Objective: To determine the endothelial-dependent control of decreased peripheral vascular resistance in skeletal muscle microvessels during evolving sepsis.

Materials and Interventions: Acute (4 hours, n = 7), established (24 hours, n = 7), or chronic (72 hours, n = 8) infection was induced in Sprague-Dawley rats (150-175 g) by injecting Escherichia coli and Bacteroides fragilis (1 × 10^9 colony-forming units for both) into a subcutaneous sponge. Control animals were injected with an isotonic sodium chloride solution and analyzed at the same time points: (n = 6-8 per group). Dilation in response to the topically applied endothelial-dependent agonist acetylcholine (ACH) (1 × 10^-9 to 1 × 10^-5 mol/L) was measured in inflow first-order (A1) and precapillary fourth-order (A4) arterioles in cremaster muscle in vivo with videomicroscopy. Acetylcholine dose-response curves were used to determine vascular reactivity by calculating the concentration of ACH necessary to elicit 50% of the maximal dilator response.

Main Outcome Measures: In vivo reactivity of striated muscle microvessels to the dilation agonist ACH during acute, established, and chronic infection.

Results: A1 vessels were unresponsive to all doses of ACH at all time points. A4 vessels showed an increased dilator response during short-term treatment, which deteriorated over time to depressed dilation during chronic infection.

Conclusions: Precapillary A4 vessels have increased dilator reactivity during early sepsis, which progresses to depressed levels with chronic infection. A1 microvessels remain dilated and are not substantially influenced by endothelial dilator mechanisms initiated by ACH. Maximum dilation of the large A1 vessels appears to contribute to the decrease in peripheral vascular resistance noted during systemic infection.

Arch Surg. 1998;133:1335-1342

Acutely sepsis manifests a hemodynamic state in which the cardiac output (CO) and heart rate are increased in an attempt to adequately supply peripheral perfusion. This high-output state occurs concomitantly with a decrease in systemic vascular resistance and oxygen consumption. These cardiovascular and metabolic changes reflect a failure to deliver or use oxygen at the cellular level, resulting in metabolic acidosis. Microvascular blood flow abnormalities, such as an increased number of capillaries with no flow, occur in striated muscle during sepsis. These metabolic, cardiovascular, and blood flow alterations indicate a need to assess skeletal muscle microvascular function to understand changes in peripheral resistance during the evolution of sepsis. Endothelial cells control the contractility of vascular smooth muscle cells primarily by the synthesis and release of nitric oxide (NO). Endothelial release of NO is altered during sepsis. The question remains whether endogenous NO activity in microvascular beds leads to the hemodynamic changes of sepsis. In the acute phase of sepsis, large arterioles are constricted and small terminal arterioles are dilated in rat cremaster muscle. This constriction of large-inflow arterioles reduces the overall blood volume delivered to capillaries and may ultimately contribute to metabolic acidosis.

Sepsis typically begins with a hyperdynamic clinical presentation that includes tachycardia, tachypnea, acidosis, temperature elevation, leukocytosis, and early organ system dysfunction. As the infection persists, organ dysfunction, hypoperfusion, and hypotension ensue. During chronic sepsis, inducible nitric oxide synthase (iNOS) levels are increased, and constitutive NOS (cNOS) levels are decreased. These and many similar findings are the basis for iNOS inhibition as a
treatment of sepsis. Recent studies have shown, however, that a nonspecific inhibition of NOS is detrimental during the later stages of endotoxemia or septic shock. The effectiveness of iNOS inhibition may depend on the stage of sepsis, the capability of endothelial cells to release NO, and the selectivity of the antagonist. In vivo microvascular reactivity throughout the development of sepsis has not been studied. To determine the capacity of endogenous NO release by the striated muscle microvascular bed, we chose to analyze endothelial-dependent relaxation in response to acetylcholine (ACH) during the progression of infection from the acute to the chronic state. We hypothesized that the development of chronic sepsis would depress the endothelial-dependent relaxation because of a diminished capacity of the endothelial cell to release NO.

