PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **186**, 218–222 (1987)

Lack of Direct Coronary Vascular Effects of *Escherichia coli* Endotoxin in Dogs (42607)

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Abstract. This study explored the hypothesis that coronary vascular injury and dysfunction result from intracoronary administration of *Escherichia coli* endotoxin (0.025 to 0.4 mg/kg) in dogs. Peak hyperemic coronary flow following a 15-sec period of stopped flow and the maximum flow in response to adenosine were used to estimate coronary vascular reserve. The wet-to-dry ratio of myocardial tissue was used to estimate extravascular water content as an indicator of vascular leak due to endothelial injury. Intracoronary saline was used as a control. Peak reactive hyperemia and maximum flow at constant coronary pressure were not different in the animals receiving intracoronary endotoxin (n = 6) and the animals receiving saline (n = 5) during 4 hr following treatment. In addition, wet-to-dry ratios were similar in these two groups. These data fail to support the hypothesis that endotoxin, per se, produces coronary vascular injury of sufficient magnitude to produce myocardial dysfunction.

Despite aggressive medical therapy, a large fraction of patients with septic shock die (1). Several phases of septic shock are evident in the clinical setting. The initial phase is characterized by a high cardiac output and low systemic vascular resistance, and the final phase, by a low cardiac output and high systemic resistance. The cause of the transition from the initial phase to the final phase is uncertain, but myocardial failure is implicated (2–5). Coronary hypoperfusion secondary to low arterial pressure is probably a contributing factor to myocardial dysfunction, but animals made hypotensive for similar periods without endotoxin do not demonstrate the same degree of myocardial failure (6). Myocardial depressant factor, a humor released by ischemic gut or pancreas, has been implicated (7), but this mechanism is not accepted by all workers in the field (8). Myocardial ischemia is implicated in the process by studies showing patchy subendocardial ischemia and hemorrhage in the hearts of animals dying from septic shock (9). However, the cause of the ischemia has not been identified. It seems reasonable that vascular damage by endotoxin could be involved.

Pharmacologic vasodilators elicit an increase in coronary flow during the early and transitional phases of endotoxic shock, but not during the terminal phase (10). This information is consistent with the concept of vascular damage and decreased vasodilator reserve in the coronary circulation. Histologic examination of the vessels of animals dying after intravenous endotoxin provides evidence of injury (11, 12). Damage to coronary arteries has been reported to include loss of microvilli and complete exfoliation of endothelial cells (13). Injury can be observed as early as 1 hr following endotoxin (11).

The purpose of this study was to determine if *Escherichia coli* endotoxin, administered directly into the coronary circulation, results in vascular damage and dysfunction. The hypothesis was that vascular or endothelial damage might interfere with the vessel's ability to autoregulate flow or to dilate in response to a metabolic signal from the myocardial cells. Such impaired *vascular* function, combined with the increased cardiac rate and low arterial perfusion pressures seen with sepsis, might lead to the patchy myocardial ischemia that has been observed in animals dying in septic shock.

The aim of this study was to determine the direct effects of endotoxin on coronary blood flow and vasodilator reserve. Heart rate was relatively constant and arterial blood pressure was controlled during the measurement periods so that myocardial oxygen consumption would not vary. The flow response to a short period of stopped-flow and the maximum flow elicited at constant coronary pressure with a powerful vasodilator, adenosine, were used to estimate vasodilator reserve.

Endotoxin was infused directly into the

0037-9727/87 $1.50

coronary artery in a dose calculated to be similar to that resulting from intravenous administration of a uniformly lethal dose of endotoxin. Direct intracoronary administration of endotoxin was used to maximize damage to the coronary circulation and minimize systemic effects. The results fail to support a direct coronary vascular effect of *E. coli* endotoxin in this canine model in the first 4 hr following endotoxin.

