VOLUME 6

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ADVANCES IN SHOCK RESEARCH

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VOLUME 6

Proceedings of the Third Annual Conference on Shock
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Part 2

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ADVANCES IN SHOCK RESEARCH

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Preface

Volume 6 of ADVANCES IN SHOCK RESEARCH is a collection of papers presented at the Third Annual Conference on Shock at Lake of the Ozarks, Missouri, June 11–13, 1980, and represents the state of the art in basic and clinical research in the areas of septic and endotoxic shock. The studies cover a gamut of septic shock models demonstrating the physiologically and biochemically induced effects of sepsis and endotoxemia.

The volume continues the tradition established during the First Annual Conference on Shock in 1978 of disseminating to the scientific and medical communities the latest advances in the study of the complex, multisystem disease of sepsis and endotoxemia. In the last analysis, the value of such conferences depends on how effectively new knowledge is reported and transmitted to medical researchers and clinicians. The publisher, editors, and participants hope that the reader will find this information useful in the development of new therapeutic and research applications.

William Schumer

SEPTIC SHOCK

Contents

ENDOTOXIC SHOCK

Advances in Shock Research 6:1-13 (1981)

Another Unacceptable Model of Primate Septic Shock

Clayton H. Shatney and Gordon Read

The lack of a primate model suitably reproducing clinical septic shock prompted an attempt to adapt a successful canine model to the Rhesus monkey. Animals were sedated with 2 mg/kg Sernylan. Local anethesia for vascular catheter insertion was produced with 1% lidocaine. After baseline hemodynamic and blood measurements, the abdomen was entered and the cystic artery and duct were ligated and divided. A suspension of E. coli (ATCC 25922) was injected into the gallbladder, and the abdomen was closed. Sequential monitoring was performed every 12–24 hours until the animals expired. The first monkey was given 10^8 E. coli/kg, the dose used in the canine model. This animal expired during the first 24 hours with ATCC 25922 E. coli septicemia. The same outcome was seen in the next monkey with 10^6 E. coli/kg. Neither monkey exhibited a hyperdynamic circulatory status. Autopsies revealed no intraabdominal abscess or peritonitis. Three animals were given 10^4 E. coli/kg. One died within 24 hours, one died after 13 days, and one was a permanent survivor. Two had bacteremia but no abscess. None had a hyperdynamic circulatory status. In three other animals the cystic artery and duct were ligated and divided, but no E. coli was given. Survival varied from 1½ to 2½ days. None had a hyperdynamic circulatory status. All had bacteremia. Autopies revealed no abscesses. In summary, the Rhesus monkey is exquisitely sensitive to endogenous and exogenous sources of bacteremia, which produce a hypodynamic circulatory status and death before peritonitis or intraabdominal abscess can develop. Thus, the ischemic, infected gallbladder preparation is an inadequate septic shock model in the Rhesus monkey.

The views or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

INTRODUCTION

During the past 20 years various aspects of septic shock and its complications have been extensively investigated. The discovery that the cell walls of gram negative bacteria contained a substance which could produce shock in experimental animals ultimately led to the development of a model of bacterial shock. As purified endotoxins became available, numerous reproducible models of endotoxin shock appeared. In most instances the dog was chosen as the experimental animal. Since the pathophysiology of canine endotoxin shock was similar to what was being observed in human septic shock, the terms endotoxin shock and septic shock became synonymous.

In 1967, however, studies by MacLean and associates [1] and by Siegel and colleagues [2] suggested that there were significant pathophysiologic differences between early human septic shock and canine endotoxin shock. Subsequent clinical studies by numerous investigators have borne out the original impressions of these two authors. As more was learned about human septic shock, questions were raised regarding the clinical applicability of findings in not only endotoxin shock but also in the dog. Specifically, it was suggested that endotoxin shock was not an adequate representation of the human situation, and, hence, it was wrong to extrapolate findings in this form of shock to the clinical environment. Furthermore, many investigators felt that the dog was not close enough to man on the phylogenetic scale to allow for the transfer of findings in a canine model to the human condition. As a consequence of these criticisms, numerous attempts have been made during the past decade to develop a model of septic shock which more closely mimics the pathophysiology seen in the clinical arena. In order to overcome the criticisms regarding the use of the dog in experimental shock, many investigators have attempted to create models of septic shock in various primate species. To date, however, a universally accepted model of primate septic shock has not been developed.

