Evidence for Superoxide Radical-Dependent Coronary Vasospasm After Angioplasty in Intact Dogs

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Background. Active oxygen species can influence vascular tone and platelet activation through a variety of mechanisms. This study assessed the role of the superoxide anion, the hydroxyl radical, and hydrogen peroxide in vasoconstriction and mural thrombosis after coronary artery angioplasty in intact dogs.

Methods and Results. Injury was induced by inflation of a balloon catheter 50±6% above baseline arterial diameter; dogs were followed for 2 hours before death. Epicardial coronary diameters at arteriography and extent of thrombus deposition at serial histological sections were analyzed in controls (n=20) and in dogs pretreated with superoxide dismutase (SOD, a superoxide radical scavenger, n=10); other dogs were pretreated with the hydrogen peroxide scavenger catalase (n=8), the iron chelator deferoxamine (n=6), or the hydroxyl radical scavenger 1,3-dimethyl-2-thiourea (n=9). Angioplasty-induced injury was similar among groups. After angioplasty, control dogs exhibited localized and persistent vessel constriction, which was maximal at the initial 5 minutes (28.9±6.3% diameter decrease versus baseline). Corresponding arterial diameters of SOD-treated dogs were 24–69% larger (95% confidence interval, p<0.001) than controls at 5 minutes and, on average, 32% larger than controls thereafter (p<0.01). Vasoconstriction was not prevented by the other treatments. The SOD dose used accounted for inhibition of zymosan-stimulated blood cytochrome c reduction versus baseline (7±3 versus 30±6 nmol/min/10⁶ cells, respectively, p=0.003); such inhibition occurred in no other group. Prevalence of mural thrombosis was similar among all groups, but large thrombi (>15% of lumen area) were absent in SOD-treated dogs, contrary to control group (p=0.028); other groups were similar to control. In the absence of injury, SOD alone induced no change in coronary diameter, coronary blood flow, or platelet aggregation.

Conclusions. These data provide evidence implicating the superoxide radical in the genesis of vasoconstriction after coronary angioplasty in vivo. Such effects seem to be independent of its conversion to hydroxyl radicals and availability of hydrogen peroxide or catalytic iron complexes. (Circulation 1991;83:1705–1715)

Vascular response to coronary angioplasty is characterized in the acute phase by platelet deposition, mural thrombosis, and vasospasm. This response may exert homeostatic functions, such as limitation of blood leakage, remodeling of the vessel wall, and removal of necrotic debris; however, it also underlies the relatively high incidence of acute or chronic complications. In particular, the importance of vasoconstriction has recently been emphasized. This phenomenon occurs regularly after experimental as well as clinical angioplasty and may contribute to acute vessel closure and some cases of restenosis. Proposed mechanisms of vasoconstriction implicate 1) predisposing factors related to endothelial injury, with consequent loss of endothelium-derived relaxing activity, 2) triggering factors such as serotonin and other platelet-derived vasoconstrictors, 3) lipoxigenase-derived arachidonate metabolites, 4) possibly leukocyte products, and 5) myogenic stretch-dependent constriction mediated by endothelium-derived cyclooxygenase product(s).
However, the precise chemical nature of the agents mediating interactions among blood elements, endothelium, and vascular smooth muscle cells remains unclear.

Our hypothesis was that active oxygen species produced at the site of injury mediate in vivo coronary vessel spasm and thrombosis after angioplasty. These radicals originate from stepwise univalent reduction of molecular oxygen in the course of a variety of metabolic pathways, such as arachidonate degradation, NADPH-dependent oxidation in leukocytes, auto-oxidation of catecholamines, hypoxanthine oxidation by xanthine oxidase, and many others. Therefore, those highly reactive intermediates are likely produced at the site of vascular injury. Free oxygen radicals exert a range of vascular effects and have been proposed as a mechanism regulating endothelial cell secretion. The superoxide (O_2^−) radical inactivates endothelium-derived relaxing factor. Superoxide release from endothelium appears to mediate basilar artery constriction provoked by calcium ionophore. Superoxide may give rise to the hydroxyl (OH^−) radical, a potent oxidant molecule that attacks virtually every cell constituent; hydroxyl radicals and lipid hydroperoxides inhibit synthesis of prostacyclin and may influence vessel tone. In addition, superoxide enhances platelet activation in vitro.

