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Lidocaine Protects from Myocardial Damage due to Ischemia and Reperfusion in Mice by Its Antiapoptotic Effects

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Background: **Perioperative myocardial ischemia poses a vital threat to surgical patients. Means to protect postischemic myocardium are clinically not available. Lidocaine has been demonstrated to exert antiinflammatory pleiotropic effects. The authors set out to test if lidocaine protects ischemic myocardium from reperfusion injury.**

Method: **A mouse model of transient coronary artery ligation (30 min) and reperfusion (24 h) was used with animal care committee approval. Infarct size and area-at-risk were determined. Leukocyte recruitment was quantified on immunohistochemical stainings. Apoptosis was assessed using enzyme-linked immunosorbent assay to detect histone modifications and terminal deoxynucleotidyl transferase dUTP nick end labeling assays. Lidocaine effects on leukocyte-endothelial interactions were assessed *in vitro* by using a parallel-plate flow chamber or static adhesion assays.**

Results: **Infarct size per area-at-risk was reduced by 27% in mice treated with a lidocaine bolus (1 mg/kg) before a continuous infusion (0.6 mg · kg^{-1} · h^{-1}) during ischemia ($P < 0.005$). Neutrophil density in the infarct and periinfarct zone was not reduced by lidocaine, although the size of the infiltrated area was. Terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cardiomyocytes and endothelial cells were significantly reduced in the periinfarct zone by lidocaine. *In vitro*, no effect on leukocyte rolling or firm adhesion to resting or activated endothelium was demonstrable. *In vitro*, lidocaine reduced cardiomyocyte apoptosis induced by hypoxia and reoxygenation (3h/1h) significantly. Infarct size and *in vitro* cardiomyocyte apoptosis were likewise reduced when lidocaine bolus and infusion were administered after the ischemic insult.**

Conclusion: **Lidocaine exerts cardioprotective effects when administered before or after the ischemic insult. This effect is mediated through an antiapoptotic and not through an antiinflammatory pathway and may be therapeutically exploitable.**

MYOCARDIAL infarction in the perioperative phase is frequent and carries an exceedingly high mortality.[1] In postsurgical patients, the success of current therapeutical interventions like lysis therapy or percutaneous coronary interventions aiming at early reperfusion of the occluded vessel are limited because of the imminent bleeding risk that results from associated antiplatelet or anticoagulation strategies.[2] In addition, success of reperfusion therapy is limited by reperfusion syndrome. Tissue ischemia is a potent inflammatory stimulus resulting in robust endothelial activation in the periinfarct zone, where ischemia does not lead to cellular necrosis. Necrosis as well as the adjacent activated endothelium in the postischemic myocardium will attract leukocytes and lead to their extravasation.[3] Leukocyte recruitment is desirable because it is the prerequisite for removal of the necrotic tissue and consecutive scarring of the infarct area.[4] Yet, leukocyte recruitment also causes additional damage to the adjacent nonnecrotic ischemic myocardium, enlarging the size of the infarct.[3] Attenuation of the inflammatory response may thus diminish reperfusion damage and maximize the benefit of the antiischemic therapy.[5]

The search is ongoing for agents exerting such modulating effects on inflammation. Among other pleiotropic effects, local anesthetics have been described to play a role in inflammatory processes.[6,7] In 1980, Nasser *et al.* showed that lidocaine reduces infarct size in dogs[8] without offering a candidate mechanism of action. Ebel *et al.* demonstrated in their isolated leukocyte-free, buffer-perfused rat heart model that lidocaine reduces myocardial damage caused by low-flow ischemia. This group observed no effect on reperfusion injury.[9] In their porcine model, Lee *et al.* demonstrated that lidocaine reduces myocardial damage when administered retrogradely through a coronary sinus catheter before coronary reperfusion. Systemic lidocaine administration, however, had no beneficial effect. On the basis of these data, the authors suggested that endothelial cells (EC) and not leukocytes are the target for lidocaine effects.[10]

We set out to investigate whether the lidocaine-afforded cardioprotection in our very well characterized *in vivo* mouse model of myocardial ischemia with reper-

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fusion is dependent on antiinflammatory or antiapoptotic properties of lidocaine on leukocytes or ECs.

