Creatine Kinase Release Not Associated With Myocardial Necrosis After Short Periods of Coronary Artery Occlusion in Conscious Baboons

GUY R. HEYNDRICKX, MD,* JUN AMANO, MD,* TWILA KENNA, Phd,* JOHN T. FALLON, MD, PhD,† THOMAS A. PATRICK, BSEE,* W. THOMAS MANDERS, BA,* GEOFFREY G. ROGERS, Phd,‡ CLIVE ROSENDORFF, MD, Phd, FACC,‡ STEPHEN F. VATNER, MD*

Boston and Southboro, Massachusetts and Johannesburg, South Africa

The effects of 15 minute periods of coronary artery occlusion on plasma creatine kinase (CK) and CK-MB isoenzyme activity, regional myocardial function and subsequent myocardial necrosis were studied in six conscious baboons 2 to 3 weeks after recovery from instrumentation. Mid left anterior descending coronary artery occlusion induced complete loss of systolic wall thickening (ultrasound transit time technique) and decreases in epicardial (−93%) and endocardial (−96%) blood flows (microsphere technique). Reperfusion after 15 minutes resulted in complete recovery of regional function 24 hours later.

It is generally believed that short periods of coronary artery occlusion of less than 20 minutes duration do not result in permanent cellular damage (1,2). However, these short periods of regional myocardial ischemia associated with coronary artery occlusion result in a prolonged derangement in regional systolic and diastolic function (3-5), which has been referred to as the "stunned myocardium" (6). Almost none of the previous work in this field has been conducted in primates. In view of the marked differences between the responses of transmural blood flow to coronary artery occlusion in primates and dogs (7) and the similarity of creatine kinase (CK) isoenzyme distribution in the heart of baboons and humans (8-10), it was considered important to test the hypothesis that in baboons, brief periods (15 minutes) of coronary artery occlusion, which are characterized by intense transmural ischemia, might result in either: 1) myocardial necrosis, 2) permanent impairment of regional function, or 3) release of CK into the blood.

Methods

Experimental preparation. Nine male baboons (Papio ursinus, n = 7; Papio anubis, n = 2) weighing between 20 and 30 kg were operated on using sterile surgical technique. The animals were premedicated with ketamine hydrochloride intramuscularly (6 to 8 mg/kg body weight). General anesthesia was initiated with sodium methohexital intravenously (2 mg/kg) and maintained with halothane (0.5 to 1 volume %). Using a midline sternotomy, the pericardium was incised and the heart exposed. A hydraulic occluder was placed around the mid left anterior descending coronary artery. A pair of ultrasonic crystals for measure-
ment of wall thickness was implanted across the left ventricular free wall, in the center of the potentially ischemic zone. A miniature solid state pressure gauge (Konigsberg Instruments) was implanted in the left ventricular cavity. Heparin-filled catheters were implanted in the ascending aorta and left atrium. The chest was closed and all catheters and transducers were tunnelled to the back of the animal and buried in subcutaneous pouches.

**Measurements.** Left ventricular pressure was measured with the implanted miniature pressure gauge, which was calibrated in vitro as well as in vivo during the experiments, using systolic arterial pressure and diastolic left atrial pressure sampled through the catheters and measured with Statham P23ID strain gauge manometers. Regional wall thickness was measured with an ultrasonic transit time gauge previously described in detail (4,11,12). This instrument measures the transit time of acoustic signals travelling at a sonic velocity of $1.58 \times 10^8$ mm/s between two intramycocardial crystals. Although minimal (<0.01 mm in 6 hours), the drift of this instrument was effectively eliminated by repeated calibrations throughout the experiment. Radioactive microspheres (3M Company) were used to measure regional myocardial blood flow (13). Approximately $1.5 \times 10^5$ microspheres with a diameter of $15 \pm 2 \mu$ and labeled with either cerium-141 or strontium-85 were injected according to the method described in a recent report from this laboratory (7).

**Protocol.** Experiments were performed 2 to 3 weeks after operation when the animals had recovered fully from surgery and no signs of infection were present. On the day of the experiment, the animals were sedated with ketamine hydrochloride (6 to 8 mg/kg intramuscularly) and placed in a chair (designed for baboons) after catheters and transducer wires had been exteriorized using lidocaine for local anesthesia. At 6 to 8 hours after ketamine administration, that is, at a time when the effects of ketamine were no longer apparent (14,15) and the animal was fully awake in the chair, control recordings of left ventricular pressure, rate of change of left ventricular pressure (dp/dt), heart rate, phasic and mean arterial pressure, regional wall thickening and lead II of the electrocardiogram were obtained. Before the induction of coronary occlusion, at least two control blood samples were collected for enzyme determination. Coronary artery occlusion was then accomplished by inflating the hydraulic occluder. All animals developed arrhythmias and were treated with lidocaine. One animal developed ventricular fibrillation and died during the 15 minutes of occlusion. After 15 minutes of occlusion, the occluder was released slowly (over 1 minute) to reduce the incidence of reperfusion arrhythmias. Two animals developed ventricular fibrillation on reperfusion and were successfully defibrillated. Because of the episode of ventricular fibrillation, their data were not included. Two of the remaining six animals were studied twice. Over the first 24 hour period serial blood samples were taken, but the coronary artery was not occluded. After this period, the coronary artery was occluded for 15 minutes, and serial blood samples were collected for a second 24 hour period. Myocardial function was monitored continuously during coronary artery occlusion and for the first 6 hours after coronary artery reperfusion. The animals were then returned to their cages and brought back 24 hours later for final recording of the hemodynamic variables.

