Glucocorticoid Treatment Prevents Progressive Myocardial Dysfunction Resulting From Experimental Coronary Microembolization

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Background—The frequency and importance of microembolization in patients with acute coronary syndromes and during coronary interventions have recently been appreciated. Experimental microembolization induces immediate ischemic dysfunction, which recovers within minutes. Subsequently, progressive contractile dysfunction develops over several hours and is not associated with reduced regional myocardial blood flow (perfusion-contraction mismatch) but rather with a local inflammatory reaction. We have now studied the effect of antiinflammatory glucocorticoid treatment on this progressive contractile dysfunction.

Methods and Results—Microembolization was induced by injecting microspheres (42-μm diameter) into the left circumflex coronary artery. Anesthetized dogs were followed up for 8 hours and received placebo (n=7) or methylprednisolone 30 mg/kg IV either 30 minutes before (n=7) or 30 minutes after (n=5) microembolization. In addition, chronically instrumented dogs received either placebo (n=4) or methylprednisolone (n=4) 30 minutes after microembolization and were followed up for 1 week. In acute placebo dogs, posterior systolic wall thickening was decreased from 20.0±2.1% (mean±SEM) at baseline to 5.8±0.6% at 8 hours after microembolization. Methylprednisolone prevented the progressive myocardial dysfunction. Increased leukocyte infiltration in the embolized myocardium was prevented only when methylprednisolone was given before microembolization. In chronic placebo dogs, progressive dysfunction recovered from 5.0±0.7% at 4 to 6 hours after microembolization back to baseline (19.1±1.6%) within 5 days. Again, methylprednisolone prevented the progressive myocardial dysfunction.

Conclusions—Methylprednisolone, even when given after microembolization, prevents progressive contractile dysfunction. (Circulation. 2004;109:2337-2342.)

Key Words: microcirculation ▪ inflammation ▪ ischemia ▪ myocardial contraction

The frequency and importance of coronary microembolization in patients with acute coronary syndromes or those undergoing coronary interventions have recently been emphasized.1–3 Using an experimental model of coronary microembolization,4 we have demonstrated that after a rapid (ie, minutes) recovery from the immediate microembolization-induced ischemic dysfunction, a progressive (ie, hours) contractile dysfunction develops in the presence of unchanged regional myocardial blood flow.5 Such perfusion-contraction mismatch was associated with a local inflammatory response characterized by leukocyte infiltration.5 In subsequent studies, a causal role of tumor necrosis factor (TNF)-α and sphingosine in this progressive contractile dysfunction was demonstrated.6,7 These experiments were limited to a time frame of 8 hours, and it remained unclear whether the progressive contractile dysfunction eventually recovers. We have therefore now monitored the time course of the progressive contractile dysfunction until full recovery in chronically instrumented conscious dogs.

Glucocorticoids have been used for their antiinflammatory action in the treatment of a wide variety of diseases (for review, see Falkenstein et al8). More specifically, glucocorticoids attenuate leukocyte/endothelium interactions9–12 as well as the generation and release of inflammatory cytokines and mediators.13–17 Cardioprotective effects of glucocorticoids in the acute setting of myocardial ischemia/reperfusion have been shown experimentally with regard to structural and functional myocardial damage.18–24 However, the more long-term effects of glucocorticoids on the outcome of ischemia/reperfusion are more controversial.25

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In the present study, we examined the effect of methylprednisolone, given either before or after established coronary microembolization, on the myocardial inflammatory reaction and the progressive contractile dysfunction over a period of 8 hours. In addition, in chronically instrumented conscious dogs, we determined the more long-term effects of glucocorticoids, when given as a single dose after microembolization, on the progressive contractile dysfunction and its eventual recovery.

**Methods**

The bioethics committee of the district of Düsseldorf, Germany, approved the experimental protocols used in this study, and the dogs were handled according to the guidelines of the American Physiological Society.

**Experimental Preparation**

Anesthesia was induced with an initial bolus of sodium thiopental (30 mg/kg IV). After endotracheal intubation, anesthesia was maintained by ventilation using enflurane with an oxygen/nitrous oxide mixture. Acute experiments were performed in 19 anesthetized mongrel dogs (22 to 32 kg body weight) with an open-chest preparation. Arterial blood gases and rectal temperature were regularly monitored and kept within the normal range. Left ventricular and aortic pressures were measured with catheter-tip manometers (PC 350, Millar). For the measurement of regional myocardial blood flow, a Teflon catheter was placed into the left atrium, distal to an electromagnetic flow probe, for intracoronary injection of microspheres. Regional myocardial blood flow was determined with colored 15-μm microspheres. For each measurement, ~5×10^10 to 1×10^11 microspheres suspended in 5 mL saline with 0.02% Tween 80 were injected into the left atrium, followed by a flush of 5 mL saline. The withdrawal of arterial reference blood samples was started 30 seconds before injection of the microspheres and continued for 150 seconds at a rate of 5 mL/min. At the end of the experiment, the heart was quickly removed for postmortem analyses.