Materials and Methods

Myocardial Ischemia/Reperfusion

Myocardial ischemia (30 min with 24 h of reperfusion) was induced with the approval of the Animal Care Committee (Tierschutzkommission Muenster, Muenster, Germany) and in accordance with the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health.[11] The effect of lidocaine on infarct size was assessed in an outbred Swiss/SV129 strain. Lidocaine (AstraZeneca, Wedel, Germany) was administered IV as a 1 mg/kg bolus before infliction of ischemia followed by continuous infusion (0.6 $mg \cdot kg^{-1} \cdot h^{-1}$) of lidocaine until reperfusion was induced (n = 11). Control animals (n = 11) were treated according to the same protocol by using saline or phosphate-buffered saline (PBS; PAA, Coelbe, Germany) to assess infarct size. For immunohistochemistry and assessment of apoptosis, 11 additional control and 9 lidocaine-treated mice were used. An additional set of 18 mice were subjected to myocardial ischemia. Upon reperfusion, the same bolus and infusion protocol of lidocaine (n = 10) or saline (n = 8) resulting in the same cumulative dose was initiated to test whether lidocaine was required during ischemia or could be therapeutically given upon reperfusion. Infarct size in these mice was assessed at an early time point (3 h) because continuous administration of lidocaine for 24 h during reperfusion was technically and pharmacokinetically impossible. For infarct size measurements after myocardial ischemia, animals were perfused through the abdominal aorta with 0.9% saline. The coronary ligation was retied, and 2% Coomassie Blue was injected to delineate the area-at-risk. The heart was sectioned into five equal slices and immersed in 2,3,5-triphenyl-tetrazolium chloride (TTC; Sigma, Taufkirchen, Germany) at 37°C for 10 min. Left ventricular area, area-at-risk, and area of infarction were determined morphometrically by using NIH-Image as previously published.[12] Coronary ligation resulted in an ischemic area of 8.7 ± 0.6 mm^2 (n = 11), constituting the area-at-risk and an infarcted area of 4.0 ± 0.4 mm^2. Neither left ventricular area nor area-at-risk were statistically different between treatment groups. Plasma samples were taken before onset, immediately after ischemia, and before sacrifice, and they were stored at −80°C to determine lidocaine and troponin concentration. Lidocaine concentrations were determined using high-performance liquid chromatography (Laboratoriumsmedizin Dortmund Dr. Eberhard & Partner, A. Eberhard, M.D., Ph.D., Dortmund, Germany). Troponin T was assessed using the Cardiac T point-of-care kit (Roche Diagnostics, Mannheim, Germany) on 1:10 diluted individual plasma samples.[13]

Immunohistochemistry

Immunohistochemistry for polymorphonuclear leukocytes (PMN) was performed using a neutrophil-specific monoclonal antibody[14] clone MCA771G (Serotec, Oxford, England), and detection of apoptotic nuclei was performed by using commercial terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining kits (Chemicon, Temecula, CA; Calbiochem, San Diego, CA). TUNEL-positive nuclei were counted and expressed as percent TUNEL-positive/total nuclei. To analyze whether endothelial or cardiomyocyte apoptosis was prevented, additional immunohistochemical stainings were performed on TUNEL-stained sections developed with a fluorescein isothiocyanate fluorescent substrate (MBL, Woburn, MA). ECs were then detected using a polyclonal antibody against Laminin α 5 that is predominantly expressed in ECs. Cardiomyocytes were identified on the basis of phalloidin intercalating with actin (Alexa Fluor® 568 phalloidin; Molecular Probes, Eugene, OR). To determine the density of the PMN infiltrate, the number of PMN/mm^2 was counted on six sections per heart using automated morphometry software (AnalySIS, Muenster, Germany). On low magnification (4×), the infiltrate was easily distinguished, lined out, and measured because it colocalizes with the infarction and the periinfarct zone. Thus, the infiltrated area as a fraction of left ventricular cross-sectional area was used to estimate differences in affected myocardium.

Assessment of Apoptotic Cell Death after Simulated Ischemia/Reperfusion in Vitro

Ventricular cardiac myocytes were isolated from 1- to 3-day-old Sprague-Dawley rats (Charles River, Sulzfeld, Germany) by Percoll density gradient centrifugation and plated in Dulbecco Modified Eagle Medium/Medium 199 (4:1), supplemented with 10% horse serum, 5% fetal calf serum, 2 mM glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin in gelatin-coated culture dishes at a density of 4×10^4 cells/cm^2. After 24 h, the medium was replaced by Dulbecco Modified Eagle Medium/Medium 199 supplemented with glutamine and antibiotics. Cardiac myocytes were exposed to simulated ischemia and reperfusion as described.[15] In brief, the cells were switched from maintenance medium to a buffer containing 137 mM NaCl, 12 mM KCl, 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$, 4 mM HEPES, 10 mM 2-deoxy-glucose, and 20 mM sodium lactate (pH 6.2) and were incubated at 37°C in a hypoxia chamber (Modular Incubator Chamber-101; Billups-Rothenberg, San Diego, CA) flushed with 5% CO_2 and 95% N_2 (simulated ischemia). Control cells were cultured in a buffer containing 137 mM NaCl, 3.8 mM KCl, 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$, 4 mM HEPES, 10 mM glucose, and 20 mM pyruvate (pH 7.4) and incubated at 37°C in an atmosphere containing 5% CO_2 and 95% room air (normoxia). After 3 h, cardiac myocytes were

switched back to maintenance medium and kept in 5% CO_2 and 95% room air at 37°C for 60 min (simulated reperfusion). Cardiac myocyte apoptosis was assessed by a cell death detection ELISA (Roche Applied Science, Mannheim, Germany), which measures the formation of histone-associated DNA fragments. TUNEL-positive cells were detected *in vitro* using commercial TUNEL-staining kits (Calbiochem, San Diego, CA). Lidocaine (10^{-4} M) was added either before hypoxia or with the beginning of reoxygenation.