RESULTS

HEMODYNAMICS

Baseline hemodynamic values are reported in the Table. The mean arterial pressure was similar in all experimental groups, except at 24 hours in the established infection group, which showed a 24% increase ($P<.05$) when compared with controls that received isotonic sodium chloride, and a 14% increase in heart rate ($P<.05$). Although there was a trend toward a hyperdynamic cardiovascular state in the animals with chronic infection, these changes were not significant ($P> .05$). All hemodynamic variables were stable during intravital microscopy and dose-response protocols.

MICROVASCULAR RESPONSES

First-order arterioles and venules were unresponsive to increasing concentrations of ACH and sodium nitroprusside. These first-order microvessels maintained diameters within 10% of baseline values in all experimental groups, and dose-response curves were flat. In the group showing chronic sepsis after 72 hours, the baseline diameters of A1 and V1 microvessels were slightly larger than in the groups with acute or established infection.

Although the A3 microvessels in the groups with either acute or established infection showed no change in reactivity compared with those in controls, there was a shift in the $EC_{50}$ to the right in the group with chronic sepsis, indicating a substantial depression of dilator func-
tion (Figure 1). Precapillary A4 microvessels demonstrated a differential shift in reactivity to dilator stimulation, with an increased sensitivity and shift to the left of the EC50 noted in the groups with acute sepsis (Figure 2) and a definite depression of dilator function and shift to the right of the EC50 after 72 hours of infection (Figure 3). There was no change in reactivity of A4 microvessels in the groups with established infection compared with control animals. This differential shift in microvascular sensitivity to dilator stimulation is reflected in the calculated pD2 values for A3 and A4 microvessels in each group. The pD2 value was depressed in A3 vessels during chronic infection at 72 hours (Figure 4), whereas A4 vessels showed an early increase in the pD2 value during acute infection at 4 hours, indicating a shift over time in these small precapillary microvessels.

COMMENT

Several studies3,7,11,12 have documented that the synthesis and release of NO—the primary dilator mechanism in microvessels—is altered during sepsis. Opposing results exist.6,9,10,13,18 however, regarding circulating NO levels during endotoxia or sepsis. These results are most likely caused by differences in cNOS and iNOS at various stages of sepsis. The overproduction of NO is thought to be caused by the activity of iNOS in macrophages, hepatocytes, and neutrophils, which are activated during sepsis and partially released into the systemic circulation.10,13,18 A decreased production of NO has been attributed to a down-regulation of cNOS, which in the initial stages of sepsis is the major endothelial-dependent control mechanism of dilator function. The quantification of microcirculatory reactivity has not been studied in the face of changing levels of NO production by the endothelial cell. We conducted the present study to determine changes in the endothelial-dependent relaxation response during the developmental phases of sepsis.

Our results indicate that A3 and A4 striated muscle arterioles become less sensitive to endothelial-dependent relaxation during the development of chronic sepsis. These results correlate with the common finding of diminished constrictor and dilator responses during late-stage sepsis.6,7,8,12,18 These data also support the recent finding that endothelial cell–derived NO is decreased in late sepsis.7 Because ACH stimulates endogenous NO release primarily through cNOS, the decrease entry into the experimental protocol. After baseline measurements were obtained, serial doses of ACH were added to the bath (final concentrations, 1 × 10⁻⁴ to 1 × 10⁻⁵ mol/L). Each dose of ACH remained in the bath for 10 minutes. Hemodynamic measurements were taken during the first minute of exposure, and vessel diameters were recorded at 1, 3, 5, 7, and 9 minutes for each dose of ACH. Sodium nitroprusside was added after the final ACH dose, and hemodynamic and vessel diameter measurements were taken 5 minutes later to determine maximal vessel dilation.

Dose-response curves for dilation were generated by normalizing the measured vessel diameters with reference to the maximal vessel dilation. The mean baseline value was set at 0, and the maximal vessel diameters with sodium nitroprusside application were set at 100% maximal dilator capacity. The following equation was used to calculate the percentage of dilator capacity: (X – BL)/(NP – BL) × 100%, where X indicates vessel diameter; BL, vessel diameter at baseline; and NP, maximal vessel diameter. From the ACH dose-response curve of each animal, vascular sensitivity was determined by the concentration of ACH necessary to elicit 50% of the maximal dilator response (EC50). The pD2 value was determined as the negative log EC50, which represents the changes in microvascular sensitivity to ACH.

