Median lethal needle caliber in two models of experimental sepsis[1]

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ABSTRACT

PURPOSE: To estimate the median lethal needle caliber (LC50) of a new experimental sepsis model and compare it to the LC50 of the cecal ligation and puncture (CLP) sepsis model.

METHODS: Male albino Wistar rats were studied (n=22). Animals were allocated into two study groups. In Group I, experimental sepsis was induced by cecal ligation and puncture. In Group II, experimental sepsis was induced by ascending colon ligation and cecal puncture. Up-and-down method was used to determinate the LC50.

RESULTS: LC50 in Group I was 19 Gauge (Confidence Interval 17 to 22 Gauge). Determination of LC50 was not possible in Group II due to the death of all animals.

CONCLUSION: LC50 in cecal ligation and puncture is 19 Gauge. The lethality of the new model tested in this trial is very high.

Key words: Sepsis. Disease Models, Animal. Rats.

Introduction

Sepsis is a severe clinical syndrome characterized as a systemic inflammatory response to infection. Septic shock and multiple organ dysfunction syndrome are possible consequences[1]. Mortality rate due to sepsis is high and varies from 25 to 80%[2,3].

Sepsis is the consequence of a complex and multifactorial disarrangement of the host response to infection so the mechanism prepared to defeat the infection becomes the cause of severe tissue injury and dysfunction[4]. Pathophysiology of sepsis is still not completely understood. The most accepted theory is that sepsis is caused by hyperstimulation of the immune system[5,6]. An very complex treatment strategy is crucial to increase the chance of survival for septic patients[7]. Most recommendations, however, are not directed towards sepsis itself, but to secondary target-organs. Efficient and cost-effective new treatments for the handling of sepsis are required. These measures, however, must be developed and tested in animal models before they may be used in critically ill patients[8].

Experimental models of sepsis have been proposed by many authors due to the extreme variability and the difficulties in setting the stage of human sepsis. The aim of these models is to achieve reproducible studies about pathophysiology and the impact of new therapies[9-12]. Experimental models should replicate the rhythm and severity of human sepsis in the Intensive Care Unit; replicate hemodynamic and immunological stages; replicate histological features of target organs, and show variability between subjects[13].

Three general models are usually employed to produce experimental sepsis[14]:

a) Endotoxin injection;

b) Intravascular or intraperitoneal infusion of live bacteria;

c) Induction of fecal peritonitis.

The most utilized model in pre-clinical studies is cecal ligation and puncture because it reflects the complexity of human sepsis[15]. This model satisfies many criteria essential for an experimental model. It contains tissue injury, a necrotic tissue source, and a focal infection source that eventually promotes bacterial translocation, activation of the inflammatory response and septic shock[16]. Advantages of this model include its technical simplicity, polymicrobial origin after a focal infection, re-creation of human sepsis progression, prolonged and lower release of cytokines. Unfortunately, experimental models of sepsis do not perfectly represent the disease or its evolution in human beings[11]. Disadvantages of this method have been described as difficulty in standardization between laboratories; variable factors such as age, genre, and breed; technical discrepancies; and abscess formation[15].

Mortality in the cecal ligation and puncture model is influenced by needle caliber[17], number of punctures[18] and cecal ligation extension[19]. These factors are not always described in procedural details, although they are major determinants of variability in outcome.

A modification of the cecal ligation and puncture model was reported. It was used in other studies in our laboratory[20-22]. In this model, double perforation of the cecum occurs after the ascending colon is ligated.

The aim of this study was to estimate the median lethal needle caliber (LC50, lethal needle caliber in 50% of cases) of the new model and compare it to the LC50 of the traditional cecal ligation and puncture.

Methods

After approval by the local Ethical Committee of Animal Use (PP00729/2011), twenty two male albino Wistar rats, weighing between 300 and 450 grams, originating from the Central Animal Colony, Federal University of Santa Catarina, were used. In Group I, fifteen animals were tested. In Group II, seven animals were tested. Animals were kept in individual cages of 60x40x16 centimeters for a period of 12 days to adapt to the laboratory environment. Animals were maintained under controlled light conditions (12 hours cycles), at controlled temperature (20°C), and receiving proper food with ad libitum access to water.

Procedures

All animals were anesthetized with 20 mg of intramuscular Tiletamine + Zolazepam (Zoletil®). Repeated doses were used if necessary. It was considered anesthetized when there was loss of toe pinch response. Animals were immobilized in dorsal decubitus and anterior abdominal wall was shaved and disinfected with iodopovidone. A midline laparotomy was performed. The cecum was identified and exposed in a non-traumatically manner. Animals were randomly distributed into two study groups. In animals allocated to Group I, the cecum was ligated with 2-0 silk at half the distance between the distal pole and the base of the cecum. In animals allocated to Group II, the ascending colon was ligated with 2-0 silk one centimeter above the ileocecal valve. In both groups, the cecum was punctured twice (Figure 1). The cecum was then gently compressed to extrude a small amount of cecal content

and the bowel was replaced in the peritoneal cavity. Abdomen was closed in two layers using 3-0 nylon running sutures. After surgery, fluid resuscitation was administered (5 ml/100g of 0.9% sodium chloride subcutaneously) and animals were returned to cage and observed throughout a period of seven days.