The objective of this study was to investigate the role of some active oxygen species in the genesis of spasm that acutely follows angioplasty of intact canine coronary arteries. Epicardial arterial diameters at arteriography and thrombus deposition at serial histological sections were analyzed in control animals and in those given scavengers or inhibitors of superoxide anion, hydrogen peroxide, and hydroxyl radical.

Methods

Materials

Electrophoretically pure superoxide dismutase (SOD; activity = 2,432 units/mg, assayed by the method of McCord and Fridovich) was kindly provided by Profs. Leopold Flohé, Grünenthal GmbH, FRG, and Etelvino Bechara, Institute of Chemistry, University of São Paulo. Bovine liver catalase (activity = 2,800 units/mg), cytochrome c, and ADP were purchased from Sigma Chemical Co., St. Louis. 1,3-dimethyl-2-thiourea (DMTU) was purchased from Aldrich Chemical Co., Milwaukee, Wis. Deferoxamine mesylate (CIBA-GEIGY Corp., Basel, Switzerland) was obtained commercially. SOD was stored at 4–8°C; deferoxamine, at room temperature; and catalase and DMTU, at −20°C. Aliquots of these drugs were dissolved in 0.9% NaCl solution immediately before use.

Preparation

Fifty-three mongrel dogs of either sex weighing 16±5 (mean±SD) kg were anesthetized with pentobarbital (25 mg/kg i.v.), intubated, and connected to a volume-cycled ventilator. Arterial blood gases were kept within the physiological range. Additional pentobarbital was given as needed at an average rate of 8–12 mg/kg/hr. The left femoral artery was cannulated and connected to a fluid-filled transducer (model 1280, Hewlett-Packard Co., Palo Alto, Calif.). Arterial pressure and lead II of the electrocardiogram were continuously registered in a Hewlett-Packard model 8890-A recorder. The left jugular vein was cannulated for 0.9% NaCl administration at an average rate of 15–20 ml/kg/hr. An intravenous bolus of 100 IU/kg heparin was given thereafter, and 15 minutes was allowed before further manipulation. Additional heparin was given exclusively as 10 IU/ml of 0.9% NaCl solution and infused continuously into the coronary catheter or used to flush other catheters; this resulted in small additional dose rates between 30 and 50 IU/kg/hr. A 5F Sones catheter (S-cath) was then advanced through the left carotid artery into the left anterior descending coronary artery (LAD). Coronary arteriograms were obtained through injection of 3–4 ml of a mixture of meglumine diatrizoate and sodium diatrizoate (Hypaque M-76%, Winthrop Products Inc., New York, N.Y.) and recorded by cinephotofluorography at 30 frames/sec in the left anterior oblique position. To assure proper comparison among sequential arteriograms, care was taken to avoid any change in the dog’s position throughout the experiment. This study was performed according to the guidelines of the American Physiological Society for animal care and was approved by a local committee.