Materials and Methods. Eleven mongrel dogs of either sex weighing 20–28 kg were sedated with morphine sulfate (2.5 mg/kg sc) and then anesthetized with α-chloralose (100 mg/kg iv, Sigma). Anesthesia was maintained with an infusion of α-chloralose (10 mg/kg/hr). The trachea was intubated, and each animal was ventilated with oxygen-enriched room air to maintain arterial carbon dioxide tension between 35 and 45 mm Hg and arterial oxygen tension between 100 and 300 mm Hg. Arterial blood gas values were determined periodically (IL 813). Rectal temperature was held between 37 and 39°C by use of a heating pad and lamp. Arterial hemoglobin was determined periodically (IL 812). Blood coagulation in an external circuit was prevented by infusion of sodium heparin (750 μg/kg iv bolus plus 250 μg/kg/hr iv). Hemoglobin concentration tended to rise with time in the animals given endotoxin, and a solution of dextran (6%) in water was infused to counteract this rise. Serum glucose tended to fall following endotoxin, and dextrose (5% in water; 50–200 ml) was infused to keep serum glucose above 80 mg/dl (Chemstrip).

Arterial blood pressure was measured with a catheter introduced into the arch of the aorta via the right brachial artery. Pulmonary artery wedge pressure was measured with a balloon-tipped catheter. A pressurized blood reservoir attached to a femoral artery was used to stabilize blood pressure during the measurement period each hour. Phenylephrine was infused (100–400 μg/min, iv), if necessary, to raise arterial pressure during the measurement periods.

A stainless steel cannula was advanced into the root of the aorta via the right carotid artery. The tip of this cannula was wedged into one branch of the left coronary artery. Arterial blood from a femoral artery was supplied to the cannula via an external circuit that included a servo-controlled roller pump (Sarns). The speed of the pump was adjusted with an electronic feedback loop to maintain coronary pressure at the cannula tip constant. Cannula tip pressure was measured with a small internal steel tube. Coronary pressure was held constant at 100 mm Hg except during flow measurements when it was decreased to 70 mm Hg. The higher pressure between flow measurements was used to ensure adequate coronary perfusion. A coronary pressure of 70 mm Hg was chosen for the measurements because arterial pressure was held at this level during the measurements.

Coronary flow was measured by an electromagnetic flowmeter in the external circuit (Zepeda). The flowmeter was calibrated by timed collection of the dog's blood. Mean coronary flow was determined with the aid of an active RC circuit with a time constant of 2.0 sec.

All measurements were made at a mean arterial pressure of 70–80 mm Hg. Arterial pressure was adjusted either by blood withdrawal or by phenylphrine infusion for the duration of the flow measurements (5–10 min) each hour. Between flow measurements, arterial pressure was not controlled. Heart rate was not directly controlled but remained relatively stable.

Coronary blood flow was measured during controlled arterial pressure at a constant coronary pressure of 70 mm Hg. Then coronary flow was stopped for 15 sec by turning off the pump. At the end of this occlusion period, coronary pressure was rapidly returned to 70 mm Hg. The peak flow value during the reactive hyperemia response was taken as "peak hyperemic flow." After coronary flow had returned to baseline values, sufficient adenosine (20 m*M*, Sigma) was infused into the coronary cannula to achieve maximum coronary flow. The administration rate for adenosine was increased in a stepwise manner until no further flow increase occurred. Because all measurements were made at a constant coronary pressure, changes in this maximum flow directly reflect changes in minimum coronary vascular resistance.

These flow measurements were made prior to treatment and then hourly for 4 hr following treatment. Treatment consisted of intracoronary saline (five dogs) or intracoronary en-

dotoxin (*E. coli,* batch 055:B5, Difco) in a dose of 0.025 to 0.4 mg/kg over 30–60 min. Two dogs received 0.025 mg/kg, two dogs received 0.2 mg/kg, and two 0.4 mg/kg. Preliminary studies in dogs determined that intravenous infusion of endotoxin at a dose of 5 mg/kg uniformly resulted in hypotension and death.

Following the experimental protocol, the animal was killed by an intravenous injection of KCl, and the heart was removed. Samples of myocardium from the zone supplied by the cannulated coronary artery and from a nonperfused zone were obtained. These samples were blotted dry on cotton and weighed. The samples were weighed again following 6 days in a 40°C vacuum oven. The ratio of wet-to-dry weights was calculated. The coefficient of variation of these values was 1–3%.