STATISTICAL ANALYSIS

Within each experimental group, the baseline values were compared with ACH doses using repeated-measures analysis of variance, followed by the Tukey-Kramer least significant difference test. Experimental groups were compared using the 1-way analysis of variance, followed by the Tukey-Kramer least significant difference test. The null hypothesis was rejected when P < .05.
in microvascular dilator response is indicative of either a diminished or an impaired capability of endothelial cells to release NO or a decreased responsiveness of the smooth muscle cell.

Parker and Adams\textsuperscript{6} have reported that the process of relaxation by an endothelial-dependent, receptor-independent agonist was unaffected in guinea pig aortic and coronary vascular rings exposed to endotoxin, but was diminished for receptor-dependent agonists (ACH and adenosine diphosphate). In addition, the stimulation of a different endothelial receptor with substance P elicited a relaxation response similar to that in controls, leading to the conclusion that endotoxin inhibits endothelial cell cNOS activity and microvascular reactivity by disrupting receptor-coupled activation mechanisms shared by ACH and adenosine diphosphate.\textsuperscript{6} These results, coupled with our present findings, indicate that the signal transduction pathway for receptor activation of cNOS by ACH becomes disabled over time in the small precapillary microvessels when continually stimulated by sepsis. The present study documents depressed endothelial-dependent dilator function that develops over time. These endothelial cell findings thus support a diminished ACH or NOS signal transduction pathway.

The possibility that physical damage to the endothelium in the small arterioles may be responsible for the diminished response was not supported in this study. According to Furchgott and Zawadzki,\textsuperscript{20} the presence of intact and functional endothelial cells is necessary for
smooth muscle cell relaxation in response to ACH application. If endothelial cells are denuded or damaged, ACH application will result in smooth muscle cell constriction. Acetylcholine-stimulated constriction was not observed at any point during this study; thus, the endothelial lining of the cremaster microvasculature remained structurally intact. Other investigators have also ruled out endothelial cell damage as the cause of decreased sensitivity of the endothelial cell–dependent relaxation and constriction responses.

Increasing amounts of the cytokines tumor necrosis factor α and interleukin 1β have been shown to directly inhibit endothelial-dependent relaxation by reducing NO production. Myers et al also show that tumor necrosis factor α and interleukin 1β do not elicit the release of endothelium-derived relaxing factor from endothelial cNOS. Therefore, the release of cytokines induced by the systemic inflammatory response may be partly responsible for the apparent decrease in NO release and diminished endothelial-dependent relaxation noted in A3 and A4 vessels during chronic infection. Our data clearly show a reduced pD₂ value at 72 hours of infection in the precapillary microvessels, which did not occur during the earlier stages of infection.

Investigators recently reported an initial increase in endothelial-derived NO and vascular smooth muscle cyclic guanosine monophosphate following cecal ligation and puncture. The present data correlate with these findings in that an increased sensitivity to ACH was observed in A4 arterioles 4 hours after bacterial injection. Because ACH elicits endogenous NO release from the endothelial cell, it can be inferred that either NOS activity is up-regulated in the short term or that there is an initial increase in ACH receptors. An explanation for the increased sensitivity in A4 arterioles and not in A3 arterioles during early sepsis remains to be determined, but it is thought that paracrine microvascular control becomes dominant in the most distal microvessels.

Previous studies have shown substantial vasoconstriction or vasodilation in first-order arterioles during different phases of acute bacteremia. In our present study, the diameters of A1 vessels tended to be larger at baseline in the later stages of sepsis, but these differences were not significant (P > .05). The diameters of A1 vessels did not change significantly in any experimental group with increasing concentrations of ACH or sodium nitroprusside. The fact that neither of these agonists elicited a response indicates that these microvessels were maximally dilated from the onset of the experiment. Although this unresponsiveness may be an artifact caused by the surgical preparation, long-term stimulation can shift microvascular control in the vascular tree and relegate the larger vessels to passive conduits, as occurs with hypertension. Another possible explanation is that because these microvessels are under neural control, the use of an anesthetic may have masked or blunted any A1 or V1 microvascular response. The large A1 and A2 microvessels are the primary resistance vessels in striated muscle. The fact that they were maximally dilated throughout our studies could be a factor in the decrease in peripheral vascular resistance noted during sepsis.
The clinical septic state is characterized by an elevation of cardiac output, a decrease in peripheral vascular resistance, and ongoing systemic acidosis. These variables hint that nutrient microvascular blood flow to vital organs is impaired, while skeletal muscle blood flow is preserved. This present study defines an alteration in dilution control mechanisms for the large skeletal muscle microvascular bed during chronic sepsis. The enhanced endothelial cell dilator capacity noted in this study contributes to the decreased peripheral vascular resistance noted during clinical sepsis.

