ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Intestinal Sepsis Model of Small Animals

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Abstract

Objective: The goal of this paper was to develop a model of intestinal sepsis in the investigational animal. **Materials and Methods:** Sprague-Dawley male rats of 4 weeks (n = 42) were used. Initially, a pilot study (n = 12) was performed and distributed in groups with 0.8 cc inoculum of *Escherichia coli* ATCC intraperitoneally administered at concentrations of 9, 7, and 6. Subsequently, concentrations of 9 CFU are used in two groups of rats with reductions of 11 cc. Finally, a randomized trial of 28 rats was initiated in three treatment groups after intraperitoneal infection. Biological models of blood and peritoneal fluid were observed, and histopathological study of intraperitoneal tissues was done. **Results:** Demise of 95% of the rats infected with *E. coli* UFC concentration was observed. The blood culture and peritoneal fluid culture was positive for the same strain in all of them. The formation of blisters on the liver surface and polymorphonuclear infiltration in organs was detected. **Conclusion:** The fatal dose of *E. coli* should be diluted for intraperitoneal injection.

Keywords: Animal, Model, Rat, Sepsis

Asian Pac. J. Health Sci., (2020); DOI: 10.21276/apjhs.2020.7.3.11

Introduction

Throughout history, experimental animals have been used to create different models that help in igniting the causes, diagnosis, and treatment of diseases that affect humans and animals. They have also served as a contribution to biological teaching, development, production, and control of medicines and food.[1]

In the context of sepsis, there are a great number of animal models that try to replicate the physiopathology of human sepsis. However, we find insignificant differences between the two species and the development of septic process does not normally reproduce the conditions of human sepsis. First of all, sepsis in humans is a pathology of gradual and in animals is an insidious presentation. Sepsis is usually much more acute.[2] Second, experimental intervention happens in the early stages of sepsis, when the levels of inflammatory cytokines are elevated and organic and vascular damage is minimal, difference from the human being, where therapeutic intervention usually occurs when the cytokine response pro-inflammatory is changing to anti-inflammatory and the organic damage is already apparent. Third, it is common for the susceptible human population which is at the extremes of life (children and older adults), instead the animals that used for experimentation are young adults with no other comorbidities. Fourth, animals in most models do not receive full support treatment that includes mechanical ventilation, fluid therapy, non-toxic drugs tropics, antibiotherapy, enteral or parenteral nutritional support, and renal replacement therapy. Finally, the elapsed timed from the onset of symptoms to organ failure is much shorter in animal models, in which the process is completed in a few days. In humans, corresponding time for curing translates to weeks and organic failure is observed usually in weeks. All these severely affect the evaluation of drug therapists sepsis.[3] And hence, there is no suitable model, each model has advantages and disadvantages depending on the parameter to be studied and the experimental animation employee. One of the most widely used animal experimentations modelized is created from injection, both local and systemic live bacteria, often *Escherichia coli* or of bacterial products or endotoxins such as LPL.[4] The use of endotoxins presents a large amount of limitations in rodents, the main one being the high dose needed to produce a state of shock, which is between 12 and 90 times higher than in humans. On the other hand, there are clinical differences that endotoxin induces in rodents and in humans.[5,6] Other models are generated from the manipulation of the intestine and discharge of fecal content into the peritoneal cavity generating a polymicrobial peritonitis and as a consequence a systemic inflammatory response occurs. The most widely used procedure to generate model of rodent peritonitis is ligation and puncture. This model is suitable for evaluating therapies that act on changing pathophysiological bios produced during the septic process. The frequently used approach has been considered the "gold standard" technique.[7,8] Given the few reproducible sepsis models in the literature, we aim to develop and standardize a model of abdominal sepsis in an experimental animal by inoculation of *E. coli* with a single puncture in the peritoneal cavity.