Protocol

After the first baseline LAD arteriography, a steerable guide wire (USCI Division, CR Bard Inc., Billerica, Mass.) was advanced under fluoroscopy through the Sones catheter into the proximal LAD. The S-cath was then removed and a Gruentzig catheter (G-cath) (USCI; balloon size, 25.0 mm in length and 3.0 mm in diameter) was advanced over the guide wire into the LAD, as distally as possible. Angioplasty was performed with four balloon inflations, 30 seconds each, with a 60-second interval; the first two were at 10 atm, and the last two were at 8 atm. These pressures were chosen in preliminary experiments as those uniformly promoting rupture of the medial muscle layer; lesser degrees of balloon inflation were associated with increased variability in vasospasm. The S-cath was then placed again into the proximal LAD and flushed continuously with 0.3 ml/min heparinized saline. Arteriograms were then performed at 5, 15, 30, 60, and 120 minutes after angioplasty. Immediately after the last arteriogram, the dogs were killed with pentobarbital and KCl. The heart was excised; the injured area was easily visualized through vessel dilation and small periarterial hematoma. Diagonal branches and the distal LAD were tied; the proximal LAD was perfused through its ostium with 4% glutaraldehyde in phosphate-buffered saline, pH 7.4, for 20–30 minutes at low.
pressure and flow. The myocardial fragment containing the injured area, plus its proximal and distal 2.0-cm segments, was then immersion-fixed in glutaraldehyde, cut into 2-mm slices, and stained for light microscopy by the Verhoeff–Van Gieson method, as well as with hematoxylin and eosin. In 18 dogs (11 controls and eight treated with SOD), every third of those slices was prepared for scanning electron microscopy. These arterial slices were open and cut into smaller fragments; large visible thrombotic material was carefully removed. Specimens were fixed with cyanoacrylate over appropriate supports and submitted to critical-point dehydration, followed by sputtering with gold.

**Treatment Groups**

Treatment for each dog was chosen at random. Intracoronary infusions were given either through the S-cath or through a special adaptation of the G-cath lumen, consisting of a large oval hole 3 mm proximal to the balloon. Because this hole offered much less mechanical resistance to injection than the original orifice distal to the balloon, most of the material injected through the catheter lumen immediately reached the area of balloon-induced injury; this was confirmed in several dogs by contrast injection.

**Control group** (*n*=20). Control dogs were given heparinized saline: 20 ml i.c. (G-cath) for 5 minutes before angioplasty, 10 ml i.c. (G-cath) during angioplasty, and 0.3 ml/min i.c. (S-cath) for the remaining 2 hours of the experiment.

**SOD group** (*n*=10). A total dose of 100 mg SOD was given: 25 mg i.c. (G-cath) for 5 minutes before angioplasty, 25 mg i.c. (G-cath) during angioplasty, and 25 mg/hr i.c. (S-cath) subsequently.

**Catalase group** (*n*=8). A total dose of 100 mg deferoxamine catalase was given: 25 mg i.c. (G-cath) for 5 minutes before angioplasty, 25 mg i.c. (G-cath) during angioplasty, and 25 mg/hr i.c. (S-cath) subsequently.

**Deferoxamine group** (*n*=6). A total dose of 500 mg deferoxamine was given: 300 mg i.c. (S-cath) for 30–40 minutes (terminating 20 minutes before angioplasty), 50 mg i.c. (G-cath) during angioplasty, and 75 mg/hr i.c. (S-cath) subsequently.

**DMTU group** (*n*=9). A total dose of 500 mg/kg i.c. DMTU (S-cath) was given for 30 minutes (terminating 20 minutes before angioplasty). Saline was given subsequently as in the control group.

Those agents were given assuming their properties as antagonists of formation or action of active oxygen species. SOD is a specific enzymatic scavenger of the superoxide radical. Catalase scavenges hydrogen peroxide and thus decreases hydroxyl radical formation. Deferoxamine, an iron chelator, decreases iron-dependent hydroxyl radical formation through Fenton’s reaction. DMTU is a scavenger of the hydroxyl radical. Choice of doses for each of these agents was based on previous in vivo dog studies showing their effect against postischemic myocardial dysfunction or extent of necrosis. In particular, a single 500 mg/kg i.v. infusion of DMTU was shown to provide adequate blood and myocardial tissue concentrations for prolonged periods.

