in the treated rabbits and by the visual recording of perfused hearts at time points from 15 min to 2 h after ischemia (see Movie 1, which is published as supporting information on the PNAS web site).

Given that mitochondria are known to generate ROS under certain conditions, our first hypothesis for the mechanism of cardioprotection by chloramphenicol was based on the known inhibitory potential of chloramphenicol on mitochondrial protein synthesis and our previous findings implicating mitochondrial elongation factor Tu (3, 14). However, perfusion with chloramphenicol for 30 min was not sufficient to alter the levels of mitochondrial-encoded proteins or to affect mitochondrial respiratory chain activity (Fig. 3 *A* and *B*), suggesting that, under

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Fig. 4. Effect of various CYP inhibitors on ischemia/reperfusion in the Langendorff model. Adult rat hearts were perfused in Langendorff mode for 20 min, then subjected to 30 min of no-flow ischemia followed by 2 h of reperfusion (I/R). Inhibitors were added for the entire procedure or only at reperfusion (Sulfaphenazole Given After Ischemia), at the specified concentrations. (*A*) After 2 h of reperfusion, hearts were frozen and assessed for infarct size. Coronary effluent was collected for 15 min immediately before and after ischemia and assessed for CK release. Coronary flow was measured during the first 15 min of reperfusion. Error bars denote SEM. $*$, $P < 0.01$; $**$, $P < 0.001$ (comparison to I/R). (*B*) Representative heart sections stained with triphenyl tetrazolium chloride.
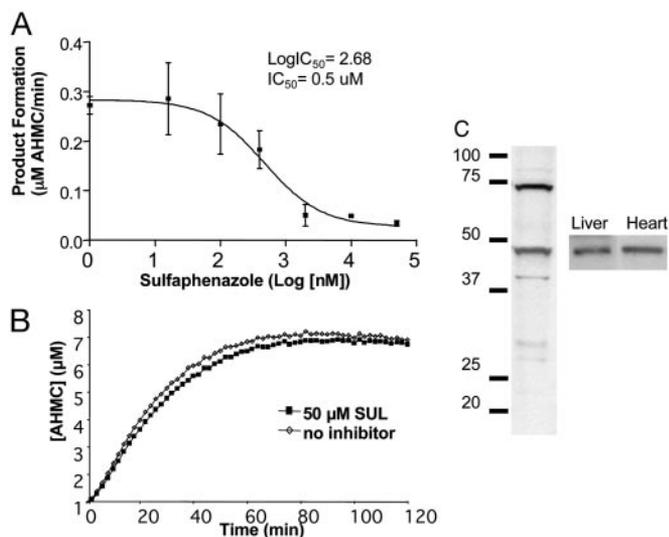
these conditions, chloramphenicol did not have an appreciable effect on mitochondria.

Chloramphenicol is also known to inhibit CYPs (15). To determine whether chloramphenicol administration during ischemia/reperfusion in the Langendorff model was inactivating cardiac CYP activity, cardiac microsomes from pooled hearts (two per group) were isolated immediately after reperfusion in the presence and absence of chloramphenicol and CYP activity was measured. CYP activity was determined for the NADPH-dependent demethylation reaction of the fluorogenic substrate AMMC (100 μM). We found that CYP AMMC *O*-demethylase activity was inhibited by 95% in the microsomes prepared from chloramphenicol-treated rat hearts (Fig. 3*C*).

To confirm that the cardioprotective effect of chloramphenicol was caused by inhibition of CYPs, we used additional CYP inhibitors that did not affect mitochondrial protein synthesis, in the Langendorff perfusion model. Cimetidine (200–600 μM) conferred a dose-dependent reduction in infarct size and CK release (Fig. 4). It should be noted that postischemic coronary flow was also enhanced in the treated hearts. Ketoconazole, a potent inhibitor of CYP3A4 and a weak inhibitor of several other CYP isozymes including CYP2C9, conferred little or no benefit at a concentration of 20 μM (data not shown).

Because chloramphenicol and cimetidine are both known to inhibit CYP2C9 (15, 16), the selective CYP2C9 inhibitor, sulfaphenazole (IC$_{50}$ = 0.6 μM; ref. 17), was also tested. Sulfaphenazole (10 μM and 300 μM) treatment resulted in a significant reduction in CK release and infarct size after 2-h reperfusion (Fig. 4). Sulfaphenazole was also tested for its ability to reduce infarct size when administered after ischemia and was found to be as effective as pretreatment (Fig. 4).