Statistical analysis

The up-and-down method was used to estimate the median lethal needle caliber[23]. This method was proposed by Massey and Dixon, and it was chosen because it requires fewer individuals to determine the Median Lethal Dose (LD50) than traditional methods in pharmacological studies[24,25]. It method consists of a series of sequential essays. The initial caliber used to puncture the cecum in both groups was 22G, which is the gauge caliber reported to be the LC50 in traditional cecal ligation and puncture[26]. If the first specimen survived for a week, the second specimen was tested with a larger needle. If the specimen died in the observation period, the needle used in the next animal was thinner. If an animal was moribund in the observation period, it was submitted to euthanasia through anesthesia overdose and outcome was recorded as death, for ethical reasons. Needle caliber was increased or decreased preferentially according to the even degrees of the Standard Wire Gauge scale.

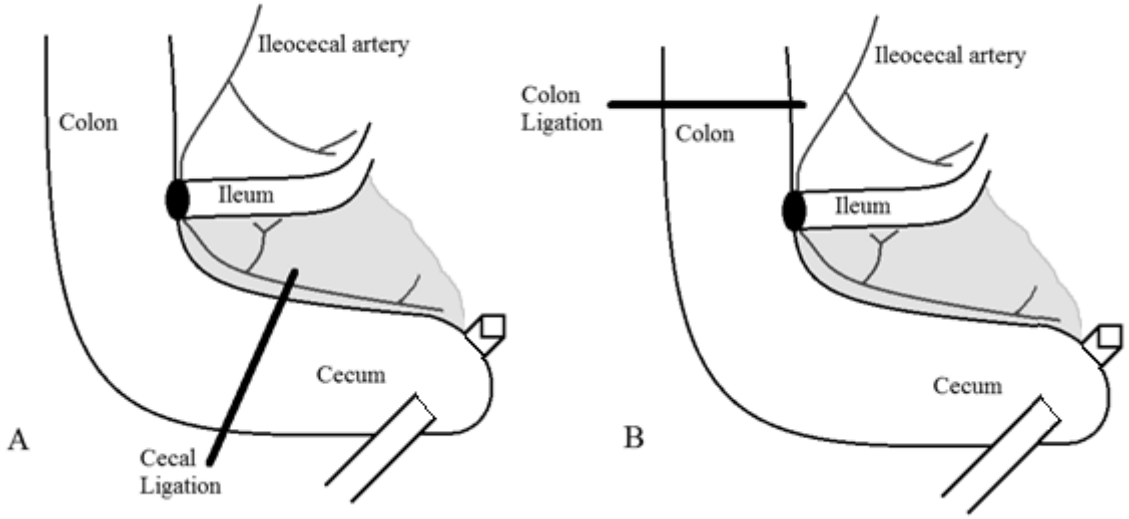

FIGURE 1 - Diagram of both models of experimental sepsis. In the traditional model, cecal ligation was performed (**A**). In the modified model, ascending colon ligation was performed (**B**). In both models, the cecum was punctured.

Tests were interrupted if there were five reversions in six consecutive animals; or if there were three consecutive animals who survived or died after testing the superior or inferior limit of needles (14G and 29G respectively); or a maximal 15 animals in each group was accepted. Data were tested to normal distribution using the Shapiro-Wilk test. SPSS v 17.0 was used to perform statistical analysis.

Results

Needle calibers used to puncture the cecum in both groups are shown in Figure 2. In Group I, LC50 was estimated to be 19G (Confidence Interval 5% 17 to 22). In Group II, estimation of LC50 was not possible. The test was interrupted due to death of three consecutive animals whose cecum was punctured with the thinnest available needle (29G).