Additional experiments were performed in 8 chronically instrumented conscious dogs that were accustomed to human handling and trained to lie quietly in a “sphinx-like” position. The dogs were instrumented with a micromanometer (model P3.5X, Konigsberg Instruments) in the left ventricle and ultrasonic crystals in the anterior and posterolateral ventricular wall.

Microembolization was performed during anesthesia under fluoroscopy through an intracoronary catheter (Cordis RapidTransit 3/2.3F, Johnson & Johnson Medical) placed in the LCx. Dogs were euthanized when wall thickening had fully recovered in placebo dogs and at the same time in methylprednisolone-treated dogs.

**Experimental Protocols**

**Acute Studies**

 Coronary microembolization was performed with slow manual injection of 3000 white-stained microspheres (42 μm Dynospheres, Dyno Particles) per mL/min coronary inflow into the LCx, as described previously.

 Three groups of dogs were studied. Placebo dogs (n=7) received 10 mL 0.9% NaCl solution IV. A second group of dogs (n=7) received 30 mg/kg methylprednisolone in 10 mL 0.9% NaCl solution IV 30 minutes before microembolization, and the third group of dogs (n=5) received 30 mg/kg methylprednisolone in 10 mL 0.9% NaCl solution 30 minutes after microembolization. Measurements of systemic hemodynamics, regional myocardial blood flow, and function were taken at baseline and repeated after 1, 4, and 8 hours after microembolization.

**Chronic Studies**

Baseline values of heart rate, left ventricular pressure, and regional myocardial function were determined on 4 consecutive days before microembolization. For coronary microembolization, white-stained microspheres (42 μm) were given slowly through the catheter placed in the LCx. The injection was stopped when the reduction in posterior regional myocardial function was of similar magnitude as in the acute studies, i.e., by 80% to 90%. Two groups of dogs were studied. Placebo dogs (n=4) received 10 mL 0.9% NaCl solution IV, and the second group of dogs (n=4) received 30 mg/kg methylprednisolone in 10 mL 0.9% NaCl solution IV 30 minutes after microembolization. Regional myocardial blood flow was measured before and 10 minutes after microembolization as well as before the dog was euthanized.

**Hemodynamic Data**

Systemic hemodynamics and regional myocardial wall thickness were monitored on an 8-channel forced-induced chart recorder (MK 200 A, Gould Inc) and stored directly to the hard disk of a computer. Hemodynamic and functional parameters were digitized and recorded on paper at 20-second intervals for each intervention.

**Postmortem Analysis**

The heart was sectioned from base to apex into 5 slices in a plane parallel to the atrioventricular groove. The slices were immersed in 0.09 mol/L sodium phosphate buffer (pH 7.4) containing 1.0% tripolyphosphate (TTC, Sigma) and 8% dextran (MW 77 800) for 20 minutes at 37°C to macroscopically identify infarcted tissue.

**Regional Myocardial Blood Flow**

Regional myocardial blood flow was analyzed as described previously. Transmural blood flows at the site of the ultrasonic crystals are reported.

**Infarct Size and Leukocytes**

In addition to TTC staining, microinfarction was quantified microscopically, as described previously. Briefly, transmural formaldehyde-fixed specimens from the anterior and posterior walls were embedded in paraffin and sectioned into slices 5 μm thick. Four such sections (total area, 2.3±0.1 cm²) each from the anterior and posterior walls were examined. The area of all necrotic foci was expressed as percentage of the total area of all analyzed tissue sections. In the same sections, inflammatory cells were counted in 10 fields (each with an area of 190 000 μm²) from each section. Most inflammatory cells, which could be clearly identified, were polymorphonuclear leukocytes.

**Myocardial TNF-α concentration**

The myocardial concentration of TNF-α was measured in ~200 mg each of the ventricular myocardium stored at −70°C from the anterior and posterior wall using the WEHI-163 clone cytotoxic activity assay, as described previously. The concentration of TNF-α was expressed in units per gram; 1 U is the reciprocal of the dilution necessary to cause 50% cell destruction.