Flow Chamber Studies and Static Adhesion Assays

Endothelial adhesiveness for mouse peritoneal macrophages and bone marrow-derived polymorphnuclear neutrophils was determined using a parallel plate flow chamber model or static adhesion assays as described previously.[11,12] Thioglycollate-elicited peritoneal macrophages or PMNs (200,000/ml) were labeled using Cell Tracker Green (Molecular Probes) and perfused (100 s^{-1}) across tumor necrosis factor α (TNFα)–activated (Biosource, Solingen, Germany) immortalized murine ECs (fEnd.5). The number of rolling cells was determined from 3-min video streams captured on a confocal microscope (UltraView; Perkin Elmer, Juegesheim, Germany). Firm adhesion was quantified on pictures taken from 15 high-power fields (hpf) at predefined locations after 3-min cell perfusion followed by 3-min buffer wash. Static adhesion assays were carried out as described elsewhere.[16] Briefly, 200,000 Cell Tracker Green–labeled PMNs or macrophages were incubated on resting or TNFα-activated fEnd.5 for 30 min on a rocking plate (20/s). Immediately after the assay, nonadherent cells were removed by decanting and buffer rinse, and the adherent Cell Tracker Green–labeled leukocytes were video microscopically counted in 15 hpfs at predefined locations (Eclipse TE300, 10× magnification; Nikon, Melville, NY).

Statistical Analyses

The more conservative approach of nonparametric testing was employed because the groups displayed significantly different SDs, precluding parametric testing without data transformation. As a result of their normal distribution, data are nevertheless reported as mean ± SEM. Kruskal-Wallis test was performed. If $P < 0.05$, a Mann–Whitney U test was used to identify significant differences between groups at $P < 0.05$ (InStat; GraphPad Inc., San Diego, CA). In experiments with comparison of multiple groups Dunn test was applied if multiple groups were compared to correct for multiple testing. All authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

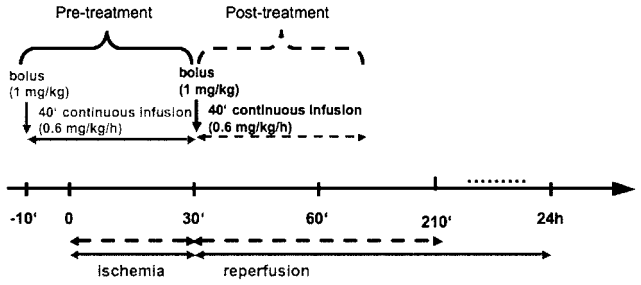

Fig. 1. Study protocol for *in vivo* experiments. A bolus of 1 mg/kg followed by a continuous infusion of 0.6 mg · kg^{-1} · h^{-1} lidocaine was administered intravenously either before and during ischemia (*solid line*) or starting with reperfusion in the 3-h reperfusion group. At the respective end of the study, blood was drawn from the caval vein for determination of lidocaine plasma levels and troponine T in the 24-h group. Animals were perfused with saline, the coronary ligation was retied, and Coomassie Blue or paraformaldehyde was infused before infarct size was determined by 2,3,5-triphenyl-tetrazolium chloride staining or paraffin embedding was performed.

Results

Lidocaine Reduces Myocardial Damage

The study protocol is schematically depicted in figure 1. Lidocaine administration led to plasma levels of 13.4 ± 1 μg in lidocaine-treated animals. The infarction area expressed as fraction of the area-at-risk was significantly reduced by lidocaine (46.3 ± 2.1% *vs.* 34.3 ± 0.9%, control *vs.* lidocaine; $P < 0.01$; n = 11; fig. 2A). The level of troponin T was likewise significantly lower in the lidocaine-treated group (1.67 ± 0.26 ng/ml *vs.* 0.85 ± 0.08 ng/ml, control *vs.* lidocaine; $P < 0.05$; n = 12/13; fig. 2B).

Lidocaine Exerts Antiapoptotic But No Antileukocyte Effects in Vivo

Reflecting a cardioprotective effect of lidocaine, the fraction of apoptotic nuclei of total cells was significantly reduced (15.4 ± 3.2% *vs.* 2.4 ± 0.9%, control *vs.* lidocaine; $P < 0.01$; n = 11/9; fig. 3, A and B). Double

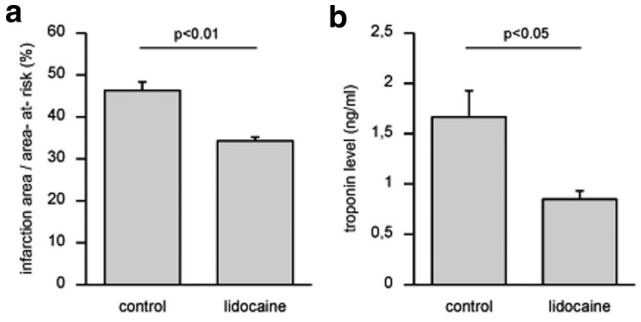

Fig. 2. Lidocaine reduces myocardial damage. (*a*) Infarct size expressed as fractions of the area-at-risk (%) was significantly reduced by lidocaine bolus administration followed by a continuous infusion until reperfusion was reinstituted (n = 11). (*b*) Troponin T levels (ng/ml) are likewise decreased by lidocaine treatment (n = 11). Troponin T values are reported as absolute values and not normalized to the area-at-risk. Area-at-risk was extremely stable between individual experiments and experimental groups.