Recognizing the need for an experimental model of septic shock which closely approximates the clinical setting, we initially evaluated an ischemic, infected gallbladder preparation in the dog. As previously reported [3], the model entailed ligation and division of the cystic artery and duct, followed by the instillation of 10^8 E. coli per kg into the ischemic gallbladder. Subhepatic abscesses and/or peritonitis were observed in almost all animals. As in clinical septic shock, two distinct patterns of hemodynamic responses were observed. About 25% of the animals developed a normo- to hypodynamic circulatory status, and 75% responded with an increase in cardiovascular dynamics. The mor-

tality rate was 100% in the low-flow group, with a mean survival of two days. The high-flow subset experienced a mortality rate of 90%, with a mean survival of five days. The improved survival in the high-flow group agreed with clinical observations [4] in patients with a hyperdynamic response to septic shock. In addition to the systemic hemodynamic changes, the responses of the respiratory system and the heart were similar to those seen in human septic shock. In sum, the ischemic, infected gallbladder model of canine septic shock had many similarities to its human counterpart. Nevertheless, in order to overcome the potential drawback of the use of the dog in this experimental preparation, it was decided to apply the ischemic, infected gallbladder technique to a primate species. This report concerns our experiences with this model of septic shock in the Rhesus monkey.

MATERIALS AND METHODS

The studies were conducted in eight fasting Rhesus monkeys weighing between 4.2 and 8.6 kg (mean weight, 6.0 kg). There were six males and two females in the series. The animals were sedated with 2mg/kg of intramuscular Sernylan. All subsequent invasive procedures were performed under 1% lidocaine anesthesia. Following sedation the neck, abdomen, and groins were shaved, and the animals were transferred to the operating room. Under local anesthesia polyethylene catheters (PE 160) were introduced into the right femoral artery and vein and advanced to the abdominal aorta and right atrium, respectively. A Swan-Ganz catheter (5 French) was introduced into the right internal jugular vein and advanced to the pulmonary outflow tract. These catheters were used for pressure measurements and for arterial and venous blood sampling. The Swan-Ganz catheter was also used for determination of cardiac output using a Columbus Instruments thermodilution cardiac output meter (Model 72-8-SX).

Following placement of the vascular catheters baseline hemodynamic measurements were made of the following parameters: heart rate, mean blood pressure, mean pulmonary artery and wedge pressures, central venous pressure, and cardiac output. Baseline arterial and venous blood samples were obtained and analyzed for pH, PO_2, PCO_2, hemoglobin, oxygen saturation, oxygen content, and P_{50} using an Instrumentation Laboratories Co-oximeter (Model 182), a Corning Blood Gas Analyzer (Model 161), and an Instrumentation Laboratories tonometer (Model 137). In addition, arterial glucose and lactic acid concentrations, a coagulation profile, and white blood cell and platelet counts were

determined. All blood removed for analysis was replaced with twice the volume of normal saline. Throughout the procedure the vascular catheters were periodically flushed with 1–2 ml of heparinized saline solution (100 units per liter) to maintain patency. Only animals with normal baseline values were used in the study. All animals were allowed to spontaneously breathe room air throughout the experiment.