**Statistics**

Data are reported as mean±SEM. Changes in hemodynamics, regional myocardial blood flow, and function in either the acute or the chronic experiments were estimated by 2-way ANOVA for repeated measures. Fisher’s least-significant difference tests were then performed to compare single mean values. Infarct size, number of leukocytes, and TNF-α concentration in the anterior and posterior walls were compared by unpaired t tests. A P value of <0.05 was taken to indicate a significant difference.
Results

Acute Studies

Systemic Hemodynamics, Regional Myocardial Blood Flow, and Function

Systemic hemodynamics remained constant throughout the protocol in all groups and were not different between groups. Anterior systolic wall thickening and blood flow were unaltered throughout the protocol in all groups. Posterior systolic wall thickening decreased immediately with coronary microembolization and recovered back to baseline within minutes in all groups. Posterior systolic wall thickening then decreased progressively over 8 hours in placebo dogs but remained unchanged in methylprednisolone-pretreated and methylprednisolone-posttreated dogs (Figure 1).

Coronary blood flow was not significantly different among groups, but posterior regional myocardial blood flow was higher in the 2 methylprednisolone-treated groups than in placebo at 4 and 8 hours after microembolization.

Infarct Size and Leukocytes

There was no evidence for myocardial infarction in the anterior wall. In the posterior microembolized wall, there were \( \approx \) 2500 microspheres/g tissue and a transmurally homogeneous distribution of small acutely necrotic foci in all dogs; infarct size in the posterior wall was 2.2 \( \pm \) 0.8% in placebo, 2.2 \( \pm \) 0.4% in methylprednisolone-pretreated dogs, and 2.0 \( \pm \) 0.2% in methylprednisolone-posttreated dogs. The number of cells infiltrating the posterior embolized wall was greater than that in the anterior control wall in placebo and in methylprednisolone-posttreated dogs but not in methylprednisolone-pretreated dogs (Figure 2).

Myocardial TNF-\( \alpha \) Concentration

The TNF-\( \alpha \) concentration was increased in the posterior embolized wall over that in the anterior control wall in placebo but not in methylprednisolone-pretreated and -posttreated dogs (Figure 3).

Chronic Studies

Systemic Hemodynamics, Regional Myocardial Blood Flow, and Function

All dogs had decreased left ventricular pressure and systolic wall thickening and increased heart rate during the acute anesthesia when the microembolization was performed. Also, all dogs developed ventricular tachycardia between 4 to 6 and 36 hours after microembolization. Posterior myocardial blood flow remained unchanged throughout the protocol and was not different between groups (Figure 4). Anterior systolic

Figure 1. Posterior systolic wall thickening (PWT) at baseline, with immediate coronary microembolization, and 5 to 10 minutes and 1, 4, and 8 hours later. In placebo group (PLA), posterior systolic wall thickening after an initial recovery from microembolization was progressively decreased, whereas it remained unchanged in groups receiving methylprednisolone before (MPpre) or after (MPpost) microembolization. Data at bottom of figure indicate regional transmural blood flow in posterior wall (mL/min per g). Data are mean \( \pm \) SEM.

Figure 2. Number of infiltrating leukocytes in anterior and posterior walls. In acute studies, number of infiltrating cells was greater in posterior embolized wall in placebo (PLA) and methylprednisolone-posttreated dogs (MPpost) but not in methylprednisolone-pretreated dogs (MPpre). In chronic studies, 6 days after microembolization, number of infiltrating cells was greater in posterior embolized wall in both groups (placebo, cPLA; methylprednisolone-treated, cMP). Data are mean \( \pm \) SEM.
wall thickening and blood flow were unaltered throughout the protocol in both groups. Immediately after microembolization, posterior systolic wall thickening decreased in both groups and recovered within minutes (Figure 4). At 4 to 6 hours after microembolization, systolic wall thickening of the posterior wall was again decreased in the placebo group, whereas it had recovered further in the methylprednisolone-treated group. Two days after microembolization, all dogs had recovered stable sinus rhythm. In the placebo group, posterior systolic wall thickening remained depressed up to day 4 after microembolization and had recovered back to baseline on days 5 and 6. In contrast, in the methylprednisolone-treated group, systolic wall thickening of the posterior wall was back to baseline 4 to 6 hours after microembolization (Figure 4).

Infarct Size and Leukocytes
There was no evidence for myocardial infarction in the anterior wall. In the posterior microembolized wall, there was a transmurally homogeneous distribution of small foci of necrosis/early fibrosis in all dogs. The number of embolizing microspheres was $4000/g$ tissue, and infarct size in the posterior wall was $5.7 \pm 1.5\%$ in placebo and $5.4 \pm 1.9\%$ in methylprednisolone-treated dogs, not different between groups. In both groups, the number of cells infiltrating the posterior embolized wall was greater than that in the anterior control wall (Figure 2) and was not different between groups.
Myocardial TNF-α Concentration

Six days after microembolization, myocardial TNF-α concentration in the posterior embolized wall was not different from that in the anterior control wall, and there was no difference between placebo- and methylprednisolone-treated dogs (Figure 3).