**Data Analysis**

Films and pathological specimens were coded in a way to prevent identification of the dog, so that all data analysis was totally blinded to the observer. Arteriographic images were projected directly over tracing paper attached to a glass plate, and the arterial contours were drawn with a sharp pencil. Care was taken to analyze all images at the same phases of the cardiac cycle. The sequential LAD tracings were divided in segments, identified by their position relative to the emergence of diagonal branches. The injured segment was defined as that within the main balloon shaft, as well as by the specific arterial alterations. Distal to this segment, the LAD was divided into 5-mm segments, named sequentially from D-1 to D-4 (Figure 1). The D-1 segment contained the tapered balloon extremity. Mural thrombosis was quantified through computer-assisted planimetry, and results were expressed as percent of lumen area. Absolute thrombus volume was also estimated; since results with this variable were similar to the former, we reported only the relative thrombus size. Vasospasm at the proximal tapered balloon extremity was also observed in some dogs. However, we chose not to analyze this variable, since it was less pronounced and less consistent than distal spasm, probably because of increased size and variability of coronary diameters proximally and less definition of images due to oblique x-ray beam incidence.

**Quantification of Angioplasty-Induced Lesion**

Proper comparison of vascular responses among the different treatment groups should assume similar degrees of angioplasty-induced lesion. Such injury was quantified 1) at arteriography, as the average and
maximal percent diameter stretching induced by the inflated balloon versus baseline and 2) at histological analysis, as a score calculated as the average of values attributed to each serial section according to the following findings: 0, no evidence of medial lesion; 1, medial thinning of less than 25% of arterial circumference; 2, medial thinning of 25–50% of arterial circumference; 3, medial thinning of more than 50% of arterial circumference; and 4, complete or nearly complete medial rupture.

**Assay for Blood Cytochrome c Reduction**

Blood cytochrome c reduction was evaluated after each intervention in 31 dogs according to the method of Bellavite et al. Briefly, 100 μl heparinized dog blood was incubated at 37°C for 5 minutes with opsonized zymosan in the presence of CaCl₂ and MgCl₂. Cytochrome c type IV (3.0 mg/ml in Hanks' balanced salt solution) was added; the reaction was allowed to proceed at 37°C for 10 minutes and was then interrupted with an ice bath plus ice-cold Hanks' balanced salt solution. The mixture was then centrifuged, and the supernatant was read spectrophoto- metrically at 550 and 468 nm. Each blood sample was analyzed in the absence and presence of exogenous SOD; final results were expressed as SOD-inhibitable production of reduced cytochrome c.

**Effects of SOD on In Vitro Platelet Aggregation**

Platelet aggregation was induced in platelet-rich plasma by ADP or platelet-activating factor (1-O-alkyl-2-acetyl-sn-3-glyceryl-phosphorylcholine) according to standard techniques for transmission aggregometry. Briefly, citrated blood was centrifuged at 1,100 rpm for 5 minutes; platelet-rich plasma was separated and diluted with saline until a final concentration of 3×10⁸ platelets/μl was reached; aliquots were placed on an aggregometer (Chrono-Log model 440, Linear Instruments Inc., Irvine, Calif.) and challenged with increasing concentrations of the agonists until a threshold aggregating concentration was reached. The assay was then sequentially repeated with the same concentration of each agonist, in the absence and presence of SOD, in concentrations of 10, 50, and 500 units/ml.

**Direct SOD Effects on Coronary Flow**

In two additional open-chest dogs, the LAD was instrumented with an electromagnetic flowprobe (Statham, Medical Division, Oxnard, Calif.) and a Tygon infusion catheter (Herd-Barger technique). Intracoronary SOD bolus injections were given in doses of 1, 3, 10, and 30 mg, separated by a 20–30-minute interval; injection time was 1–2 minutes, with a standard technique to maximize reproducibility. As a control, 10 minutes after each injection, reactive hyperemic flow response to a 20-second artery occlusion (induced with a snare) was assessed.

**Statistical Analysis**

All data are mean±SEM, unless stated otherwise. Differences among groups were tested by one-way analysis of variance or two-sample Student’s t test. Dunnett’s test was used for multiple comparisons between control and treatment groups. Extent of thrombosis was assessed by χ² test.

