Myocardial Capillary Permeability After Regional Ischemia and Reperfusion in the In Vivo Canine Heart

Effect of Superoxide Dismutase

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This study assesses the effect of the superoxide anion scavenger superoxide dismutase on myocardial capillary permeability–surface area (PS) products for small hydrophilic molecules after ischemia and reperfusion. Open-chest dogs underwent a 20-minute occlusion of the left anterior descending coronary artery followed by 1 hour of reperfusion. Myocardial plasma flow rate and capillary extraction of chromium 51-labeled EDTA or technetium 99m-labeled diethylenetriaminepentaacetic acid were measured by the single-injection, residue-detection method before ischemia and 5 and 60 minutes after the start of reperfusion. In 13 dogs, no scavenger treatment was given (nonprotected control group), whereas eight dogs were treated systemically with 15,000 units/kg superoxide dismutase during 1 hour, starting 20 minutes before ischemia. In the control group, three dogs developed reperfusion ventricular fibrillation in contrast to none in the superoxide dismutase group. Before ischemia, plasma flow rate, myocardial capillary extraction fraction, and PS values were similar in the two groups. Five minutes after the start of reperfusion, plasma flow rate increased significantly (p < 0.01) in both groups. In the control group, capillary extraction fraction increased by 12% (p = NS) in spite of the higher plasma flow; these increases in capillary extraction fraction and plasma flow induced a 69% increase in PS (p < 0.01). In the superoxide dismutase–treated group, capillary extraction fraction decreased by 32% (p < 0.05) in accordance with the increased plasma flow rate, resulting in an unchanged PS (p = NS). Sixty minutes after reperfusion, plasma flow rate, capillary extraction fraction, and PS returned to preocclusion values in both groups (p = NS). The increased capillary extraction fraction and PS values seen in the control group suggest an increased capillary permeability after ischemia and reperfusion. Superoxide anions seem to participate, directly or indirectly, in this response. (Circulation Research 1991;68:174–184)

Capillary permeability to solutes is influenced by several modalities of stimuli both during normal conditions and in pathophysiological conditions such as inflammation.1,2 In several organs and tissues such as intestine,3,4 liver,5 kidney,6 and skeletal muscle,7,8 ischemia and reperfusion alter capillary permeability.

Oxygen-derived free radicals have recently been demonstrated in reperfused myocardium.9–11 These substances, including the superoxide anion (•O₂⁻) and the hydroxyl radical (•OH), are highly reactive, short-lived oxygen species. Several studies12–14 have shown that tissue injury after ischemia and reperfusion may be mediated in part by these reactive molecules. Protection against the potential damage from these radicals can be obtained by enzymes that inhibit the synthesis of free radicals or their precursors (such as allopurinol) or by substances that eliminate the free radicals (such as superoxide dismutase [SOD], which dismutates the superoxide anion).

Capillary permeability–surface area (PS) products can be measured by methods based on indicator diffusion. When the in vivo working heart is under study, the single-injection, residue-detection (SIRD) method15,16 is simple to use, since coronary venous blood sampling is not required. After curve
resolution, it is possible to determine both capillary extraction fraction and plasma flow rate in the same experiment to allow calculation of the capillary PS product. To determine the capillary permeability (i.e., the permeability coefficient P) from the PS product, the value of the capillary surface area (S) must be known or kept constant, and under these circumstances, changes in PS can be taken to represent changes in P. The SIRD method has previously been used in a variety of organs and tissues, including the in vivo heart preparation, using small hydrophilic solutes and [111]albumin. A modification of the method with radiolabeled albumin has been used in an ischemia-reperfusion study applied to an in vitro non–blood-perfused rabbit heart preparation.

Previous studies of myocardial capillary permeability changes after ischemia and reperfusion have dealt with transcapillary exchange of macromolecules. Subtle alterations in capillary permeability will probably first be manifested in the permeability to small solutes and later on to macromolecules. The aims of the present in vivo canine study were 1) to determine whether 20 minutes of regional myocardial ischemia and subsequent reperfusion would induce an altered capillary permeability to small hydrophilic solutes when measured early (at 5 minutes) and late (at 60 minutes) after the start of reperfusion and 2) to examine the involvement of superoxide anions on changes of the microvascular exchange after ischemia and reperfusion by quantitating the effect of the superoxide anion scavenger SOD. A 20-minute period of ischemia followed by reperfusion was chosen since it has been shown to induce minimal (or no) myocyte necrosis and no morphological signs of endothelial cell injury.