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How to cite this article: Reddy RB, Rao S. Intestinal Sepsis Model of Small Animals. Asian Pac. J. Health Sci., 2020; 7(3):44-46

Source of support: Nil

Conflicts of interest: None

Received: 15/05/2020 **Revised:** 20/06/2020 **Accepted:** 07/07/2020

Materials and Methods

Experimental Animals and Conditions

An experimental study (trial therapeutic) of intra-abdominal infection where the Sprague Dawley® rat is experimental animal, Harlan Laboratories Models SL, male, 4 weeks and 90–110 gm. After adaptation week and before experimentation, all animals reach weights between 245 and 275 g. Experiment is carried out at the same time, to avoid possible inflow of the circadian cycle in the results of the work. All the animals were healthy and did not receive prior treatment. Animals are kept on food and water *ad libitum*, with a ½ day cycle of light and ½ day cycle of darkness,

and ambient temperature of 21 ± 3°C with relative humidity of the air of 55–74% and with 16–21 renovations/hour without recur air flow. They are housed in accordance with RD 52/2012 and in group to promote group behavioral skills. They also stay a week in these environmental conditions, to allow its adaptation, before the start of the study. A permanent marking is made on the tail of rats, to be able to do individual monitoring of the welfare state of each one of them. The localization of animals and their housing is carried out in the animal cited Translational Research Unit. All experimental procedures are performed in according to the guidelines of the European regulations for the protection of experimental animals. The study has been approved by the Ethical Committee for Animal. Conventional and biological waste is removed and disposed of regularly, safely, and in accordance with the institutional protocols.

Animal Working Model

Determination of lethal dose (pilot study)

Initially, a pilot study is carried out with $n = 12$, distributing them in groups with .9 ml inoculum of *E. coli* ATCC intraperitoneal in concentrations of 9, 7, and 6 CFU. In a second study (n = 8) with distribution in two groups, 1 ml of *E. coli* 11 UCF are used, being diluted in 12 and 16 ml of distilled water for vaccination. For the creation of the peritonitis model, an intraperitoneal section in rats with weights between 270 and 280 g, after anesthesia with ketamine.

The preparation of the inoculum is carried out in distilled water with a suspension of *E. coli* ATCC at different concentrations of colony-forming units per milliliter. Initially, the inoculum concentration is compared on a spectrophotometer and check with a spectrometer DADE. Starting from this suspension, many tubes are prepared for rats to undergo vaccination, adding to 0.9 ml of the suspension to account for the milliliters of distilled water to be tested. It is checked that the bacterial concentration adjusts to what is expected using serial dilutions. After extension, control and reading are performed at 19–23 at 36°C.

The time elapsed between the preparation of the inoculation and immunization is always <2 h in all cases under the study.

Therapeutic study

Once the lethal dose has been found, randomization is performed in three groups of animals: Group I (with physiological serum), Group II (with ceftriaxone), and Group III (with ceftriaxone plus allicin). Inclusion of nine rats in group without (knowledge of the lethal dose and application of the principle of the three Rs.) and 12 rats in Groups II and III. For the calculation of sample size, studies of contemporary models are taken into account.[4,7,8] and the standards on which the principles are based and ethics to minimize the use of animals in research: The three "Rs:" Reduce, Replace, and Refine. A model of peritonitis is generated in all groups. Rats from different groups are never housed in the same shelf. During the test, when the animal dies within the first 30 h, microbiological culture of liquid peritoneal and samples are taken from the liver, kidney, intestine, and peritoneum for histopathological evaluation. With the rest of the animals are expected on the 7th day of treatment. To observe the effects, MR scans on a low intensity 0.35T system are taken to account for better soft-tissue contrast of MRI scans compared to CT scans.[9] The parameters evaluated are congestion liver, polymorphonuclear (PMN) in liver sinusoids, liver and peritoneal surface PMN, and colonization of bacteria on the surface of the liver and peritoneum.

Results

Pilot Study

Twelve rats are used to conduct the study gave a pilot (four for each of the indicated concentrations). With these concentrations and dilution, it is observed that all rats survive after intraperitoneal injection despite the absence of antibiotic treatment. In this study, there is a possibility of increasing the dilution volume to 11 ml using the highest concentration of 9 CFU/ml. Of the analyzed cases, only one of them dies before 24 h. Finally, it is established that the optimal volume to produce 95% of deaths in the first 5 h of the animal is 17.