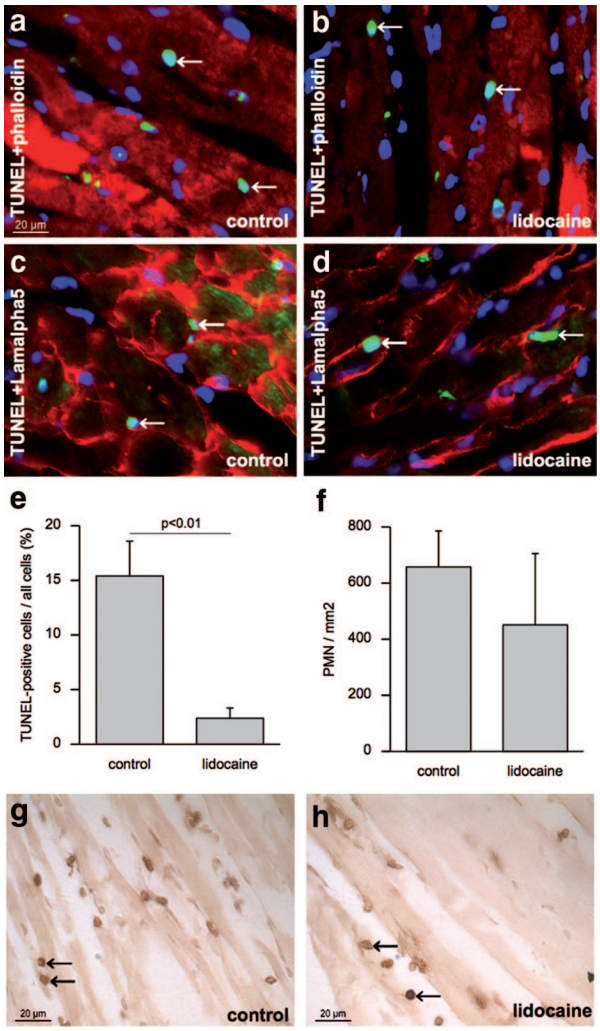

Fig. 3. PMN and TUNEL stainings of the peri-infarct zone. a) and b) Double staining for apoptotic nuclei (TUNEL, green, arrows indicate nuclei of apoptotic cells) and cardiomyocytes (phalloidin, red) and nuclei (DAPI, blue) in control (*a*) and lidocaine treated mice (*b*). Phalloidin stains the actin fibers that are partly still present after infarction. The left panel demonstrates abundant apoptosis in cardiomyocytes (red and nuclear shape) and non-cardiomyocytes (unstained and green nuclei). These cells are according to their cellular and nuclear shape likely endothelial cells. Cardiomyocyte apoptosis is reduced in the lidocaine-treated mice (*b*). c) and d) Double staining for apoptotic nuclei (TUNEL, green, arrows indicate apoptotic nuclei), endothelial cells (laminin alpha 5, red) and nuclei (DAPI, blue). Non-cardiomyocyte apoptosis (green nuclei) in control animals largely occurs in endothelial cells (red) and is likewise reduced by lidocaine treatment (*d*), indicating that lidocaine reduces apoptosis in endothelial cells and cardiomyocytes (*a–d*). e) TUNEL-positive cells expressed as the fraction of all cells (n = 11/11, p < 0.05. Lidocaine application leads to a significant decrease of TUNEL-positive cells. f) Density of polymorphonuclear leukocytes (PMN) infiltrate (cells/mm^2) is not significantly reduced despite a sufficiently large number of experiments (n = 11 and 9). g) and h) PMN-stain, arrows indicate PMNs stained by the neutrophil specific monoclonal antibody. PMNs are recruited to the postischemic myocardium and are adherent to the endothelial lining of intercardiomyocyte capillaries or extravasate into the tissue of control mice (g). PMN density is not reduced by lidocaine treatment, while the size of the infiltrate area decreases secondary to reduced cell death and myocardial loss (text). Hearts were perfusion fixed at physiological pressures to achieve total removal of blood from the tissue.

stainings for ECs and cardiomyocytes with apoptotic nuclei demonstrated that both ECs and cardiomyocytes underwent apoptosis and that the antiapoptotic effect extends to both cell types (fig. 3A). We have shown previously that in our model about 50% of the infarct size depends on leukocyte recruitment.[12] Using a neutrophil-specific antibody, we demonstrate that, despite smaller infarctions and reduced apoptosis, PMN recruitment to a given infarct area (neutrophil density) was not reduced in lidocaine-treated animals (657.5 ± 128.3 PMN/mm^2 *vs.* 451.2 ± 254.1 PMN/mm^2, control *vs.* lidocaine; P = NS; n = 11/9; fig. 3, C and D). On the basis of our previous observations, we expected a variation of PMN densities of about 25%. With the observed effect sizes and SDs, we expected successful detection of a significant difference of 15% with an α error of 0.05 and a 95% power with nine animals per group. With the actual SDs that we encountered, we achieved a statistical power of 92.6% to reject our hypothesis, suggesting that a meaningful difference in PMN recruitment is possible but highly unlikely (7.4%). To estimate the total number of PMNs recruited, we measured the area of the infiltrate. This area was reduced in lidocaine-treated hearts (18.5 ± 4.4% *vs.* 5.3 ± 3.1% of the left ventricular area, control *vs.* lidocaine; $P < 0.05$; n = 11/9), resulting–given the similarities of infiltration–in a net reduction of overall PMN recruitment.

Lidocaine Protects Cardiac Myocytes from Apoptosis during Hypoxia and Reoxygenation

To test the effect of lidocaine on cardiomyocyte apoptosis *in vitro*, we used a well-established cell culture model of hypoxia and reoxygenation to simulate ischemia/reperfusion. As shown by TUNEL stainings and histone ELISA, simulated hypoxia for 3 h followed by reoxygenation for 60 min promoted apoptosis in neonatal cardiac myocytes (fig. 4). Incubation of cardiac myocytes with lidocaine (10^{-4} M) cultured under normoxic conditions showed no significant effect on the apoptosis rate of these primary neonatal cardiomyocytes. Lidocaine (10^{-4} M) present during hypoxia reduced the percentage of TUNEL-positive cells back to control levels ($P < 0.05$; n = 4; fig. 4B), and the formation of oxygen-deprivation induced histone-associated DNA fragmentation significantly by 43% ($P < 0.05$; n = 4; fig. 4C).