Unpaired *t* tests were used to compare data from animals receiving saline and those receiving endotoxin. A regression analysis determined that the response to intracoronary endotoxin was not dose dependent, and so data from all animals receiving endotoxin were combined. Multiple regression analysis (method of least squares) was used to analyze the effects of time and treatment on coronary flow. Paired *t* tests were used to assess differences from control in hemodynamic data. A *P* value of less than 0.05 was considered significant.

Results. The results are summarized in Tables I and II. Coronary perfusion pressure was held constant at 70 mm Hg during all coronary flow measurements. Arterial blood pressure was held constant in each dog between 70 and 80 mm Hg during the flow measurements. Heart rate was not controlled but was comparable between groups and relatively constant over time in both groups (Table I). These findings suggest that the major determinants of coronary blood flow (oxygen demand and coronary perfusion pressure) were similar at all stages of the experiment. The high heart rate probably resulted from the anesthetic used. Control of hemoglobin was not as effective. Hemoglobin concentration rose 10–20% over the course of the experiment in both groups. Arterial hemoglobin concentration contributes to myocardial oxygen delivery and influences minimum vascular resistance through an effect on viscosity. Pulmonary wedge pressure was unchanged (Table I).

Autoregulated coronary blood flow was similar in both groups at the start of the experiment and did not change as a function of time or treatment (Table II). Peak reactive hyperemic flow following a 15-sec total occlusion was approximately three-fold resting flow. Hyperemic flow declined progressively over time ($P < 0.001$) but was not influenced by endotoxin. Maximum coronary flow during adenosine infusion was four-fold higher than resting flow. No significant effects of time or endotoxin were noted.

The experimental design allowed either sa-

TABLE I. HEMODYNAMIC VALUES BEFORE (TIME 0) AND FOLLOWING INTRACORONARY SALINE OR *E. Coli* ENDOTOXIN

Time (min):		0	60	120	180	240
Heart rate (b/min)	S	153 ± 14	148 ± 13	155 ± 14	152 ± 15	171 ± 5 (n = 3)
	E	140 ± 18	127 ± 16	137 ± 16	142 ± 18	142 ± 18
Pulmonary artery wedge pressure (mm Hg)	S	8 ± 2	8 ± 1	8 ± 1	9 ± 1	10 ± 1 (n = 3)
	E	8 ± 2	7 ± 1	8 ± 1	8 ± 1	9 ± 1
Hemoglobin (g/dl)	S	13.4 ± 0.5	13.9 ± 0.7	14.8 ± 0.7*	15.0 ± 1.0*	16.0 ± 1.8 (n = 3)
	E	13.8 ± 0.9	13.7 ± 0.7	15.1 ± 0.7*	15.4 ± 0.8*	15.1 ± 1.0*

Note. Values are means ± SEM. S, intracoronary saline control (n = 5); E, intracoronary endotoxin (n = 6).
* $P < 0.05$ versus time 0 by paired *t* test.

TABLE II. CORONARY FLOW DATA

Time (min):		0	60	120	180	240
Autoregulated flow (ml/min)	S	28 ± 4	26 ± 5	26 ± 3	27 ± 3	37 ± 9 (n = 3)
	E	24 ± 2	23 ± 3	22 ± 2	25 ± 2	25 ± 3
Peak reactive hyperemia flow (ml/min)	S	83 ± 9	63 ± 7	62 ± 7	58 ± 10	69 ± 11 (n = 3)
	E	86 ± 11	86 ± 14	70 ± 8	68 ± 8	68 ± 11
Maximum coronary flow (ml/min) adenosine	S	114 ± 9	98 ± 12	86 ± 8	89 ± 5	101 ± 2 (n = 3)
	E	97 ± 11	95 ± 14	86 ± 11	86 ± 8	84 ± 8

Note. Values are means ± SEM. S, saline (n = 5); E, intracoronary endotoxin (n = 6); administered between t_0 and t_{60}.

line or endotoxin to be delivered to approximately 40% of the heart by direct injection into the coronary perfusion system. The remainder of the heart was perfused by blood delivered from the aorta in the normal fashion. The hypothesis that endotoxin would cause myocardial edema was tested by comparing the wet-to-dry ratio in the perfused zone of animals receiving endotoxin (4.55 ± 0.21 SEM) with the ratio in animals receiving saline (4.82 ± 0.14). No significant difference in ratios was found. In addition, the wet-to-dry ratios of the normally perfused zones were not different from the ratios observed in cannula-perfused zones for either the animals receiving endotoxin (4.46 ± 0.08) or the animals receiving saline (4.60 ± 0.08).