Sulfaphenazole is a potent and selective inhibitor of human CYP2C9 and was recently shown to inhibit a CYP2C9-related enzyme in rat mesenteric arteries. This sulfaphenazole-sensitive enzyme activity was shown to be responsible for the generation of the arachidonic acid metabolite 11,12-epoxy-eicosatrienoic acid (11,12-EET) (9). Therefore, after observing the dose-dependent protection of sulfaphenazole on ischemia–reperfusion injury, we examined whether sulfaphenazole could inhibit rodent P450 isozyme activity. The effect of sulfaphenazole concentration on rat hepatic microsome, isozyme-mediated modification was investigated with the fluorogenic substrate AMMC. The ability of the CYP2C9-like activity in rat mesenteric arteries to demethylate AMMC is unknown. However, we found that sulfaphenazole inhibited AMMC demethylation in rat liver microsomes (IC$_{50}$ = 0.5 μM) (Fig. 5*A*). AMMC is described by the manufacturers as a selective substrate for CYP2D2 in rat and CYP2D6 in humans. To determine whether CYP2D2 was the AMMC demethylase inhibited by sulfaphenazole, the effect of sulfaphenazole (50 μM) on AMMC demethylase activity was assessed by using CYP2D2 supersomes (microsomes isolated from baculovirus-infected insect cells that specifically express CYP2D2). Sulfaphenazole did not inhibit CYP2D2 activity, suggesting that sulfaphenazole is inhibiting an as yet unidentified CYP AMMC demethylase (Fig. 5*B*),

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Fig. 5. Effect of sulfaphenazole on AMMC demethylase activity. (*A*) The rate of AHMC product formation was assessed, and the IC_{50} value was calculated as described. (*B*) AHMC product formation was measured over 2 h in baculovirus-infected CYP2D2-specific supersomes treated in the presence or absence of sulfaphenazole (50 μM) (SUL). (*C*) Rat cardiac microsomes were resolved by SDS/PAGE and immunoblotted with anti-CYP2C9 antibody. (*Right*) Three micrograms of liver and 10 μg of cardiac microsomes.

Fig. 6. Effect of chloramphenicol on superoxide generation. Rat hearts were perfused in Langendorff mode with or without chloramphenicol (300 μM) (CAP) for 15 min and then subjected to 20 min of ischemia and 15 min of reperfusion with the same buffer. Superoxide levels were assessed by measuring dihydroethidium conversion to ethidium as described. The asterisk represents a P value <0.05. Error bars represent the standard error for ischemia/reperfusion ($n = 5$) and ischemia/reperfusion plus chloramphenicol (300 μM) ($n = 4$).

which we suggest is the recently reported rodent CYP2C9-like enzyme (9).

To further test this possibility, we obtained antibody to human CYP2C9, which also crossreacts with rat 2C family members, and examined microsomes prepared from rat heart tissue. We detected several immunoreactive bands in rat heart microsomes corresponding to CYP2C isozymes (Fig. 5*C*). The ≈50-kDa band was approximately one-third as abundant as in rat liver microsomes (data not shown). This finding is consistent with the observations of Earley *et al.* (9), who described an ≈56-kDa 2C isozyme in rat mesenteric arteries.

Ischemia and reperfusion injury are associated with increased production of ROS, such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), singlet molecular oxygen ($^1O_2^*$), and hydroxyl radical (HO$^{\cdot}$). CYP2C9 has recently been shown to be a significant source of superoxide in human coronary arteries (8). To assess whether the cardioprotective effect of chloramphenicol could be attributed to a reduction in the production of ROS, superoxide generation was measured in heart slices obtained after 30-min ischemia and 15 min of reperfusion (Fig. 6). The oxidation of dihydroethidium to ethidium, a quantifiable chemical marker of ongoing superoxide anion production, was reduced ≈3-fold in chloramphenicol-treated hearts vs. ischemia/reperfusion without drug ($33.6 \pm 5.0\%$ vs. $10.5 \pm 1.6\%$, $P < 0.05$, $n = 4$).