At the conclusion of the control monitoring period, a midline laparotomy was made under local anesthesia. The cystic artery and duct were identified, ligated, and transected. A suspension of E. coli (type ATCC 25922) in broth culture containing a specific number of organisms per kilogram of body weight was then injected into the gallbladder. The pressure of the gallbladder was brought to 20 cm of water by instillation of normal saline. Following the introduction of bacteria into the gallbladder lumen the abdomen was closed, and the animal was returned to the cage. The monkeys were fed a standard kennel diet and were allowed ad libitum activity. Hemodynamic and metabolic studies were repeated on a daily basis until the animals expired. The animals were sedated with Sernylan and the cannulas were reinserted under local anesthesia for the sequential studies.

RESULTS

The first monkey was given 10^8 E. coli per kg, the same dose used in the successful canine model. This animal expired during the first 24 hours. Autopsy revealed a gangrenous, but non-perforated, gallbladder. Blood cultures demonstrated type ATCC 25922 E. coli (Table I). The gallbladder lumen was also positive for E. coli.

Because the first animal expired so soon after the bacterial insult, the second monkey was given a dose of 10^6 E. coli per kg. This animal survived slightly under two days. Hemodynamic measurements performed 24 hours after the introduction of bacteria revealed no change in the blood pressure or heart rate. The pulmonary artery pressure and wedge pressure were elevated, and the cardiac output was reduced, compared to baseline measurements. Respiratory alkalosis was present, but there was no hypoxemia. Autopsy of this animal revealed a necrotic and perforated gallbladder with serous fluid in the subhepatic space. Blood cultures were positive for type ATCC 25922 E. coli. The fluid found in the subhepatic space was also positive for E. coli.

Since the second monkey died fairly soon after the introduction of the septic focus, the third animal was given 10^4 E. coli per kg. This monkey survived less than one day. At autopsy the gallbladder was gangrenous but not perforated. A blood culture was positive for Enterobacter. The gallbladder fossa was positive for type ATCC 25922 E.

coli. The short survival time of this animal, plus the finding of Enterobacter on blood cultures, suggested that this animal died from endogenous bacteremia, possibly due to the ligation of the cystic artery and duct. Accordingly, it was elected to subject the next three animals to sham shock.

Three monkeys underwent ligation and division of the cystic artery and duct, but only normal saline was injected into the lumen of the gallbladder. Pertinent hemodynamic and metabolic findings in these monkeys are presented in Figures 1 and 2. Survival and culture data are displayed in Table I. In addition to subjecting these animals to sham shock, samples of bile were removed from the gallbladder and cultured for bacteria prior to ligation of the cystic artery and duct. No growth was obtained in two cultures, but in one monkey Acinetobacter was cultured from the bile. The survival of these three monkeys varied from 1½ to 2½ days. None of the animals developed a hyperdynamic circulatory status. Progressive hypocarbia and venous hypoxemia were observed, but arterial hypoxemia did not occur. Lacticacidemia and slight hyperglycemia were noted, and the P_{50} shifted to the right. The total leukocyte count fell progressively, as did the platelet count. The fibrinogen level doubled during the course of sepsis. Blood cultures became positive for bacteria in all three monkeys (Table I). Two animals exhibited Enterobacter bacteremia. In two monkeys more than one organism was cultured from the blood. Acinetobacter, which was cultured from the bile of one monkey prior to cystic artey and duct ligation, was not isolated from the blood. All animals underwent postmortem examination. No intraabdominal abscesses or peritonitis was found in any of these monkeys.

Despite the demonstration that ligation of the cystic artery and duct produced lethal septic shock in the Rhesus monkey, it was elected to study two more animals with a dose of 10^4 E. coli per kg. The rationale

TABLE I. Survival of Septic Monkeys

Monkey no.	Dose E. coli/kg	Blood culture results	Survival (days)
R038	10^8	E. coli	1
P130	10^6	E. coli	< 2
R518	10^4	Enterobacter	< 1
R535	10^4	E. coli	13
F133	10^4	negative	permanent
R547	0	Enterobacter	2
R519	0	Citrobacter, Streptococcus	< 3
F134	0	Enterobacter, Streptococcus	< 2