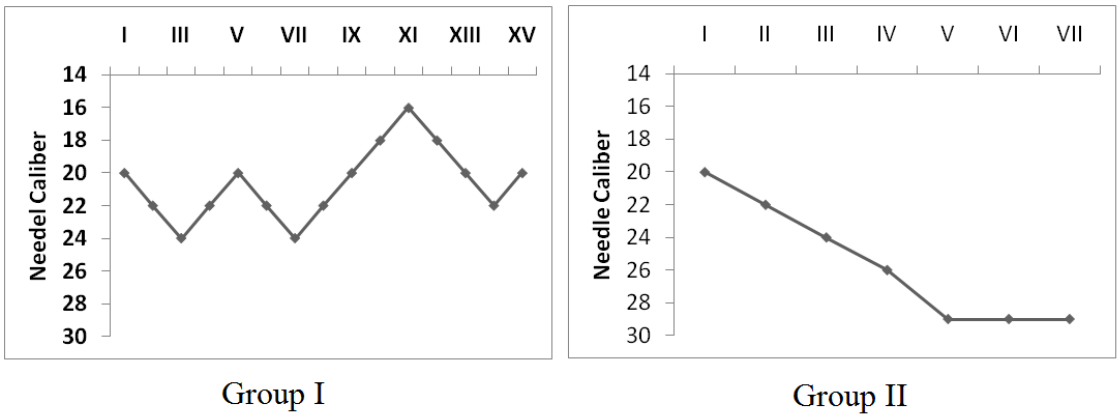

FIGURE 2 - Needle calibers used to puncture the cecum in two models of experimental sepsis. In Group I, the cecum was ligated. In Group II, the ascending colon was ligated.

Discussion

Experimental models for the study of sepsis have been proposed since the end of the nineteenth century. At that time, it was believed that bacteria causing peritonitis migrated from the abdomen into systemic circulation by the thoracic duct. In 1898, Noetzel injected *Streptococci* originating form a sick cow in the peritoneal cavity of rabbits and guinea pigs. The mortality rate of animals with thoracic duct ligation was similar to the control group, but he showed that abdominal toilet increased survival rate. He also detected live bacteria in blood ten minutes after peritoneal injection[27].

In 1922, Constain also studied the effect of thoracic duct ligation on the peritoneal sepsis evolution in dogs. He ligated the appendix and mesoappendix with chromic catgut and returned it to the abdomen. Necropsy showed the appendix had become gangrenous, and before sufficient walling off could take place, had ruptured into the peritoneal cavity. There was a diffuse septic peritonitis with abundant bloody exudate.

Despite reports of experimental models of sepsis including the puncture of the ligated cecum in the twenties[28], models of injection of intraperitoneal bacteria and cecal ischemia were the most popular until 1980, when Wichterman standardized the cecal ligation and puncture method[9]. The advantage of puncturing the cecum is to create a more predictable onset than waiting for spontaneous rupture of the residual cecum[14].

Appendicitis was a cause of great concern before the development of the surgical treatment of peritonitis. Mortality consequential to appendicitis at the end of the nineteenth century was almost 100%[29]. Nowadays death after appendicitis occurs in 0.07 to 2.4 per thousand surgeries due to the advance of surgery-anesthesia-asepsis[30].

Cecal ligation and puncture is a model of an inflammatory acute abdomen (caused by appendicitis or diverticulitis). In the new model, there is simulation of an obstructive acute abdomen complicated by intestinal perforation. Mortality in this scenario is significantly higher, even today. Recent reports have demonstrated a mortality rate of 5.9 and 6.9%, despite endoscopic-surgical or surgical treatment respectively[31].

It is reasonable to assume that intestinal lumen obstruction is responsible for the death of animals allocated to Group II. Pathophysiology of intestinal obstruction is well known. Air and liquid swallowed accumulate onto the obstruction, predisposing it to bacterial multiplication. Gas production is increased, which worsens the case and predisposes to systemic complications by bacterial translocation. If the process is not resolved, there is loss of absorptive capacity of the bowels and fluid shift to intestinal lumen. Such combination leads to electrolytic disturbances and state of shock. There is also loss of blood supply favoring necrosis and perforation[32]. Since 1968, Clowes argued in favor of digestive tract permeability maintenance as essential to the development of sepsis models in the dog[33].

In rats, Wichterman found 90% lethality when an 18G needle was used to perform a double puncture of the cecum and 77% when a 22G needle was used. He also reported no mortality if the specimen survived until the fifth post-operative day[9]. Another study reported a 7-day mortality of 50% when an 18G needle was used[34]. There is also variation in mice. Reports of LC50 in mice vary from 21 to 18G[26,35]. Cecal ligation and puncture may be considered a reproducible method between laboratories despite this variation.[15]

Animal models that lead to significant mortality within the first six to 12 hours may not describe an outcome that is relevant to human sepsis[36]. There must be time enough before death to

allow sepsis features to be studied. In addition, it is expected a reasonable survival rate. Injuries that are too severe may prevent the appearance of sepsis signs or hinder an efficient therapeutic measure to increase survival rate of milder injuries. The lethality of the new model tested in this trial is very high.

Future studies will define the role of the model "Intestinal Obstruction and Perforation" in the research about physiopathology and treatment of acute sepsis and intestinal obstruction.

Conclusions

Median lethal needle caliber in cecal ligation and puncture is 19 Gauge. The lethality of the new model tested in this trial is very high and its median lethal needle caliber could not be determined.

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