**Results**

**Hemodynamic and Hematological Variables**

For all dogs, baseline arterial pressure was 112±3 mm Hg, and heart rate was 115±2 beats/min. Arterial pressure changed by no more than 10 mm Hg throughout each experiment. Platelet count was (280,000±17,000)/μm³, total leukocyte count was (7,740±443)/μm³, and hematocrit was 32±1%. These variables were similar among all treatment groups. After heparin administration, thrombin time and activated partial thromboplastin time were prolonged in every dog at least fourfold and threefold, respectively.

**Angioplasty-Induced Injury**

Baseline coronary diameters and their percent stretching induced by the inflated balloon were similar among all groups; histological injury scores were equally similar (Table 1). Neither of the two indexes of arterial injury was found to correlate with the severity of postangioplasty vasospasm (r=0.10, p=NS, for each correlation).

**Degree of Coronary Vasospasm**

Arteriographic evolution of the main injured coronary arterial segment after angioplasty is shown in
Table 1. Initially, there was mild arterial retraction, relative to the inflated balloon, which was similar in all groups. Later, however, the injured arterial segment remained essentially unaltered for the remaining of the study in each dog.

Temporal evolution of D-1 segment diameter after angioplasty for the control and SOD-treated groups is shown in Figure 2. After coronary injury, control dogs exhibited spasm of the D-1 segment, which was maximal at the 5-minute arteriogram and decreased to stable values thereafter. SOD significantly prevented coronary vasoconstriction. This effect was persistent throughout the 2-hour period of observation. The maximal SOD effect was noted at 5 minutes after angioplasty: diameter changes compared with baseline were \(-28.9 \pm 6.3\%\) for the control group and \(+17.9 \pm 9.6\%\) for the SOD-treated group; the 95% confidence interval for the difference between these two groups was 24.0–69.0%. Catalase, deferoxamine, and DMTU induced no change from control at any time period. Figure 3 depicts the changes in the D-1 segment diameter averaged for the 2-hour period of observation. Arterial diameters were, on average, 32% larger in SOD-treated dogs than in controls, which averaged 18.4% constriction compared with baseline. Mean D-1 segment diameters with catalase, deferoxamine, and DMTU were similar to control.

Figure 4 shows SOD effects in arterial segments D-2 to D-4. As opposed to segment D-1, in control dogs, there was no significant constriction of these more distal segments; diameter changes compared with baseline were \(4.6 \pm 5\%\) (D-2), \(8.4 \pm 5.7\%\) (D-3), and \(10.7 \pm 5.5\%\) (D-4). Such diameters were not further enhanced by SOD. These data indicate that the SOD effect was more pronounced at the D-1 segment and not due to diffuse epicardial coronary vasodilation. Catalase, deferoxamine, and DMTU had no significant effect on segments D-2–D-4, inducing average pooled diameter changes of \(10.8 \pm 6.6\%\), \(-2.3 \pm 5.9\%\), and \(-3.7 \pm 5.9\%\), respectively.

**Figure 2.** Graph showing arteriographic evolution of the diameter of D-1 segment of left anterior descending coronary artery after angioplasty in control and superoxide dismutase (SOD)–treated dogs. Data are percent variations from diameter of the same arterial segment before angioplasty. SOD completely prevented vasoconstriction throughout the 2-hour period of study.