The time points of 4, 24, and 72 hours for investigation were chosen based on previous models to represent a progression from acute to chronic infection. Inducible NOS is thought to appear in substantial levels 6 hours after an inflammatory stimulus. Thus, the 4-hour point during early stages of bacteremia was chosen to study the short-term effects of this model on eNOS activity exclusively. The 24-hour point was chosen to represent established infection with the development and stimulation of eNOS and iNOS activity on the microvascular endothelium. The 72-hour point was selected to represent the chronic inflammatory state so that the temporal changes of the endothelial-dependent relaxation response could be studied over time.

Results of this study support the use of this model in the investigation of chronic sepsis. Previous studies have used either short-term septic challenge or intraperitoneal abscess formation. The sponge implantation on the back was used to avoid a local peritoneal reaction, which could affect cremaster muscle blood flow. This model has been well documented and closely resembles the signs and symptoms of chronic clinical sepsis with intermittent aerobic and anaerobic bacteremia, increased white blood cell counts, elevated CO sustained over time, and a protein catabolic anaerobic bacteremia, increased white blood cell counts, and ongoing systemic acidosis. These variables hint that nutrient microvascular blood flow to vital organs is impaired, while skeletal muscle blood flow is preserved. This present study defines an alteration in dilution control mechanisms for the large skeletal muscle microvascular bed during chronic sepsis. The enhanced endothelial cell dilator capacity noted in this study contributes to the decreased peripheral vascular resistance noted during clinical sepsis.

Our data suggest that a differential response to septic challenge develops over time in striated muscle tissue. It is suggested that NOS activity is increased initially, but NOS activity becomes depressed in precapillary arterioles during the development of chronic sepsis. Large A1 arterioles, the primary resistance vessels in large striated muscles, are maximally dilated, which may account for the depression in peripheral vascular resistance noted during chronic hyperdynamic sepsis. Because microvessel baseline diameters were similar in the control groups and in those with sepsis, a balance between dilation and constriction is apparently maintained. Our data, however, indicate the development of a depression in the dilator response of precapillary arterioles and support the idea that this depression of endothelial-dependent relaxation is a result of a disruption in the ACH or NOS signal transduction pathways in intact endothelial cells.

This study was supported in part by the Office of Research and Development, Department of Veterans Affairs Merit Review, Washington, DC.


Reprints: R. Neal Garrison, MD, Department of Surgery, University of Louisville School of Medicine, Louisville, KY 40292.
Fred A. Luchette, MD, Cincinnati, Ohio: This well-thought-out and -conducted study is a continuation of this group’s research efforts in describing the alterations in the microcirculation during sepsis. An increasing number of studies indicate that the vascular endothelial cell contributes to the control of vascular smooth muscle contractile function by synthesis and release of NO under normal as well as adverse circulatory conditions. Acetylcholine, a receptor-dependent agonist, on the endothelial cell membrane leads to an increase in intracellular calcium stores from the endoplasmic reticulum. This elevation in cytoplasmic calcium concentration activates cNOS, which induces vascular relaxation. New literature contains many reports that contradict each other regarding the impact of endotoxemia or sepsis on endothelial NO production.

Some reports claim that sepsis enhances production, and others demonstrate a significant reduction in production during endotoxemia. These opposing results may be because these studies were conducted at different times after the initiation of the septic insult. A major difference in the various study designs is the use of vessel rings for in vitro assessment of vascular reactivity with topical application of Ach and nitroglycerin. In contrast, the work presented here uses an in vivo model of skeletal muscle microcirculation to determine the endothelial-dependent relaxation response during the progression of infection from an acute to a chronic state. I have several questions for you, Dr Garrison.