**Plasma CK assays.** Serial samples of blood (5 ml) were withdrawn from the animals over a 24 hour period. The samples were taken at 20 minute intervals for the first hour, then every hour for 8 hours, every 2 hours for the next 8 hours and every 4 hours for the final 8 hours. The samples were collected in tubes containing ethylene glycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetae and centrifuged. The plasma was decanted and frozen immediately at $-70^\circ$C. Creatine kinase in plasma was assayed spectrophotometrically, as described by Rosalki (16). Creatine kinase-MB isoenzyme was measured using a modified microbatch filtration method, using anion exchange glass beads, as described by Henry et al. (17). The lowest MB isoenzyme activity that could be reliably measured was 8 U/liter using the Gilford 3401 system.

**Histologic studies.** All animals were sacrificed 48 hours after the experiment with a lethal dose of sodium pentobarbital. Autopsy was performed and the heart was removed. Patency of the coronary artery was verified. In four animals, the heart was cut into 1 cm thick transverse slices from apex to base and stained with triphenyltetrazolium chloride, a method shown to be sensitive and specific for myocardial necrosis (18). The rings were subsequently placed in formalin for 2 days. Samples from the ischemic zone, confirmed by blood flow determinations with microspheres, as well as from a normal zone were prepared for histologic analysis as well as for radioactive counting. In two animals, the heart was immediately placed in formalin. After fixation, these hearts were cut into 1 cm thick transverse slices from apex to base, and histologic sections prepared from the basilar 2 mm of each slice. The remainder of each slice was analyzed for radioactive counts.

**Tissue enzyme analysis.** Normal heart tissue samples from three baboons were assayed for total CK and CK isoenzymes. Six skeletal muscle samples were also analyzed for CK isoenzymes by electrophoresis (45 minutes at 225 volts). The cellulose acetate strips were developed for 20 minutes at 37°C with Gilford Diagnostics reagent (CK 14 creatine kinase), dried, visually inspected and pen-recorded under ultraviolet light (366 nm).

**Analysis of data.** During the experiments all signals were stored on a tape recorder (Honeywell 101) and played back on two multichannel direct-writing oscillographs. Mean and standard error of the mean (SEM) were calculated. Significant changes from control values were determined using analysis of variance (19).
Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, revised 1978).

**Results**

**Left ventricular function** (Fig. 1). With coronary artery occlusion, no significant changes occurred in mean arterial pressure left ventricular systolic pressure and dP/dt. Left ventricular end-diastolic pressure increased (p < 0.05) from 7.0 ± 0.1 to 12.5 ± 1.0 mm Hg and heart rate increased from 112 ± 6 to 127 ± 5 beats/min. With coronary artery reperfusion, these values returned to control and no further changes occurred in these hemodynamic variables over the subsequent 24 hour period.

With coronary artery occlusion, systolic wall thickening decreased by 164 ± 16% from 1.99 ± 0.15 mm and was replaced by holosystolic thinning. With coronary artery reperfusion, systolic wall thickening resumed immediately but remained depressed for at least 3 hours. At that time, systolic wall thickening was still depressed by 33 ± 12% from control (p < 0.01). At 24 hours after reperfusion, systolic wall thickening had returned to preocclusion levels.

**Regional myocardial blood flow.** During coronary artery occlusion, blood flow decreased by 96 ± 2% in the endocardium and by 93 ± 3% in the epicardium, reflecting homogenous transmural ischemia.

**Serial blood creatine kinase** (Fig. 2). After reperfusion was initiated at 15 minutes, CK in plasma increased gradually from 71 ± 11 U/liter to a peak of 976 ± 158 U/liter at 9.3 ± 1.4 hours. Creatine kinase increased from levels too low to be measured accurately to 21.4 ± 2.9 U/liter. Time to peak CK-MB was shorter (4.3 ± 1.1 hours) compared with the time to peak total CK (9.3 ± 1.4 hours). In two animals not subjected to a coronary occlusion, no significant change in plasma enzyme activity was observed over a 24 hour period.