**Materials and Methods**

**Animal Preparation**

The experiments were performed on mongrel dogs. Anesthesia was induced intravenously by 0.75 mg · kg⁻¹ diazepam, 0.5 mg fentanyl, and 4 mg pancuronium bromide. The dogs were intubated and ventilated artificially with oxygen and nitrous oxide to maintain normal arterial blood gases. Intravenous fentanyl (0.025 mg · kg⁻¹ · hr⁻¹) and pancuronium bromide (0.060 mg · kg⁻¹ · hr⁻¹) were given continuously. A catheter for blood pressure recording was introduced from the femoral artery, and the tip was placed in the ascending aorta. A Swan-Ganz flow-directed thermodilution catheter was inserted through the right external jugular vein, and the tip was placed in the pulmonary artery. A left thoracotomy was made by incision in the fifth intercostal space, and the pericardium was incised and sutured to the thoracic wall to limit the movements of the heart. The left anterior descending coronary artery (LAD) was isolated distally to the first major diagonal branch, and a silk suture was placed around the artery, to be used for short occlusions during indicator injections and to induce temporary coronary occlusion. To reduce the trauma to the artery at the site of occlusion, the silk suture passed through a small, plastic twin tube. Distal to the snare, a polyethylene catheter with an outer diameter of 1.0 mm was inserted into a small diagonal branch from the LAD. This catheter was connected to a pressure transducer (American Edwards Laboratory, Irvine, Calif.) by a saline-filled line and used for recording of the intracoronary blood pressure and for intracoronary indicator injections. The left atrial appendage was cannulated for infusion of saline or SOD solution. The dogs were given heparin (2,500 IU · hr⁻¹) and were allowed to rest for 30 minutes before measurements.

Throughout the experiments, aortic and pulmonary blood pressures, electrocardiogram, body temperature, arterial blood oxygen saturation, arterial blood oxygen tension, arterial blood carbon dioxide tensions, arterial pH, hematocrit values, and cardiac output were recorded. Coronary artery blood pressure was measured continuously from 10 minutes before the occlusion, during ischemia, and after reperfusion.

**Study Protocol**

Regional myocardial blood flow rate and capillary permeability of technetium 99m–labeled diethyleneetriaminepentaacetic acid ([⁹⁹mTc]DTPA) (molecular weight, 485.0; molecular radius, 0.5 nm) or chromium 51–labeled ethylenediaminetetraacetic acid ([⁵¹Cr]EDTA) (molecular weight, 340.2; molecular radius, 0.47 nm) were measured before LAD occlusion (measurement I). After this measurement, the dogs received either saline (control) or SOD infused into the left atrium. The SOD (copper-zinc SOD, synthesized from yeast [Saccharomyces cerevisiae], Carbiotech, Denmark; specific activity, 2,700 IU · mg⁻¹; 15,000 units/kg body wt) was dissolved in 90 ml isotonic saline and filtrated through a micropore filter (Millipore Corp., Bedford, Mass.) with a pore diameter of 0.22 μm. The SOD dose given in this study is similar to that used in several other canine myocardial ischemia-reperfusion studies. In the nonprotected control group, 90 ml saline was given. The infusion of saline or SOD was given for a total period of 60 minutes. Twenty minutes after the start of the infusion, myocardial ischemia was induced by tightening the snare around the LAD. After 10 minutes of ischemia, collateral blood flow rate was measured by the local ¹³³Xe washout method. The total duration of the ischemic period was 20 minutes, and reperfusion was achieved by instantaneous release of the snare. If necessary due to low count rates, a new ¹³³Xe deposit was injected 1–2 minutes before reperfusion to measure the reactive hyperemic response. After 5 minutes of reperfusion (measurement II), myocardial plasma flow rate and capillary permeability were measured. Sixty minutes after release of the LAD occlusion, the last measurements of myocardial plasma flow rate...
and capillary permeability were performed (measurement III).

Arterial plasma SOD concentrations were determined in blood samples before surgery, immediately before SOD infusion, 4, 8, 12, 16, 20, 30, 40, 50, and 60 minutes after the start of SOD infusion, and 15, 30, and 45 minutes after the SOD infusion was terminated. After the final blood sample had been taken, the animal was killed. Simultaneous venous and arterial blood samples were taken in one experiment for evaluation of SOD concentration in the blood and to exclude peripheral removal of SOD.