Discussion

We have shown that chloramphenicol, cimetidine, and sulfaphenazole are able to reduce infarct size, with sulfaphenazole being the most potent inhibitor. The properties of CYP inhibitors toward human liver microsomes have been extensively characterized, and selective inhibitors for some isozymes have been identified. The only human isozyme that is inhibited by chloramphenicol, cimetidine, and sulfaphenazole is CYP2C9 (15, 18–21). Considering that a CYP2C9-like activity has been demonstrated in rat arteries, it is reasonable to hypothesize that inhibition of this enzyme is the basis of protection by chloramphenicol, cimetidine, and sulfaphenazole.
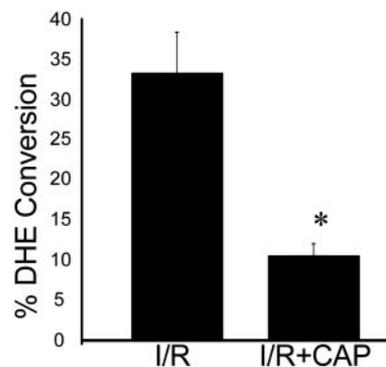
Arachidonic acid is a substrate for CYP epoxygenases, CYP ω-hydroxylases, cyclooxygenases, and lipoxygenases. These products have widely different physiologic effects; thus, inhibition of a subset of CYP enzymes may have complex consequences, potentially shifting the equilibrium between epoxides (EETs), ω-hydroxyls (HETEs), and other products (prostaglandins, prostacyclins, leukotrienes). The calcium-independent phospholipase A2 (iPLA2) liberates arachidonic acid, and inhibition of iPLA2 reduces infarct size (22). These observations suggest that the participation of CYP enzymes in the metabolism of arachidonic acid is of physiologic importance in the myocardium. It is becoming increasingly clear that CYP enzymes play a key role in the modulation of vascular homeostasis through the conversion of arachidonic acid to vasoactive eicosanoids (4, 5). It is of interest, therefore, that these CYP inhibitors preserved coronary flow upon reperfusion. Eicosanoids can serve as intracellular second messengers for protein kinases (23, 24). CYP-generated eicosanoids have been shown to regulate ATP-sensitive K$^+$ channels on the plasma membrane as well as the large-conductance calcium-sensitive potassium channel (BK_{Ca}) (25). It is also possible that CYP-generated eicosanoids could modulate the mitochondrial K_{ATP} channel that has been demonstrated to be essential for protection by preconditioning (26). In this regard, it is noteworthy that overexpression of CYP2J2 has been shown to confer protection against hypoxia/reoxygenation through the production of epoxygenated metabolites (EETs), an effect that is blocked by the mitoK$_{ATP}$ channel inhibitor 5-hydroxydecanoate (27). A sulfaphenazole-sensitive CYP2C9-like enzyme has been demonstrated in rat arteries and is implicated in the production of the eicosanoid 11,12-EET (9). Interestingly, 11,12-EET was shown to exacerbate contractile dysfunction after ischemia and reperfusion (28). Another metabolite, 14,15-EET, has been shown to be responsible for the negative inotropic effect of bradykinin (29). This metabolite opens L-type calcium channels, thereby causing a brisk influx of calcium (30). CYP 2C9 has recently been shown to also be a significant source of superoxide in human coronary arteries, and the superoxide production could be inhibited by pretreatment with sulfaphenazole (8). Our observation that chloramphenicol largely abrogated dihydroethidium conversion suggests that one or more of the heme-containing CYP monooxygenase enzymes are the main culprits in the generation of superoxide during reperfusion of the heart. This is surprising, considering the relatively low abundance of CYP enzymes in heart tissue.

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However, dysregulated CYP activity could lead to mitochondrial damage and secondary production of ROS from the electron transfer complexes (31).

Chloramphenicol and sulfaphenazole are strongly cardioprotective even when administered at reperfusion, suggesting that reperfusion injury is a major determinant of tissue damage in the Langendorff model. Administration of chloramphenicol or sulfaphenazole at reperfusion did not significantly reduce CK release when measured during the first 15 min of reperfusion, although they reduced infarct size substantially. In part, CK release may reflect damage sustained during the ischemic phase as well as injury sustained during the first minutes of reperfusion. Free radicals are generated in the first minute of reperfusion with a peak 4–7 min later, but ROS production continues at lower sustained levels for quite some time after that (32). The reduction in injury by ischemic postconditioning has been shown to be associated with diminished ROS production (33). We suggest that inhibition of a CYP2C9-like enzyme suppresses ongoing ROS production during reperfusion and thereby reduces tissue damage as assessed by tetrazolium staining. Inhibition of ROS production may be more beneficial than a free radical scavenger, because such antioxidants must compete with cellular targets to protect tissue from ongoing ROS production. In contrast, CYP inhibitors may prevent the formation of ROS in the first place.

The finding that CYP play a deleterious role in ischemia/reperfusion injury is not completely novel. Cimetidine was used to inhibit CYP and attenuate reperfusion injury in the rabbit lung (34). Cimetidine was also shown to diminish arrhythmias during reperfusion (35). That this was due to inhibition of CYP rather than antihistamine effects is suggested by a later study comparing cimetidine to ranitidine, which does not inhibit CYP and was not protective (36). Clotrimazole was shown to confer some benefit (37). Clotrimazole, like ketoconazole, is an inhibitor of some CYP isozymes, including CYP 2C8 (but not 2C9) and CYP 3A4. We found that ketoconazole (20 μM) was of marginal benefit (data not shown). CYP were shown to be a source of catalytic iron in primary cultures of renal cells subjected to hypoxia/reoxygenation, and CYP inhibitors were protective in that model (38). Interestingly, in that study, CYP inhibitors attenuated hydroxyl radical formation, but had no effect on superoxide production, in contrast to our observation that chloramphenicol attenuated superoxide production in the heart. These observations support the hypothesis that cytochrome P450 enzymes in the heart play a significant role in ischemia/reperfusion injury.

The finding that CYP monooxygenases mediate myocardial reperfusion injury has important implications for the treatment of cardiovascular disease. For instance, cigarette smoking upregulates CYP enzymes and increases the risk of a fatal myocardial infarction, whereas cholesterol-lowering drugs of the statin family are, for the most part, CYP inhibitors and reduce cardiovascular risk independently of their cholesterol lowering effects. Many cardiovascular drugs are, in fact, CYP inducers. Perhaps the most immediate impact of the present study is the realization that a variety of agents already in clinical use, which share the widespread pharmacological property of inhibiting CYP monooxygenases, may serve to protect organs, including the heart, against the damage that accompanies ischemia and reperfusion.

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