Lidocaine Does Not Influence Leukocyte Adhesion

Because a reduction in the amount of inflammatory cells recruited can be the result of alterations in endothelial adhesiveness, leukocytic adhesiveness, or reduced tissue susceptibility to inflammatory activation, we analyzed the effects of lidocaine on leukocytes and ECs in *in vitro* adhesion systems. The number of firmly adherent PMNs in the static adhesion assays was 679.1 ± 84.0 cells per 15 hpfs (n = 12, control

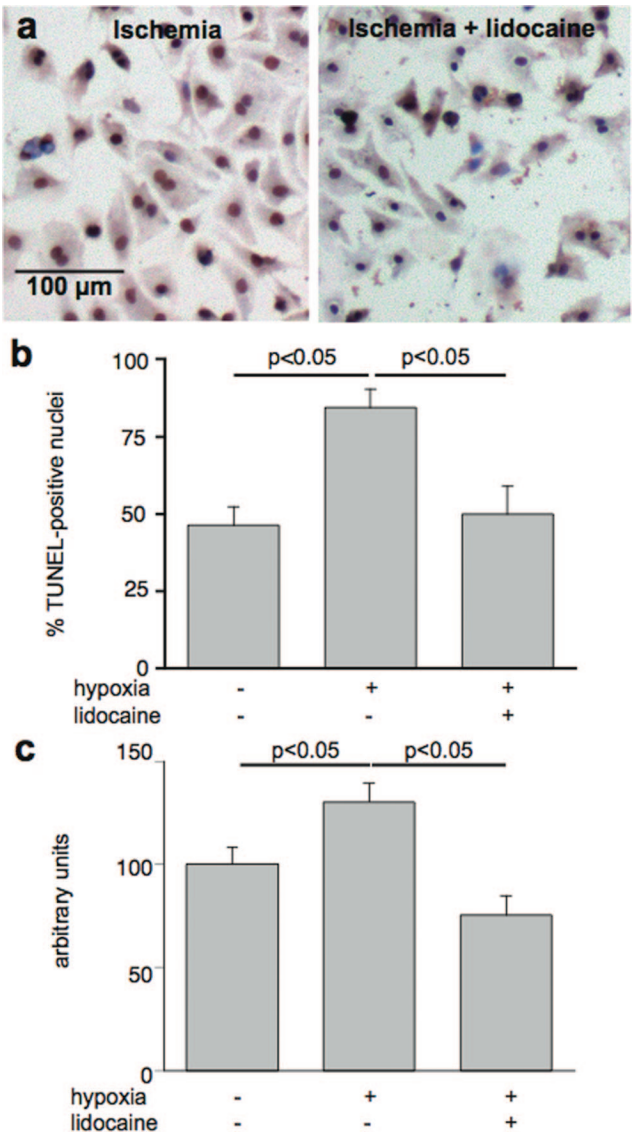

Fig. 4. ***In vitro*** **antiapoptotic effects of lidocaine on cardiomyocytes. (*a*) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) stainings of primary neonatal rat cardiomyocytes reveal a prominent reduction of TUNEL-positive nuclei. (*b*) Morphometric quantification of 10 high-power fields per well revealed a prominent increase in TUNEL-positive nuclei by ischemia that was in part prevented by lidocaine coincubation (n = 4; $P < 0.05$). (*c*) For confirmation of the antiapoptotic effect of lidocaine, histone methylation was assessed by enzyme-linked immunosorbent assay (ELISA; n = 4; $P < 0.05$) and revealed a significant reduction of apoptosis-specific DNA modifications.**