The capillary extraction depends on myocardial plasma flow rate in such a way that decreasing capillary extraction fraction values are seen at increasing flow rate values, which is expected if one thinks of blood flow in terms of mean transit time. If the intravascular mean transit time is low (i.e., blood flow is high), only a small fraction of the indicator can traverse the capillary membrane during the organ transit, whereas if the mean transit time is high (i.e., blood flow is low) a large proportion of the indicator molecules will have time to pass the capillary membrane, giving a high capillary extraction fraction. Since the plasma flow rate at the three time points of SIRD measurements demonstrated an altered flow rate, it was necessary to determine the relation between the capillary extraction fraction and plasma flow rate in our model. This was accomplished by simultaneous measurements of the two variables during physiological control conditions.

Experimental Procedure

Measurement of the regional myocardial blood flow rate. Regional blood flow rates were measured with the local 133Xe washout technique. Five to 10 μl 133Xe (370 MBq·mL⁻¹, The Radiochemical Center, Amersham, England) in isotonic saline was injected into the anterior free wall of the left ventricle at a depth of 6 mm from the epicardial surface. The gamma radiation of 133Xe was recorded by a NaI(Tl) scintillation detector placed 5 cm above the heart, and the window of the gamma spectrometer was adjusted around the 14 keV photopeak of 133Xe. A sampling time of 5 seconds was used.

The regional myocardial plasma flow rate (fₚ) was obtained from $fₚ = kₚ Xₑ Aₑ (1 - Hₑ) \cdot 100$ [mL·(100 g·min)⁻¹], where $kₚ$ is the rate constant (min⁻¹) of the recorded washout curve of 133Xe, $Xₑ$ is the tissue to blood partition coefficient of 133Xe, and Hₑ is hematocrit. A $Aₑ$ value of 0.7 was used.

The single-injection, residue-detection experiments. Capillary permeability was studied applying the SIRD method with the small, hydrophilic indicators $[^{51}Cr]EDTA$ or $[^{99mTc}]DTPA$. From 30 to 100 μl $[^{51}Cr]EDTA$ (370 MBq·mL⁻¹, New England Nuclear, Boston) or $[^{99mTc}]DTPA$ (TCK-6 kits, 200 MBq·mL⁻¹, CIS Bioindustries, Gif-sur-Yvette, France) was placed in the conus of a syringe containing 0.2 ml isotonic saline and injected as a bolus into the LAD through the polyethylene catheter. The injection lasted less than 1 second, during which time the LAD was occluded proximal to the site of injection by the silk snare. Activity was recorded by a NaI(Tl) scintillation detector placed about 5 cm above the heart, and the window of the gamma spectrometer (Scandetronic, Hadsund, Denmark) was adjusted around the 320 keV photopeak of $^{31}$Cr or the 143 keV photopeak of $^{99mTc}$. A sampling integration time of 1 second was used. The activity was recorded for 2 minutes, and then the syringe connected to the diagonal branch catheter was removed for background correction.

Activity was plotted as a function of time after bolus injection in a semilogarithmic diagram $[q_{total}(t)]$. The capillary extraction for the injected indicator in the myocardium (E) was obtained from

$$E = [q_{total}(t_{peak})]^{-1} \cdot a_e \cdot \exp(-k_e \cdot t_{peak})$$

where $a_e$ is the intercept with the ordinate at time zero of $k_e$, which is the rate constant of $q_{total}(t)$ in the time interval from 20 to 50 seconds after the bolus injection, and $t_{peak}$ is the time where peak activity of $q_{total}(t)$ occurs. In the time interval from 20 to 50 seconds from indicator injection, the transmitted indicator fraction, that is, the fraction of the indicator amount that has not crossed the capillary membrane and thus remains intravascularly, has been removed from the area seen by the detector by intravascular convection, and activity contribution due to recirculation is negligible. Previous myocardial studies have shown that in this time interval, $q_{total}(t)$ is monoexponential; that is, the fractional loss of indicator from the interstitial space to the vascular space is constant.

Myocardial plasma flow rate as determined kinetically by this method was taken as $f_p = V_p f_{intr}$, where $V_p$ is the intravascular plasma volume and $f_{intr}$ is intravascular mean transit time. $V_p$ was calculated from the hematocrit fraction and the myocardial blood volume fraction of 0.136 mL blood·g tissue⁻¹. In the calculations, we used large-vessel hematocrit values, although tissue hematocrit fractions, which are known to be less than large-vessel hematocrit values, would have been strictly more correct. We assume that the microvascular hematocrit fractions and the myocardial vascular volume is unchanged at the three measurements in each dog. $f_p$ was taken as area divided by height of the curve obtained after subtraction of the extrapolated line, $k_e$ from $q_{total}(t)$. The plasma flow rate calculated in this way is measured within the same area as the capillary extraction fraction.