Discussion. Intracoronary endotoxin did not affect coronary autoregulation nor did it reduce coronary vascular reserve in the first 4 hr following administration. In addition, endotoxin did not influence myocardial tissue water content, as reflected in wet-to-dry ratios. These data suggest that endotoxin did not directly injure the coronary arteries.

The dose of endotoxin ranged from 1 to 16% of an LD_{80} dose (2.5 mg/kg). Because coronary flow into the cannulated zone was approximately 1% of cardiac output, these doses represent 1 to 16-fold increases above the level of endotoxin that would be encountered with intravenous administration of an LD_{80} dose. Thus, it seems unlikely that the lack of effect was caused by an inadequate dose of endotoxin.

The study followed animals for only 4 hr after endotoxin infusion. It is possible that an effect might have been seen if observations had been carried out to 8–10 hr. On the other hand, if vascular injury were an important aspect of myocardial dysfunction, then some early effect on vascular reserve should have been present. Ischemia would be unlikely unless coronary reserve were exhausted.

Maximum coronary flow during adenosine infusion was 15–25% lower in both the saline and the endotoxin-treated animals at 4 hr. This finding suggests a progressive impairment of flow that was most likely caused by time, anesthesia, increasing blood viscosity, and the coronary perfusion system. It is possible that an endotoxin effect on coronary reserve might be observed in another experimental preparation that avoided these effects. In addition, the animals were heparinized, and this anticoagulation may have obviated an endotoxin effect on the coagulation process.

The present results appear to rule out a direct effect of endotoxin on coronary tone or the ability to dilate in response to adenosine. The present results do not, however, exclude indirect effects of endotoxin when given to the entire animal rather than just into a coronary artery. The existence of such an indirect mechanism leading to vascular injury or dysfunction is supported by a recent study (14).

Artman and co-workers studied isolated rabbit hearts perfused with blood at constant flow. They observed no change in calculated coronary resistance when endotoxin was administered directly into the perfusion apparatus. In contrast, a 20–50% increase in coronary resistance occurred when the perfusate was switched to blood from a donor animal that had received endotoxin 30–360 min previously. This finding suggests that a blood-borne factor produced in the periphery in response to endotoxin has a constrictive effect on coronary vessels. Possible constrictors include catecholamines, leukotrienes, vasopressin, and angiotensin (15).

In summary, intracoronary *E. coli* endotoxin in doses from 1- to 16-fold the dose achieved from intravenous administration of an LD_{80} dose failed to produce vascular injury as manifest in changes in autoregulated or maximal coronary flow. In addition, wet-to-dry ratios failed to support the concept of endothelial injury causing extravascular accumulation of water. Thus, it appears unlikely that direct coronary vascular injury by endotoxin plays a major role in the depressed myocardial function observed in animals and humans dying of septic shock.

Dr. Buffington was supported by Clinical Investigator Award HL-00695 from the National Heart Lung and Blood Institute. Dr. Lechner was supported by Medical Scientist Training Program Grant GM-07337 from NIH. Funds for this study were provided by the American Heart Association, Washington Affiliate; the Research Fund of the Graduate School of University of Washington; and the Department of Surgery, University of Washington School of Medicine, Seattle, Washington.

The authors thank Mr. Rod Gronka for his valuable technical assistance and Ms. Barbara Pearson for typing the manuscript. The authors are especially indebted to Dr. Warren Guntheroth for the *E. coli* endotoxin and much helpful advice.

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Received May 27, 1986. P.S.E.B.M. 1987, Vol. 186.
Accepted July 9, 1987.