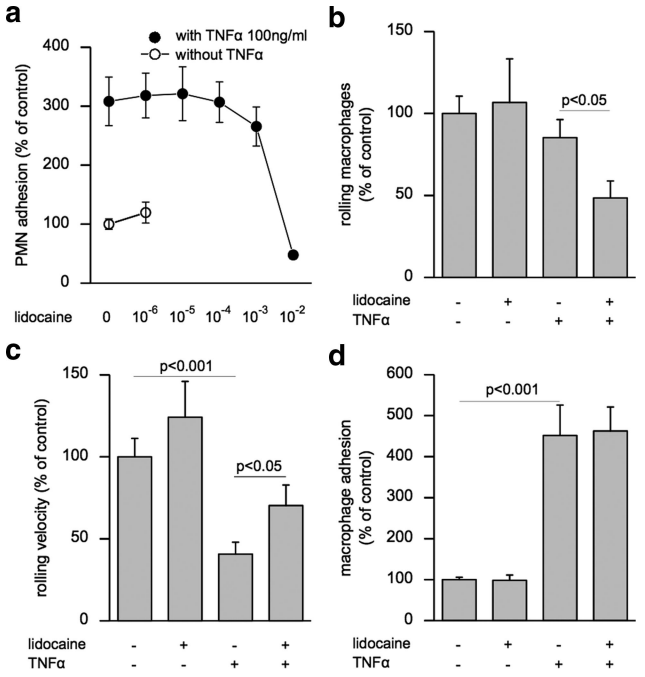

Fig. 5. ***In vitro*** **antiinflammatory effects of lidocaine are subtle. (*a*) Adhesion of polymorphonuclear leukocytes (PMN) in the static adhesion assay as percent of control. Lidocaine influences neither adhesion to resting nor tumor necrosis factor α (TNFα)–activated endothelial cells (EC); 10^{-2} M lidocaine is toxic to ECs and leads to their rounding and detachment. (*b*) The number of rolling macrophages on TNFα-activated ECs under flow conditions is significantly decreased by lidocaine. (*c*) TNFα significantly decreases rolling velocity, which can be partly reversed by lidocaine (n = 24). (*d*) Macrophage adhesion in the flow chamber assay. Data are presented as percent of control. Lidocaine has no effect on adhesion to resting or TNFα-activated ECs.**

group). Activation of ECs with TNFα (100 ng/ml) increased PMN adhesion threefold (308.3 ± 41.2%; $P < 0.001$; n = 12; fig. 5A). Pretreatment of ECs with lidocaine (10^{-6} M) altered adhesion to neither resting ECs (119.7 ± 17.8%; n = 12) nor TNFα-activated ECs (318.1 ± 38.0%; n = 12). Higher concentrations of lidocaine (up to 10^{-3} M) had no antiadhesive effect on activated ECs, whereas a dose of 10^{-2} M lidocaine exerted a cytotoxic effect leading to rounding and detachment of the ECs (fig. 5A). The calculated power to detect a difference of 25% with an SD of 15% in groups of n = 12 at $\alpha = 0.05$ was 0.99.[17] Similar results were obtained using alternative cell lines (human immortalized cell line EAHy926 and/or murine macrophages; data not shown). Numerous groups have demonstrated antiadhesive effects of lidocaine; we therefore asked whether the antiinflammatory potential of lidocaine depended on the activating stimulus and its signaling pathway. Neither was EC activation induced by the thromboxane-A2-(TXA_2) receptor agonist U-46619 (Calbiochem, Bad Soden, Germany) nor lysophosphatidic acid (LPA) or Phorbol 12-myristate 13-acetate (PMA; both Sigma-Aldrich, Taufkirchen, Germany) amenable to lidocaine-mediated suppression of leukocyte adhesion (data not shown). In addition, no effect of lidocaine on leukocyte-endothelial interactions was observed when leukocytes or ECs were pretreated with lidocaine separately or if lidocaine was present during the assay.

Lidocaine Increases Rolling Velocity of Leukocytes on Activated EC

We hypothesized that lidocaine may affect the dynamic interactions of leukocytes with ECs and used a parallel plate flow chamber model, allowing for direct

visualization of dynamic interactions between endothelium and superfused leukocytes. The number of rolling macrophages was 28.8 ± 3.5 cells in 3 min (n = 8; fig. 5). TNFα (100 ng/ml) did not increase the number of rolling cells per hpf (100.0 ± 10.6% *vs.* 85.2 ± 11.0%, control *vs.* TNFα; P = NS; n = 8; fig. 5B). Preincubation of resting ECs with lidocaine (10^{-6} M) had no effect on the number of rolling macrophages (100 ± 10.6% *vs.* 106.7 ± 26.6%, control *vs.* lidocaine; P = NS; n = 8/5; fig. 5B). However, on TNFα-activated ECs, lidocaine did reduce the number of rolling macrophages (48.5 ± 10.4%; $P < 0.05$; n = 7; fig. 5B). Rolling velocity of macrophages on resting ECs was 22.7 ± 2.8 μm/s (n = 26). TNFα (100 ng/ml) significantly reduced the rolling velocity (100 ± 11.2% *vs.* 40.7 ± 7.3%, control *vs.* TNFα; $P < 0.001$; n = 26/25; fig. 5C). Lidocaine increased the velocity of rolling leukocytes on resting ECs (100 ± 11.2% *vs.* 124.2 ± 21.8%, control *vs.* lidocaine; P = NS; n = 26/24; fig. 5C). Even more pronounced was the increase of rolling velocity on TNFα-activated ECs (70.3 ± 12.5%; $P < 0.05$; n = 24; fig. 5C). This subtle antiadhesive effect did not, however, result in differences in leukocyte recruitment to ECs. The number of firmly adhering macrophages on resting EC at the end of the flow chamber studies was 56.6 ± 8.2 cells/15 hpfs (n = 8). TNFα increased firm adhesion to 451.6 ± 74.3% ($P < 0.001$; n = 7) with no effect of lidocaine (10^{-6} M) on both untreated ECs (98.3 ± 12.9%; n = 5) and TNFα-activated ECs (462.7 ± 58.5%; n = 7; fig. 5D).

Therapeutic Administration of Lidocaine During Reperfusion

In a subset of animals lidocaine was administered with reperfusion commencing using the same protocol as for the preventive protocol. In order to yield comparable drug doses and to avoid larger cumulative doses and differences in plasma levels caused by 24 h lidocaine application we chose to assess infarct-reducing effects of lidocaine after 3 h of reperfusion. Infarcts were overall smaller, but infarct size in the lidocaine-treated animals was reduced by 25% ($P < 0.001$, n = 8/10, fig. 6A). In addition, after simulated ischemia/reperfusion *in vitro* cardiomyocyte apoptosis was reduced significantly if lidocaine (10^{-4} M) was added to the cells with the start of reoxygenation (18 ± 1% *vs.* 12.3 ± 0.7%; control *vs.* lidocaine, $P < 0.05$, n = 4, fig. 6B).