PS was calculated according to Renkin as

$$PS = -f_p \cdot 0.89 \cdot \ln(1 - E)$$

in milliliters plasma water·(100 g·min)⁻¹. The constant 0.89 is used to convert plasma flow to plasma water flow and to take into account the transmembrane Donnan effect.

Determination of superoxide dismutase. Xanthine (grade V, 99–100%) and xanthine oxidase (buttermilk, grade III, 14.7 units/ml) were from Sigma Chem-
ical Co., St. Louis. Hydrogen chloride (Titrisol), sodium hydroxide (Titrisol), hydroxylammonium chloride, potassium hydrogen sulfate, sulfanilic acid, and N-(1-naphthyl) ethylenediammonium dichloride, acetic acid (96%), all reagents “for analysis,” were from Merck, Darmstadt, FRG. EDTA “for analysis” was from BHD Chemicals Ltd., Parkstone, England, and copper-zinc SOD, identical to that used for infusion, was from Carbiotech.

The SOD assay used was based on the principles suggested by Elstner and Heuple and further developed by Bjerrum. The SOD concentration was determined indirectly from the reducing effect of a sample on the steady-state concentration of superoxide produced by a xanthine/xanthine oxidase system. The reaction steps of the superoxide producing system are 1) production of superoxide from the xanthine/xanthine oxidase reaction, 2) reaction of formed superoxide with hydroxylammonium, forming nitrite, and 3) quantitative determination (in acidic solution) of the produced nitrite after formation of diazotized sulfanilic acid, which further reacts with the dye reagent N-(1-naphthyl)ethylenediammonium dichloride. The reaction produces an azodye with a broad absorption maximum at 550 nm.

**Determination of SOD concentration in plasma.** Blood samples (5 ml) were taken in EDTA vials (25 μmol). The plasma was isolated within less than 1 hour by centrifugation at room temperature (1,000g for 10 minutes) and stored at −80°C for later analysis. The samples taken before SOD infusion were analyzed without dilution, whereas samples taken after SOD infusion were diluted 50- or 100-fold with 100 mM potassium hydrogen phosphate (pH 7.8). Samples (100 μl) of SOD standards (0–100 ng), diluted or undiluted plasma, were initially vigorously mixed at room temperature with stock solutions and buffer with a total volume of 900 μl (containing 83 mM potassium hydrogen phosphate, 0.56 mM EDTA, 0.56 mM xanthine, 0.56 mM hydroxylammonium chloride, and 2.5 milliunits xanthine oxidase, pH 7.8), giving a total reaction volume of 1 ml. The production of nitrite was initiated by addition of the hydroxylammonium stock solution and interrupted after 30 minutes by addition of 1 ml sulfanilic acid (5 mM in 4 M acetic acid and 0.2 M HCl), followed by the addition of 1 ml N-(1-naphthyl)ethylenediammonium dichloride (0.25 mM in 4 M acetic acid). All samples were run in duplicates. Each sample was also performed without xanthine oxidase present to be able to correct for nitrite produced by spontaneous oxidation of hydroxylammonium. The produced azodye that corresponds to the produced nitrite during the 30-minute incubation (and which is thus proportional to the “steady-state” superoxide concentration) was determined at 542 nm by using an automatic sample–reading spectrophotometer (Ultron System 2074 calculating absorbtiometer, LKB, Bromma, Sweden). The SOD content in the plasma samples was finally determined from the SOD standard curve and expressed in SOD international units.

**Indicator preparation studies.** Before experiments using the indicator [99mTc]DTPA, 99mTc and DTPA were allowed to bind for at least 30 minutes. Content of free unbound 99mTc was controlled by thin-layer chromatography demonstrating a content less than 0.1%. Previous studies of [51Cr]EDTA have revealed a content of unbound Cr less than 0.1% (authors’ unpublished data). Accordingly, the contribution to the activity curves from unbound indicator was considered negligible.

**Statistics.**

All results are presented as mean±SEM. When values within the control and the SOD group were measured repeatedly (such as plasma flow rate, capillary extraction fraction, and PS), the data were analyzed by the Friedman test (nonparametric variance analysis) in analogy with the suggestions of Wallenstein and coworkers. When a significant overall difference was found by the Friedman test, pairwise comparisons were performed with the Wilcoxon test. If no significant difference was found by the Friedman test, no further paired analysis was carried out. When values from the control group and the SOD group were compared, the differences between the baseline value and the 5- or 60-minute postreperfusion value were analyzed with the Mann-Whitney test for unpaired observations. A value of p<0.05 was considered statistically significant. In the analysis of the correlation between −ln(1−E) and the reciprocal plasma flow, linear regression analysis was applied.