**Tissue creatine kinase isoenzymes.** Total myocardial CK averaged 11,599 mU/mg protein. Myocardial isoenzymes expressed as a percent of total tissue isoenzymes averaged 0.9 ± 1.1, 83.5 ± 0.7 and 7.5 ± 1.1% for BB, MB, MM and mitochondrial fraction, respectively. The six skeletal muscle samples exhibited 100% CK-MM isoenzyme and 0% of the other isoenzymes.

**Figure 2.** Time course of serial plasma total creatine kinase (CK) (circles) and CK-MB isoenzyme (triangles) curves after a 15 minute coronary artery occlusion (CAO).
**Myocardial pathology.** Coronary artery patency was demonstrated in all hearts from the six occlusion experiments. Staining with triphenyltetrazolium chloride revealed no evidence of myocardial infarction. Histologic sections from the six animals showed no evidence of acute (48 hour) myocardial infarction or localized myocyte necrosis. However, microscopic foci of mononuclear cells and fibrosis were present in sections from ischemic and normal zones in both control animals and animals with coronary occlusion, which could have been due to the thoracotomy and implantation of instrumentation.

**Discussion**

In clinical practice, demonstration of elevated plasma enzyme activity has been used as an important criterion for diagnosing myocardial infarction. This practice stems from the assumption that once intracellular enzymes start leaking from myocardial cells as a result of altered cellular permeability, the cellular injury has reached a stage of irreversibility (20,21). In addition, enzymatic estimation of infarct size experimentally, as well as clinically, is based on the premise that release of enzyme from ischemic myocardium reflects irreversible injury (22–24).

The present experiments performed in conscious baboons demonstrate that short periods (15 minutes) of coronary artery occlusion, which do not result in demonstrable myocardial necrosis or permanent derangement in regional myocardial function, are associated with a significant increase in plasma CK as well as in CK-MB. The peak increases in plasma total CK and CK-MB activity occurred at 9.3 and 4.3 hours, respectively. The shorter time to peak activity for CK-MB may be due to the shorter half-life of CK-MB as compared with total CK (25).

It was surprising to note that no early peak plasma enzyme activity was observed with the onset of reperfusion at 15 minutes as is systematically observed with reperfusion after coronary artery occlusions of longer duration in dogs (26). This would suggest that either the brief ischemic insult or the reperfusion resulted in a derangement of membrane permeability, which was gradual in onset and, although reversible, slow to recover. The finding that adenosine triphosphate (ATP) and the adenine nucleotide pool remains depressed for a prolonged period after a 15 minute occlusion (5,27) suggests that the integrity of cellular membranes, which require high energy phosphate, is still impaired long after the ischemic insult is over. A recent study by Piper et al. (28) showed that there is a gradual release of cytosolic enzymes from reversibly injured myocardial cells in culture, which is consistent with our results in conscious baboons. It is also conceivable that the enzymes released from myocardial cells are preferentially drained by the lymphatic channels, which have a relatively low flow rate and, therefore, delay the appearance of enzyme in the blood (29).

Our results, however, are at variance with the data presented by Ahmed et al. (21), who demonstrated that short episodes of ischemia (<30 minutes) in conscious dogs did not result in elevated plasma CK-MB activity or histologic evidence of myocardial necrosis. However, there are several important consequences due to differences in the species studied. Species differences in resistance to ischemia have been shown by Hearse et al. (30). In addition, the percent of MB isoenzyme activity in the heart varies among species. In dogs, the MB fraction accounts for approximately 2 to 3% of total CK, whereas, according to Yasmineh et al. (8), the MB fraction accounts for approximately 17% in baboons. With the technique utilized in the present study, the MB fraction in the baboon heart was 9% of the total CK. It is also important to note that the reduction in blood flow was more intense and was evenly distributed across the myocardial wall in the baboon. The greater severity of the ischemia could have been the major difference. However, a recent study by Michael et al. (31) found CK release in the cardiac lymph in conscious dogs after brief coronary artery occlusions insufficient to induce necrosis.

**Clinical implications.** Clinical studies (32) in which short episodes of ischemia during anginal attacks were associated with increased plasma enzyme activity lack the pathologic data to confirm the presence or absence of cellular necrosis. There is, however, indirect evidence that transient ischemic episodes not associated with necrosis may be associated with enzyme leakage. Chiong et al. (33) reported small but significant increases in CK release in the coronary sinus during pacing-induced ischemia in patients with coronary artery disease. It is also important to note that even under normal conditions, basal amounts of enzyme leak from myocardial cells (34). This basal leakage can be increased by stress, for example, tachycardia (35,36) and severe muscular exercise (37,38).

The findings of the present investigation have potential clinical significance because plasma enzyme activity is routinely used as an indicator of myocardial infarction. From our results, we may speculate that in patients with coronary artery disease not all episodes of ischemia associated with modestly elevated plasma levels of CK in blood reflect myocardial necrosis.

We acknowledge the excellent animal care under the supervision of John Austin, DVM, the dedicated technical help during surgery of Andres Ndou and the assistance during the experiments of Ann Coull.

**References**