**Figure 3.** Bar graph showing average percent changes in arteriographic diameter of D-1 segment of left anterior descending coronary artery. Vessel caliber was significantly larger in superoxide dismutase (SOD)–treated dogs vs. controls; other treatments designed to antagonize hydrogen peroxide or hydroxyl radicals were ineffective. DMTU, dimethylthiourea.
Extent of Mural Thrombosis

Mural thrombi that adhered to the injured vessel were verified by light microscopy in 73% of all dogs. The prevalence of such thrombosis was similar among all treatment groups. Figure 5 shows the extent of mural thrombosis for all groups, quantified as the maximal encroachment in arterial lumen area. In the control group, 50% of the dogs exhibited thrombi greater than 15% of the lumen area (a value arbitrarily defined for purposes of analysis); growth of thrombi beyond this size was completely prevented by SOD but not by other treatments. Figures 6 and 7 depict scanning electron microscopy findings for control and SOD-treated groups. There was no detectable alteration induced by SOD in platelet density at injured wall sites. As expected from the light microscopic findings, there was an increased amount of fibrin that adhered to the wall of control dogs compared with SOD-treated dogs.

Blood Cytochrome c Reduction

The aim of this assay was to verify whether the administered SOD dose was sufficient enough to account for superoxide radical scavenging effects in circulating blood. Results were expressed as the portion of cytochrome c reduction inhibitable by exogenously added SOD. There was no significant difference in superoxide radical production before versus after angioplasty in control dogs (23±4 versus 32±6 nmol/min/10^6 cells, respectively, n=8). In contrast, blood from SOD-treated dogs significantly inhibited cytochrome c reduction compared with baseline (7±3 versus 30±6 nmol/min/10^6 cells, respectively, n=7, p=0.003). Superoxide production was not affected by catalase (n=5), deferoxamine (n=6), or DMTU (n=5).

Direct SOD Effects on Coronary Flow and Epicardial Coronary Diameters

In three additional intact dogs not given any previous treatment, arteriographies were performed at baseline twice (separated by a 15-minute interval) and at 2 and 10 minutes after an intracoronary 30-mg SOD bolus. Arteriographic images were divided from LAD origin into five equally spaced segments. There were no differences in epicardial coronary diameters for each condition.

In the two additional open-chest dogs instrumented to measure coronary flow, bolus injections of SOD did not alter coronary flow (0±0% change) from the respective baseline values of 24 and 31 ml/min. On the other hand, coronary flow could be increased at least 2.7 times by postischemic reactive hyperemia (used as a control).

Thus, SOD lacked a direct effect on epicardial or small-vessel coronary tone in our preparation.

SOD Effects on In Vitro Platelet Aggregation

To examine the possibility of direct SOD effects on platelets, influence of increasing concentrations of exogenous SOD (10, 50, or 500 units/ml) on in vitro platelet aggregation induced by ADP or platelet-activating factor was analyzed. Threshold aggregating concentrations for these two agonists were, respectively, 11.7±3.8 µg/ml (range, 0.7–29.5) and 1.0±0.4 µg/ml (range, 0.5–5.0). SOD induced no significant change in the velocity of platelet aggregation with either agonist. The amplitude of aggregating responses was similarly unaffected by the enzyme. Similar results occurred in plasma obtained after
systemic heparinization (n = 12). In four dogs, threshold aggregating concentrations for platelet-activating factor and ADP were unchanged after systemic SOD administration.

**Discussion**

The major finding of this study was the significant prevention of coronary vasospasm after angioplasty in dogs treated with SOD. Nonspecific direct effects of this enzyme on platelets and coronary vessels are unlikely to primarily explain our findings, since this enzyme alone induced no change in coronary flow and epicardial artery diameters in vivo and platelet aggregation in vitro. SOD is a specific superoxide radical scavenger and has been extensively used in vitro and in vivo as a pharmacological probe to assess superoxide activity. Also, SOD doses used in the present study were sufficient to account for scavenging of substantial amounts of superoxide radical in circulating blood. Therefore, the present data constitute significant evidence implicating the superoxide radical in vasoconstriction after angioplasty in vivo. This effect can be mediated through a variety of mechanisms.