**Results.**

Twenty-one dogs entered the study (nonprotected control series, n=13; SOD series, n=8). Three dogs from the control series were excluded due to development of ventricular fibrillation immediately after reperfusion (23.1%; 95% confidence limits, 5.0–53.8), as compared with none in the SOD-treated group (0%; 95% confidence limits, 0–36.9).

Measurements of capillary extraction fraction and plasma flow rate obtained before LAD occlusion (measurement 1) in the protocol above supplemented with measurements from dogs participating in other protocols (same anesthesia) served to determine the relation between these two variables during physiologic al conditions (n=39).

**Hemodynamic Data.**

Arterial blood gas values and pH were kept within the physiologic range. Table 1 presents mean±SEM values for heart rate, arterial blood pressure, coronary artery blood pressure, pulmonary artery blood pressure, and cardiac output before LAD occlusion, during occlusion (measured after 10 minutes of ischemia), and 3 minutes after reperfusion in the two groups.

**Myocardial Plasma Flow Rate.**

Table 1 presents plasma flow rates determined by the local 133Xe washout technique before ischemia, during ischemia (collateral plasma flow rate), and during the
initial phase of reactive hyperemia (maximum plasma flow rate) after the release of the occlusion.

A representative activity versus time response curve after intracoronary $[^{99m}\text{Tc}]{\text{DTPA}}$ bolus injection (SIRD experiment) is shown in Figure 1. Myocardial plasma flow rates determined by the SIRD method before LAD occlusion (measurement I), 5 minutes after the start of reperfusion (measurement II), and 60 minutes after the start of reperfusion (measurement III) are presented in Figure 2. Before

![Figure 1. Plot showing single-injection, residue-detection experiment. The response curve (in counts per second as a function of time, plotted in a semilogarithmic diagram), obtained by external activity registration after intracoronary bolus injection of $[^{99m}\text{Tc}]{\text{DTPA}}$ is shown. In the time interval from 20 to 30 seconds, the linear regression line was calculated and extrapolated to the time of peak activity. The capillary extraction fraction ($E$) was then calculated from this extrapolated value at peak time divided by the value of peak activity of the response curve. The indicator fraction that stays intravascularly is described by the open circles obtained by subtracting the values on the extrapolated line from the response curve values. From the curve of the intravascularly transmitted indicator fraction, the plasma flow rate ($f_p$) was determined. The capillary permeability-surface area product (PS) is calculated as $PS= -f_p \cdot 0.89 \cdot \ln(1 - E)$.](image-url)
ischemia (measurement I), plasma flow was 87.9±5.9 ml·(100 g·min)^{-1} in the control group and 92.7±5.3 ml·(100 g·min)^{-1} in the SOD group (p=NS). After 5 minutes of reperfusion (measurement II), plasma flow rate increased significantly in both groups: control, 130.7±17.8 ml·(100 g·min)^{-1}, p<0.01; SOD, 143.6±15.9 ml·(100 g·min)^{-1}, p<0.01. Sixty minutes after the start of reperfusion (measurement III), plasma perfusion rates had returned to values that were not statistically different from preocclusion in both groups: 89.1±6.1 ml·(100 g·min)^{-1} versus 92.1±8.9 ml·(100 g·min)^{-1}. No differences between the two groups were seen at any of the three measurements.

**Myocardial Capillary Permeability**

In the present study, [51Cr]EDTA was used as an indicator in five dogs, and [99mTc]DTPA was used in 13 dogs. We have previously shown that the two small hydrophilic indicators [51Cr]EDTA and [99mTc]DTPA have similar characteristics of capillary permeability in the myocardium. The values of the capillary extraction fraction are presented in Figure 3. Measurements before ischemia (measurement I) gave a mean capillary extraction fraction value of 0.459±0.035 in the control series versus 0.452±0.041 in the SOD series (p=NS). Five minutes after the start of reperfusion, (measurement II, Figure 3) a mean capillary extraction fraction value of 0.523±0.053 was found in the control series (p=NS), whereas in the SOD-treated group, capillary extraction fraction decreased to 0.306±0.045 (p<0.05). At measurement II, a significant difference between the control group and the SOD group was seen (p<0.05). Sixty minutes after the start of reperfusion (measurement III, Figure 3), no significant change in capillary extraction fraction in the two series (control, 0.508±0.047; SOD, 0.479±0.047) was detectable.