First, you demonstrate with your hemodynamic measurements that the animals do develop an increased heart rate and mean blood pressure only at 24 hours or the established infection group. At 72 hours, or the chronic state, they did not demonstrate the hemodynamic measures consistent with a hypoperfusion or hypotensive state. You also did not report any data about the acid-base status of these animals. This data would help support that the animals were in a chronic or low-CO septic state. I am not convinced that this model represents chronic sepsis seen in clinical practice. Would you comment on these 2 points?

DISCUSSION

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sponse beds, and with the exception of the acute infusion of epinephrine or direct stimulation of the sympathetic system, those vessels probably do not contribute much to the change in peripheral vascular resistance that we see. The A1 vessels are the resting vessels; the A3 and A4 vessels are the reactive vessels that locally determine blood flow.

We used a topical application because that avoids the systemic effects of the drug. That is a powerful reason for using this technique where you can study local tissue reactivity without changing blood flow or pressure or whatever the perfusion is to that particular tissue. Admittedly, the vessel is exposed to ablumin, but in all of the other studies we have done, we have never seen a problem with exposure eventually to the vessel as the tissue preparation is very thin.

The local tissue is maintained in an environment-controlled tissue bath, so acidosis is not an issue. It may well be in a whole animal where you have the lactic acid or other acidic compounds accumulate and secondary pH changes, but the tissue in this local bath is controlled, and so you eliminate the effects of pH. That would be a different study where you would change temperature or pH as the study variable.

Finally, we have not done studies with adrenomedullin, but I suspect that that is an acute response, and therefore, the effect would be seen in the A1 and A2 vessels in the acute situation, and in the chronic situation the compound would not be of issue. I don’t know this for a fact, though.

Richard H. Turnage, MD, Dallas, Tex: Could you comment on this phenomenon in other vascular beds, in particular the splanchnic vascular bed? Have you made any of these observations in these vessels in that bed?

Dr Garrison: As you know, we have done considerable work in the intestine. We have in our laboratory an intestinal microvascular preparation, a kidney preparation, and a liver preparation, all of which we can look at microvessels. We have done a significant amount of work in the intestinal bed, and there is, similarly, a depressed dilator response in the intestines in a 2-hit model. We have not done anything in this chronic abscess model, which is the model in the current study reported, but in a 2-hit model where the initial physiologic insult is hemorrhagic shock followed by a bacteremic incident, you can demonstrate that in the small A3 vessels, which are the premucosal blood vessels that serve the blood flow to the mucosa, there is a depressed dilator response.

ARCHIVES OF INTERNAL MEDICINE

Are Therapeutic Decisions Supported by Evidence From Health Care Research?

Gaëtane Michaud, BS, BEd, MD; Jessie L. McGowan, BMus, MLIS; Richard van der Jagt, MD, FRCPC; George Wells, PhD; Peter Tugwell, MD, MSc, FRCPC

Background: One of the most common decisions physicians face is deciding which therapeutic intervention is the most appropriate for their patients. In recent years much emphasis has been placed on making clinical decisions that are based on evidence from the medical literature. Despite the emphasis on incorporation of evidence-based medicine into the undergraduate curriculum and postgraduate medical training programs, there has been controversy regarding the proportion of interventions that are supported by health care research.

Objective: To investigate the proportion of major therapeutic interventions at our institution that are justified by published evidence.

Methods: One hundred fifty charts from the internal medicine department were reviewed retrospectively. The main diagnosis, therapy provided, and patient profile were identified and a literature search using MEDLINE was performed. A standardized search strategy was developed with high sensitivity and specificity for identifying publication quality. The level of evidence to support each clinical decision was ranked according to a predetermined classification. In this system there were 6 distinct levels, which are explained in the study.

Results: Of the decisions studied, 20.9% could be supported by placebo-controlled randomized trials and 43.9% by head-to-head trials. Half of these were shown to be significantly superior to the treatment against which it was being compared. For 10 of the 150 clinical decisions, evidence was found demonstrating alternative therapies as being more effective than that selected.

Conclusions: Most primary therapeutic clinical decisions in 3 general medicine services are supported by evidence from randomized controlled trials. This should be reassuring to those who are concerned about the extent to which clinical medicine is based on empirical evidence. This finding has potential for quality assurance, as exemplified by the discovery that a literature search could have potentially improved these decisions in some cases. (1998;158:1665-1668)

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