Discussion

Coronary microembolization in patients with coronary heart disease has received more attention recently. Using an experimental model of coronary microembolization, we have demonstrated that progressive myocardial contractile dysfunction develops within hours after an initial rapid recovery from the immediate microembolization-induced ischemic dysfunction. The present experiments confirmed this progressive myocardial dysfunction, and eventual full recovery was observed after 5 to 6 days. In both the acute and chronic studies, the initial ischemic dysfunction with the immediate microembolization was of similar magnitude. However, to achieve this degree of dysfunction, the number of embolizing microspheres and the resulting infarct size in the chronic studies were approximately twice those in the acute studies. As observed previously and supported by our data with a trend to lower TNF-α values in the chronic studies, the acute open-chest preparation sensitizes for the release and effect of inflammatory cytokines and possibly also for the mediators of ischemic contractile dysfunction.

The developing progressive myocardial dysfunction after microembolization in the placebo dogs occurs at unchanged blood flow (perfusion-contraction mismatch) and is associated with a local inflammatory reaction. We have recently established a causal role of TNF-α and sphingosine in this progressive contractile dysfunction. The inflammation in early myocardial ischemia is characterized by infiltration with leukocytes, a process involving the expression of L-selectin, CD11/CD18, and adhesion molecules (for review, see Gumina et al and Jordan et al). Glucocorticoids decrease the expression of L-selectin and CD11/CD18 on leukocytes and the expression of endothelial-leukocyte adhesion molecule-1 and the intercellular adhesion molecule-1. Accordingly, in the present study, nonspecific antiinflammatory treatment with methylprednisolone before coronary microembolization prevented leukocyte infiltration. However, when administered 30 minutes after established coronary microembolization, methylprednisolone no longer prevented leukocyte infiltration, possibly because initial steps of leukocyte activation and leukocyte/endothelium interaction had already occurred.

Glucocorticoids have previously been shown to inhibit the expression of TNF-α mRNA in immunologically activated rat peritoneal mast cells, to suppress the production of TNF-α in serum and myocardium of lipopolysaccharide-stimulated rats, and to abolish the release of TNF-α into serum of humans during cardiac surgery. Glucocorticoids also attenuate the infiltration of TNF-α–producing macrophages/monocytes after coronary microembolization in pigs. Also, in the acute experiments of the present study, glucocorticoids prevented an increase of TNF-α in the microembolized myocardium when given before and even when given 30 minutes after coronary microembolization. Accordingly, in the acute experiments, both glucocorticoid administration regimens, before and 30 minutes after coronary microembolization, prevented the progressive contractile dysfunction resulting from coronary microembolization (Figure 1). This finding was also confirmed in the chronically instrumented dogs, in which glucocorticoid administration 30 minutes after microembolization prevented progressive contractile dysfunction (Figure 4). Six days after microembolization, the TNF-α concentration in microembolized myocardium was no longer different from that in nonembolized myocardium in both placebo- and methylprednisolone-treated dogs.

Apparently, in our model, the glucocorticoid effect on TNF-α is of greater functional impact on contractile function than its effect on leukocyte infiltration, which was prevented only by pretreatment. Leukocyte infiltration was also still enhanced in microembolized myocardium after 6 days, possibly reflecting an ongoing repair process, whereas both TNF-α and contractile function were already back to normal then.

To what extent our experimental model of coronary microembolization reflects the clinical scenario has been critically discussed previously with regard to the size and number of the microemboli, their lack of chemoattractant properties, and the open-chest preparation in the acute experiments. In the past, glucocorticoids have been used clinically for the treatment of acute myocardial infarction, but such treatment was abandoned because of their deleterious long-term effects on scar stability and aneurysm formation. However, our results from the chronically instrumented dogs suggest that antiinflammatory treatment by a single dose of glucocorticoids in the presence of small, patchy microembolization-induced infarcts exerts no adverse effects.

The potential benefit of such short-term glucocorticoid treatment in the clinical scenario of coronary microembolization in patients with acute coronary syndromes or undergoing coronary interventions remains to be established, but given our results, it will merit consideration. In particular, coronary microembolization is characterized by reduced coronary reserve in our experimental model and in patients who have troponin release after coronary interventions. In patients undergoing stenting of a de novo stenosis, the incidence of periprocedural myocardial injury, as assessed by analysis of creatine kinase and troponin T, was reduced by preprocedural treatment with HMG-CoA reductase inhibitors. This beneficial effect is most likely attributable to antiinflammatory properties of HMG-CoA reductase inhibitors. Thus, patients at risk of coronary microembolization may indeed benefit from acute antiinflammatory treatment with glucocorticoids.

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References


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