The relation between myocardial plasma flow rate and capillary extraction fraction is dealt with in Figure 4. The figure shows data from 39 measurements where values of −ln(1−E) are plotted as a function of the reciprocal plasma flow rate. A linear relation with a correlation coefficient of 0.77 and a slope of 65.5 was obtained. Such a linear relation would be expected from the Renkin formula (see "Materials and Methods") if the PS product (equal to the slope of the experimental line in Figure 4 multiplied by 0.89) is constant within the examined interval of plasma flow rate. It can be deduced that extrapolation of the line (under this condition of constant PS product) should pass through the origin. Extrapolation of the line toward the ordinate gave an intercept of −0.095±0.112, which is not significantly different from zero. This result indicates that the PS product is constant within the plasma flow range observed under preocclusive conditions and that the observed data show concordance with the Renkin formula. When the graph of −ln(1−E) versus the reciprocal plasma flow rate was transformed to show the relation between capillary extraction fraction and the plasma flow rate (Figure 5), decreasing extraction fractions are seen, as expected, at increasing plasma flow rates.

Values of the reciprocal plasma flow rate and −ln(1−E) obtained from the measurement 5 minutes after the start of reperfusion (measurement II) in the absence (control series) and the presence of
SOD are shown in Figure 6. The slopes of the two regression lines drawn through the origin represent the mean value of the PS product multiplied by 0.89 in the two groups. The slope of the line obtained with SOD present is similar to the line presented in Figure 4, which was obtained during preocclusion conditions, whereas the line obtained without SOD has a much steeper slope (p<0.01). This result indicates a significant increase in the PS product 5 minutes after the start of reperfusion, as discussed below. In addition, the effect of SOD on the capillary extraction fraction 5 minutes after the start of reperfusion at a given plasma flow rate can be determined from the relation in Figure 6. At similar plasma flow rate (x axis value), a higher value of $-\ln(1-E)$, indicating a higher value of capillary extraction fraction, is seen in the control series as compared with the SOD series.

The PS values at the three times of measurement are presented in Figure 7. Before LAD occlusion (measurement I), no difference between control at 47.1±3.3 ml·(100 g·min)$^{-1}$ and SOD treatment at 49.0±3.8 ml·(100 g·min)$^{-1}$ was seen. In contrast, 5 minutes after reperfusion (measurement II), PS had increased to 79.5±6.2 ml·(100 g·min)$^{-1}$ in the control group (p<0.01), whereas no such increase was seen in PS values after SOD treatment of 44.9±6.8 ml·(100 g·min)$^{-1}$. After 60 minutes of reperfusion (measurement III), an average PS value of 56.8±6.7 ml·(100 g·min)$^{-1}$ was determined in the control group (p=NS) versus 52.8±3.1 ml·(100 g·min)$^{-1}$ (p=NS) in the SOD-treated dogs.

There was no significant correlation between the increase in PS and the collateral flow during ischemia.

**Plasma SOD Measurements**

No difference between arterial and venous blood samples for plasma SOD concentrations was detectable, indicating that SOD is evenly distributed in the whole plasma volume. The mean plasma SOD concentrations are presented in Figure 8. The half-time for SOD uptake during the ascending part of the curve (21.5±1.7 minutes) was slightly less than the half-time for the descending part (25.8±2.1 minutes). The mean SOD concentration at the beginning of reperfusion was 65.8 IU/ml plasma, reaching a maximum of 78.1 IU/ml plasma 20 minutes later when the infusion of SOD was stopped.

**Discussion**

In the present in vivo canine heart study, a transitorily increased myocardial capillary extraction and PS product for small, hydrophilic molecules were seen after reperfusion following 20 minutes of regional ischemia. When protection against the superoxide anion radical had been given with the ·O$_2^-$ scavenger SOD, no significant increase in capillary PS product was detectable after reperfusion. Therefore, oxygen-derived free radicals seem to be involved in this response after ischemia and reperfusion.
Whole-organ methods for measurements of capillary permeability, including the applied SIRD method, determine the PS product, and the two components of this variable (the permeability coefficient P and the capillary surface area S) cannot be separated. However, from simultaneous measurements of tissue blood flow or intravascular tissue blood volume, the constancy of the capillary surface area under various conditions can be evaluated. Myocardial plasma volume is not changed after 15 minutes of ischemia and 3 minutes of reperfusion, which was close to the time of our measurement 5 minutes after the start of reperfusion following 20 minutes of ischemia. After varying lengths of time of ischemia followed by reperfusion, the myocardial content of radiolabeled erythrocytes injected 5 minutes after reperfusion was taken as a measure of perfused capillary surface area. These measurements did not show any significant change in radiolabeled blood content. These studies indicate that the S component of the PS product is unchanged at the measurement 5 minutes after reperfusion and, therefore, suggest that the increased PS value 5

**FIGURE 6.** Plot showing the relation between values of the reciprocal myocardial plasma flow and −ln(1−E) obtained at the measurement 5 minutes after start of reperfusion in the nonprotected control series (○) and the superoxide dismutase series (△), where E is the capillary extraction fraction. The regression lines through the origin were fitted to the data points. The line representing the control series lies significantly above (steeper slope) the line representing the superoxide dismutase series (p<0.01). This implies that at similar plasma flow rate higher values of −ln(1−E) and, therefore, higher E values are seen in the control series as compared with the superoxide dismutase series.