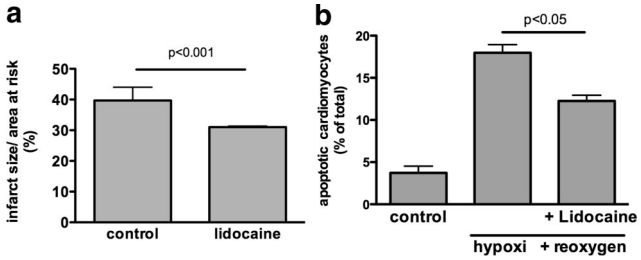

Fig. 6. Lidocaine reduces infarct size and cardiomyocyte apoptosis when administered during reperfusion. (*a*) Treatment of postischemic myocardium with lidocaine during reperfusion reduces infarct size 3 h after ischemia. The infarctions are overall smaller, yet lidocaine treatment reduces infarction by 25% compared to saline control (n = 8/10; $P < 0.01$). (*b*) Cardiomyocyte exposure to hypoxia induces apoptosis as assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Apoptosis can be significantly reduced by lidocaine during reoxygenation (n = 4; $P < 0.05$).

Discussion

The salient findings of this study are that lidocaine does exert cardioprotective effects after experimental myocardial ischemia with reperfusion, as shown by a reduction of the infarcted area and troponin T values in mice. This myocardium-sparing effect is, however, not attributable to antiinflammatory effects, but it is the result of antiapoptotic effects of the substance at the dosage used on damaged and cell death-prone cardiomyocytes. The cardioprotective effect of lidocaine is also observable if treatment starts immediately after ischemia is terminated.

Beyond their sodium-channel blocking properties, many pleiotropic effects of local anesthetics have been described.[6,7] Prominent effects are the inhibition of leukocyte-endothelial interactions and antithrombotic effects. The mechanisms by which local anesthetics exert these pleiotropic effects are still only partially clarified.

Lidocaine has been shown to exert beneficial effects in the setting of tissue ischemia.[18–21] Loss of myocardium in the course of myocardial ischemia is the result of aggregate damage caused by the initial ischemia, which induces cell death due to cellular hypoxia, and by reperfusion injury, which is partially governed by recruitment of inflammatory cells, leading to additional cellular damage.[3] Tissue loss resulting from ischemia can only be influenced if time of ischemia or tolerance to ischemia is altered, delivering the basis for the current clinical concept of early reperfusion therapy.[2]

The benefit of early reperfusion is limited during the postoperative phase because all available strategies (lysis, percutaneous coronary interventions, surgical revascularization) necessitate more or less intense antiplatelet and/or anticoagulatory interventions. These interventions are hampered by an accompanying risk increment for bleeding in surgical patient populations. Reperfusion-induced damage on the other hand is therapeutically accessible during later time points of the process. Leukocyte recruitment to infarct tissue follows immediately on reperfusion of an occluded vessel and leads to additional damage in the penumbra of the infarct.[2] Prevention or reduction of reperfusion injury salvages postischemic myocardium by reducing late myocardial death and apoptosis.[5,12] Local anesthetics, including lidocaine as reported by a multitude of groups, seemed to be a

candidate strategy to attenuate reperfusion injury by interfering with leukocyte-endothelial interactions.[22–25]

Some groups have reported that local anesthetics reduce recruitment of leukocytes from vessels into tissue, which could explain reduced reperfusion injury. Suggested mechanisms varied. Lidocaine inhibits granulocyte adherence and recruitment to inflammatory sites.[3,25,26] TNFα-induced leukocyte adhesion and tissue accumulation is reduced, whereas rolling is not.[21] Local anesthetics attenuate lysophosphatidic acid–induced priming in human neutrophils, rendering neutrophils less responsive to proinflammatory stimuli.[20] Lipopolysaccharide-induced production of monocyte chemoattractant protein 1 is reduced.[24] Cytokine-induced upregulation of adhesion molecules is reduced by lidocaine.[4,5] The effect of local anesthetics on leukocyte recruitment may, however, be more indirect. Sympatholysis induces vasodilatation and increases blood flow velocities, thereby reducing the probability of leukocytes contacting the vessel wall, which is a crucial step for leukocyte margination and extravasation. Furthermore, local anesthetics are known to exert an antiplatelet effect,[27] whereas cell emigration from the flowing blood can be assisted through deposited thrombocytes or aggregates with leukocytes.[28] We have been unable to demonstrate an alteration of endothelial-leukocyte interactions with respect to hypoxia- and ischemia-induced inflammation. We did not find a reduction or weakening of tethering or rolling of leukocytes, with the exception of an increased rolling velocity. We were likewise unsuccessful to demonstrate a difference in permanent recruitment and firm adhesion in our flow chamber model as well as in static adhesion assays. This lack of an antiadhesive effect of lidocaine was dose-independent and also independent of the stimulus used to induce endothelial activation. We also observed a lack of reduction in the density of leukocytes recruited to the infarct and the periinfarct zone. Still, the amount of leukocytes recruited to the myocardium in total was reduced, suggesting a prominent effect on myocardial tissue loss induced by ischemia and early reperfusion.