It is well known that oxygen-derived intermediates such as superoxide anion, hydrogen peroxide, and hydroxyl radicals are interrelated; their relative concentrations are dependent on the availability of endogenous scavengers and catalytic metal ions. Superoxide anion, being poorly reactive in aqueous medium and highly diffusible through intact cell membranes, is capable of diffusing outside its intracellular sources, probably through ion channels. On the other hand, the hydroxyl radical is very reactive, being extremely short-lived and unlikely to diffuse toward extracellular space. In our study, catalase, deferoxamine, and DMTU were ineffective against vasoconstriction and thrombus development. However, interpretation of results for each of those interventions alone should be cautious, considering scavenger accessibility and multiple properties. Catalase can be inefficient for scavenging low concentra-
allowing scavenger penetration into at least some cells, cannot be excluded. Therefore, our results strongly suggest that the superoxide radical is the species that triggers vascular responses after angioplasty in vivo, an effect apparently independent of its conversion to hydroxyl radicals or the presence of catalytic iron complexes. On the other hand, the above considerations do not allow us to completely exclude that superoxide effects are due to its conversion to other active oxygen species.

The superoxide radical can influence vascular responses in different ways. First, SOD enhances the half-life of endothelium-derived vascular relaxing factor \(^{21,22}\); this mediator is probably nitric oxide \(^{44}\) or a closely related precursor, such as \(\text{S-nitrosocysteine}^{45}\). It is possible that superoxide rapidly oxidizes nitric oxide to the less active nitrates \(\text{NO}_2^-\) and \(\text{NO}_3^-\) \(^{44}\) or, alternatively, oxidizes free sulfhydryl groups to the disulfide form, thereby decreasing nitrosothiol synthesis. \(^{45}\) Superoxide release by endothelium could work as an in vivo mechanism of endothelium-derived relaxing factor inactivation. \(^{18,44}\)

Also, SOD shares with nitric oxide the capacity to activate the crucial enzyme guanylate cyclase \(^{46}\); superoxide radicals are generated by in vitro preparations of guanylate cyclase, suggesting that active oxygen species might work as regulators of cyclic GMP formation. \(^{46}\) Nitric oxide, in addition to its vascular effects, also antagonizes platelet function and may be an important physiological mechanism preventing adhesion of platelets to the vessel wall. \(^{44}\) In our in vivo model of angioplasty, nitric oxide release within and near the injured zone by endothelial cells or by macrophages and possibly neutrophils \(^{44}\) could help prevent arterial constriction and thrombus formation. Therefore, prolongation of endothelium-derived relaxing factor half-life by SOD is compatible both with the observed prevention of vasospasm and reduction in thrombus size. Second, it was demonstrated that arterial distension releases a vasoconstrictor compound from endothelium \(^{14,15}\) that acts directly, independent of platelets and neural impulses. This endothelium-derived contracting factor has been designated as EDCF-2, as opposed to EDCF-1, which is endothelin. EDCF-2 appears to be a cyclooxygenase-derived arachidonate metabolite; however, the possibility that its release is dependent or enhanced by the superoxide radical \(^{15}\) or that this factor is the superoxide radical itself \(^{23,47}\) has been considered previously. Indeed, there is evidence that superoxide release in vitro from the endothelium directly contracts canine basilar arteries, \(^{23}\) although findings for this artery cannot be generalized because of some of its atypical responses. In addition, cultured endothelial cells are known to produce superoxide \(^{18,48}\) and to exhibit high activities of prostaglandin endoperoxide synthase \(^{49}\); the peroxidase activity of both this enzyme and lipoxygenase generates superoxide radicals via a side chain reaction that is dependent on the presence of appropriate reducing cosubstrates. \(^{37,50}\) In contrast to these data, some

**Figure 7.** Scanning electron microscopic findings in control dogs showing persistence of at least some endothelial cells within or close to the injured arterial segment. Panel A: Boundaries of damaged arterial wall segment, exhibiting detached endothelial cells (arrows) exposing denuded subintimal tissue (D). In the right upper corner, beginning of platelet infiltrates (P) adhered to the stretched artery. Panel B: In another dog, midportion of damaged arterial segment, with presence of endothelial cells (arrow) interspaced with denuded vascular tissue. Bars, 10 μm.
previous studies show a vasodilator effect for superoxide. However, such in vivo data refer to arteriolar resistance rather than conductance vessels and were obtained mainly in cerebral vessels, which may react differently from other vascular beds; also, it is difficult to ascertain whether these effects are due to superoxide or to one of its reaction products. In summary, the action of SOD may have been due to antagonism of direct and indirect superoxide radical vascular effects.