**FIGURE 7.** Bar graphs showing myocardial capillary permeability–surface area product (PS) of small hydrophilic solutes determined by the single-injection, residue-detection method during preocclusive conditions (I) and 5 minutes (II) and 60 minutes (III) after the start of reperfusion following 20 minutes of ischemia in the nonprotected control series and in the superoxide dismutase (SOD) series. In the control series, a statistically significant increased PS value was seen (p<0.01), whereas in the SOD-treated group, no significant difference was seen. In addition, a statistically significant difference between the control series and the SOD series was observed at measurement II (p<0.01). Bars indicate 1 SEM.

**FIGURE 8.** Plot showing the mean plasma superoxide dismutase (SOD) concentration curve obtained from six experiments during 1 hour of SOD infusion (ascending part of the curve) and after termination of the infusion (descending part of the curve). All data points were fitted to a simple monoeponential uptake curve using a nonlinear regression program (Enzfitter, R.J. Leatherbarrow, Elsevier-Biosoft, Cambridge, England). SOD was given as a constant infusion of 250 IU·kg⁻¹·min⁻¹. Reperpusion was initiated at a mean SOD plasma concentration of 65.8±7.6 IU·(ml plasma)⁻¹. During the 1-hour infusion, a mean concentration of 78.1±6.5 IU·(ml plasma)⁻¹ was reached with an uptake half-time of 21.5±1.7 minutes. After stopping the infusion, the SOD plasma concentration decreased with a half-time of 25.8±2.1 minutes. The data point at 120 minutes was the mean of two experiments only. The bars represent ±1 SEM.
minutes after start of reperfusion seen in the control series reflects an increased capillary permeability. Tissue blood flow can be increased by 1) an increased number of perfused capillaries (capillary recruitment), 2) a decreased vascular transit time, or 3) both. At the SIRD measurement 5 minutes after reperfusion, plasma flow was equally increased in both the control and the SOD group. Accordingly, we find it reasonable to assume that the contribution from the two factors determining tissue blood flow is similar in the two groups. We assume that SOD by itself (directly or indirectly) does not modify S (i.e., that capillary recruitment is not mediated by oxygen-derived free radicals). Experimental data suggest that the coronary vascular conductance is a determinant of the number of perfused capillaries (i.e., S) and that increased S is only seen at maximal vasodilatation.\(^1\) In the present study, the tone of the arterioles during the initial phase of reperfusion, evaluated by measurement of maximum plasma flow rate, was similar in the two groups, suggesting similar S in the two groups.

In the calculations, we used large-vessel hematocrit fractions whereby no correction was made for the Fahraeus effect,\(^3\) by which small-vessel hematocrit values show significantly lower values than large-vessel hematocrit values.\(^3\) However, the use of large-vessel hematocrit values introduces a “constant deviation” that will affect the plasma flow rates and the PS values similarly in the two groups. We assume that ischemia and reperfusion do not change the microvascular hematocrit fraction. A previous study\(^3\) has shown that microvascular hematocrit is not changed by pharmacological vasodilatation. Associated with pronounced hemodynamic changes during asphyxia, a reduction of microvascular hematocrit has been observed.\(^3\) In addition, we assume that the myocardial vascular volume is constant at the three measurements. Previous data\(^3\) from dog hearts subjected to similar duration of ischemia followed by reperfusion support the assumption that myocardial plasma volume is unchanged after reperfusion.