Leukocyte recruitment follows ischemia-induced cell death, which leads to synthesis and release of proinflammatory mediators.[3,11] Prevention of cell death in the postischemic myocardium would thus lead to a secondary reduction of the inflammatory response and thereby the leukocyte infiltrate. A candidate mechanism for such an effect would be the prevention of ischemia-induced cell death due to an increased hypoxic resistance or to an increased resistance to apoptosis secondary to ischemia and reperfusion-induced inflammation. We observed a very prominent antiapoptotic effect of lidocaine on ischemic myocardium *in vivo* and hypoxia-exposed cardiomyocytes *in vitro*. Support for this notion stems from a study by Ebel *et al.* demonstrating in a leukocyte-free, buffer-perfused Langendorf preparation of hypoxic rat hearts that lidocaine, when present during ischemia, can reduce myocardial damage and functional compromise during reperfusion.[9] Lidocaine and bupivacaine significantly increased ischemia tolerance of explanted hearts when added to the depolarizing cardioplegic solution.[29] Although no data about the mechanisms are available for myocardial ischemia, there is evidence that lidocaine may have an effect on neuronal death. Lidocaine attenuates apoptosis in transient focal cerebral ischemia in rats.[30] Reduced Na^+/K^+-ATPase activity translates into improved preservation of adenosine triphosphate,[18] and cell membrane stability[31] may represent beneficial effects of lidocaine.

Plasma concentrations greater than 10 μg/ml were reached in our study. These concentrations are also higher than those used to clinically exploit pleiotropic effects afforded by lidocaine (1–5 μg/ml) after systemic administration in patients[32] and 5-fold to 10-fold lower than needed for neuronal sodium channel blockade.[33] On the other hand the doses administered in our study were much lower than those identified as CD_{50} and LD_{50} for fatal convulsions and arrhythmias in mice, whereas the route of administration in those early studies were the intraperitoneal or subcutaneous routes, with a slower kinetic compared to the IV administration in our study.[34,35] We did not observe obvious toxicity of our regimen and the plasma concentrations reached.

A number of recent studies suggest a proapoptotic effect of lidocaine in neuronal cells[36–38] and other cell types.[8,39,40] Lidocaine was shown to activate the mitochondrial pathway of apoptosis at concentrations that occur in the cerebrospinal fluid during spinal anesthesia (3–6 mM).[41] At even higher concentrations, ($\geq$ 10 mM) the mechanism of cell death is predominantly necrosis. In concordance to these results, lidocaine at the highest concentration (10 mM) had a toxic effect on the EC line used here. Thus, the observed toxic effects seen in previous studies of apoptosis induction can be explained by much higher concentrations compared to those used here (1–100 μM). Along the same line, lidocaine has been demonstrated to abolish cardioprotective preconditioning effects of ischemic episodes and volatile anesthetics if administered at cytotoxic concentrations.[42,43] Taking together, lidocaine and other local anesthetics may affect mitochondrial bioenergetics in a concentration-dependent manner, leading to improved tolerance for ischemia and reperfusion at low concentrations (1–5 μM) and mitochondrial dysfunction and activation of mitochondrial apoptosis at higher concentrations (3–6 mM).

Our study has some limitations. Intravital microscopy is currently not feasible in the myocardial microvasculature. We therefore resorted to *in vitro* models that allow us to study leukocyte-EC interactions directly and independently of the surrounding tissues. Although we employed a number of different cell lines, leukocyte sources, and stimuli, we were unable to detect a reduction in leukocyte-EC interactions by lidocaine. We are

able to demonstrate robust induction of leukocyte adhesion by the inflammatory stimuli used, but we were unable to show antiadhesive effects of lidocaine in any setting except from a subtle increase of rolling velocity that did not translate into reduced firm adhesion. Extrapolation from these *in vitro* models to the *in vivo* setting is troublesome. We did however observe a very good concordance of our *in vivo* observation that leukocyte recruitment was not reduced in postischemic myocardium *per se*, but that the reduction of infarct size would secondarily cause a reduction of overall amount of leukocytes recruited. We have previously shown that leukocyte depletion or antiinflammatory strategies would reduce the size of an infarction after a given ischemic stimulus.[11,12] All these interventions caused a reduction of the density of leukocyte infiltrates. Lidocaine merely reduced the volume of myocardium infiltrated but not the density of the infiltrate itself; therefore, we indirectly conclude that the lidocaine effect is due to an improved ischemia resistance. Lidocaine was administered before ischemia and continuously until reperfusion was reinstituted or during reperfusion. But we did not investigate whether a low dose before ischemia would have been sufficient. We therefore cannot exclude that a preconditioning effect of lidocaine has contributed to the observed effect.[44]

In conclusion, we here demonstrate that lidocaine reduces infarct size preventively or therapeutically, most likely by reducing susceptibility of cardiomyocytes and ECs to hypoxia-induced apoptosis in mice. Systemic administration of lidocaine may therefore, in addition to its known beneficial effects, protect patients at risk for perioperative ischemia from myocardial damage. Administration of lidocaine has no major effects on blood coagulation or platelet function, although an inhibitory effect on the thromboxane-A2-receptor has been described. Therefore, use of lidocaine in a perioperative setting to reduce myocardial damage preventively in patients at risk for or therapeutically after having experienced myocardial ischemia could be a future option. Clinical studies to elucidate this effect in the clinical setting are therefore warranted.

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