Superoxide anion is also directly involved in platelet activation, in addition to its indirect (e.g., nitric oxide–mediated) effects. Platelets contain SOD and can produce detectable amounts of superoxide anion. Coincubation of platelets with superoxide radicals in vitro enhances platelet serotonin secretion and thrombin-induced platelet activation; also, SOD blocks platelet aggregation induced by thrombin. In the present study, mural thrombosis was less pronounced, although not less prevalent, in SOD-treated animals in relation to controls, an effect not observed in the other groups. This suggests that direct or indirect prevention of platelet activation by SOD occurred at some stage of its protective action. SOD was ineffective in our in vitro tests of platelet aggregation; however, the platelet should not be excluded as an in vivo target for protective SOD effects, because the aggregators that are used in vitro may differ from those that specifically trigger platelet activation in vivo. In addition, superoxide could be produced in vivo by sources other than platelets, a phenomenon not detected in vitro; superoxide effects on indirect platelet–endothelium interactions (e.g., via endothelium-derived relaxing factor) would also be missed by in vitro studies. We observed the lack of SOD effect on platelet deposition at the injured vessel wall; this fact is not surprising, since even substances with a proven antiplatelet effect, such as aspirin, do not reduce platelet adhesion. Neutrophils and other white blood cells could also be involved in beneficial SOD effects, since they constitute an important source of superoxide radicals, which, in turn, enhance their chemotactic responses. However, the role of those cells in acute vascular response to injury is unclear at present.

A particular feature of this model was the strict location of vasospasm at the distal tapered balloon extremity. This finding was well observed in other studies in vivo or in vitro. Specifically, Fischell et al. showed that the location of vasospasm, whether at the injured zone or at the tapered balloon edges, was dependent on the degree of balloon inflation but that the phenomenon was basically the same. In our model and in previously reported ones, medial thinning and rupture was induced by balloon dilation in each animal, similar to clinical angioplasty; the severity of angioplasty-induced injury, as assessed by angiographic and histological criteria, was similar among treatment groups. Such muscle injury induced by the main balloon shaft could cause local vessel "paralysis." In contrast, the tapered balloon extremity was associated with a spectrum of progressively less injury, as shown in Figure 7. It is conceivable that this spectrum contains the ideal combination of endothelial and muscular injury necessary to provoke local spasm. A potential limitation of this model was the use of intact rather than atherosclerotic arteries. Presence of an atheroma could further influence vascular response to angioplasty by exposing a larger vessel wall surface for platelet adhesion, by promoting activation of cellular elements such as macrophages, by generating cholesterol-loaded fragments, and by leading to formation of folds and pouches likely to promote blood stasis. However, even in intact arteries, it is reasonable to assume that platelet deposition, mural thrombosis, and vasoconstriction are likely stimulated by vessel rupture and exposure of subintimal and particularly medial tissue; this exposure is the key determinant of vascular response to injury. This assumption is supported by previous studies. Effects of angioplasty may be further determined by the type of artery chosen as a model, since platelet deposition is directly correlated to shear stress, which is higher in coronary than in carotid or femoral arterial systems.

In conclusion, this study provides in vivo evidence implicating superoxide anion activity in some aspects of vascular response, particularly vasoconstriction, after angioplasty. This radical or closely related oxygen species may represent a system mediating physiological or pathological interactions among blood elements, vascular endothelium, and smooth muscle cells. Further research clarifying mechanisms of such interactions could expand therapeutic possibilities for the control of occlusive episodes after coronary angioplasty.

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