In canine myocardium subjected to 40 minutes of coronary occlusion followed by reperfusion, no ultrastructural changes in the capillaries were seen, whereas 90 minutes of ischemia followed by reperfusion induced major morphological changes.\(^4\) Treatment with SOD and catalase protected the endocardial microvasculature after 2 hours of ischemia in a canine heart model, suggesting that oxygen-derived free radicals are involved in the morphological microvascular injury following myocardial reperfusion.\(^5\) In contrast, a 70% increase in capillary permeability for albumin has been found after 20 minutes of global ischemia in isolated rat hearts without a protective effect of SOD.\(^6\)

Oxygen-derived free radicals are produced in large amounts when ischemic myocardium is reperfused, most abundantly in the initial phase of reperfusion,\(^7,10\) but continue to be present in diminished amounts during the first 3 hours of reperfusion.\(^11\) SOD treatment reduces the amounts of free radicals produced during reperfusion.\(^47\) Even during ischemia, free radicals are produced in small amounts.\(^10,11\) Despite the fact that free radicals are predominantly released in the first minute after reperfusion, we had to postpone the first postreperfusion measurement to avoid the initial phase of reactive hyperemia with its very high blood flow rate,\(^48\) since the SIRD method requires steady-state conditions during the measurement. Five minutes after reperfusion, steady-state requirements seem to be fulfilled.\(^49\)

When intravital microscopy of the epicardial vasculature of the rat heart after ischemia and reperfusion was used, signs of increased capillary permeability of macromolecules were found, supporting the observations of an increased tissue content of labeled albumin under similar experimental conditions.\(^22,23\) In the brain, enzymatically produced free oxygen radicals induced a rapid increase in the permeability of the exposed postcapillary venules.\(^50\) At low doses of \(\cdot \text{O}_2^-\), a maximum effect was seen after about 3 minutes, with normalization within 5 minutes after termination of \(\cdot \text{O}_2^-\) administration, whereas at high doses, the change was permanent.\(^50\) In a hamster cheek pouch model, ischemia followed by reperfusion demonstrated a reversibly increased number of postcapillary venular leaks, reaching a maximum 7 minutes after the start of reperfusion.\(^51\) These studies and the present suggest that both ischemia-reperfusion and exposure to free oxygen radicals can induce a reversibly increased capillary permeability.

The used SOD product has been found practically atoxic, with an \(LD_{90}\) value after intravenous administration of about 5,000 mg/kg body wt, when given to mice. Our graph of plasma SOD concentration versus time is similar to that shown by Richard and coworkers;\(^52\) this plasma SOD concentration should give sufficient protection against oxygen-derived free radicals. Endotoxin content in SOD products has been described recently.\(^53\) We found a positive limulus reaction in the SOD infusion solution, indicating the presence of endotoxin. When quantitated, the SOD infusion solution contained approximately 100 pg endotoxin/ml. Blood samples taken before SOD infusion showed a negative limulus test, and after the infusion had been given, the test was still negative. This result was further confirmed by quantification after the infusion, which yielded a content of 0 pg endotoxin/ml blood. The negative limulus test in the blood after the SOD infusion reflects the large capacity of the liver to clear circulating endotoxin. No signs of endotoxin intoxication were seen after SOD infusion, supporting the view that endotoxin was not participating in the observed permeability changes.\(^54\) Endotoxin by itself is expected to cause the opposite effect on permeability, that is, an increased permeability.\(^54\)

The maximum plasma flow rate during reactive hyperemia measured by the local \(^{133}\text{Xe}\) washout method was similar in the control and the SOD-treated groups, and the values are in accordance with
a previous study\(^8\) of the reactive hyperemic response after shorter ischemic periods applying the same technique. The normal reactive hyperemic response reflects the maximum capacity for vasodilation of the arterioles and indicates that the ability of these vessels to dilate is not altered by 20 minutes of ischemia and the very first part of reperfusion and that superoxide anions do not participate in this response. This interpretation is supported by the similarity in myocardial plasma flow rate in the two groups determined by the SIRD method 5 minutes after the start of reperfusion (Figure 2), reflecting the late period of reactive hyperemia.

In conclusion, an increased capillary permeability to small hydrophilic solutes occurred after 20 minutes of regional myocardial ischemia followed by 5 minutes of reperfusion, which from previous studies is known not to induce morphological endothelial cell or myocyte alterations. The increased capillary permeability was normalized within 1 hour. After treatment with SOD, no evidence of increased capillary permeability was seen, indicating that superoxide anions, directly or indirectly, participate in the evolution of increased capillary permeability. The study supports the hypothesis that increased microvascular permeability to small hydrophilic indicators is an early event following ischemia and reperfusion, which probably precedes the development of morphologically detectable endothelial cell injury.

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References
37. Bjerrum MJ: Structurelle og spektrofotoskopiske studier over superoxid dismutase fra bagergaer (PhD thesis). Copenhagen, University of Copenhagen, Denmark, HCO tryk, 1988
52. Richard VJ, Murry CE, Jennings RB, Reimer KA: Therapy to reduce free radicals during early reperfusion does not limit the size of myocardial infarcts caused by 90 minutes of ischemia in dogs. Circulation 1988;77:473–480

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