Therapeutic Trial

Subsequently, we determined the optimal concentration and dilution to cause peritonitis effective cases in the pilot study the therapeutic trial is performed with the dilution of 14 ml of distilled water in Group I (control), all rats die within 5–7 h. In all of them samples of peritoneal fluid and blood verified that *E. coli* strain is found with identical antibiotic sensitivity to that used for inoculation. In Groups II and III at the same time as inoculation occurs, are treated with an antibiotic. It is observed that all survive (24 rats) except one, in which *E. coli* strain is verified in the analysis of peritoneal fluid with similar sensitivity in antibiogram. When the animal dies within the first 24 h, peritoneal fluid culture is performed. In all observed cases, the growth of *E. coli* with identical anti-sensitivity biotic to that used in inoculation. In the histopathological evaluation, formation of swellings on the surface of the liver, large infiltration of PMN cells, and bacterial colonies happens. We can also see PMN and abundant colonies of *E. coli* on the peritoneal surface.

Discussion

Some authors stress the importance of achieving a standardized model in which the septic process is induced in an easy and reproducible way. Same authors created model of peritonitis from fecal material of origin human. Feces are collected and processed as per a protocol and are injected intraperitoneally into animals.[10] Another study of the models tested the placement of a stent in the ascending colon wall (colon ascenders stent peritonitis- CASP). This solves one of the problems that can occur with CLP, which is the formation of an intra-abdominal swelling instead of septic shock from peritonitis. CASP is a relatively new and rare case of diffuse peritonitis polymicrobial that reproduces the symptoms of acute peritonitis,[11] in addition to altering the blood flow with necro-secondary effects on the intestinal wall. The main disadvantage of CASP compared to CLP is its complexity. The model developed in this study starts from the reference in the literature to generate monomicrobial peritonitis with *E. coli*. Sanchez *et al*.[11] described lethal doses in healthy rats, with cirrhosis with and without ascites, applying *E. coli* to different concentrations and dilutions in healthy rats between

192 and 225 g. They noted short-term mortality in <48 h with a .9 ml inoculum of *E. coli* with 10 and 10 CFU diluted in 18 ml of sterile water (81% and 98% *E. coli,* respectively) significantly increased mortality. However, they note that same doses in healthy rats with weights of 470–510 g do not present mortality. This leads them to conclude that mortality may be directly related with the volume injected into the peritoneal cavity.[12,13] Authors describe how rats with crisis without ascites show that short-term mortality depends on day of administered *E. coli* concentration. When concentrations are low in *E. coli* (0.9 CFU/ml), only one rat dies (1/16). However, when inoculating 0.9 CFU/ml of *E. coli,* it was observed that the mortality increases, but significantly less compared to that produced with 0.9 CFU/ml.[14] This model agrees with what was found by other authors[13] and is similar to that described in the model that we present. In our model, 0.9 ml of *E. coli* with 9, 7, and 6 CFU, based on the concentration, high tractions independent of volume were enough to produce sepsis without any mortality. In second instance, a concentration of 9 CFU is inoculated, adding a dilution volume of 12 and 16 ml of sterile water to corroborate that both concentration and volume are determinants in the development of sepsis. However, with these premises, we find a mortality of 37% in the cases received 8 ml, while those who receive 16 ml die 100% in <5 h. This coincides with what is described in literature, although in our case, the administered volume is slightly lower than that used in other works.[12] Finally, when carrying out the therapeutic trial, it is confirmed that 100% of control rats perish within the first flush 5 h by inoculation of *E. coli* with 9 CFU diluted in 16 ml. This monobacterial experimental model presents certain advantages and disadvantages. On the one hand, knowing the germ, the strain and the amount of the inoculum that we administer can be very useful in the study of pathophysiological changes of sepsis, since we are able to control the conditions under which it is produced. Some authors[14] affirm that this model in rodents is capable of reproducing various characteristic changes of human sepsis, but its clinical relevance is limited by the fact that high concentrations of the bacteria in a model with a host unable to locate the infection, unlike what has been happening in humans.[15] In addition, most models infect healthy rats without comorbidities unlike humans where the most vulnerable populations are the extremes of life with comorbidities and where the treatment of sepsis is multidisciplinary including nutrition strategies, ventilation separatory, and hemodynamic support. So far, they have described many models of sepsis in animal experimentation, but none can fully reproduce sepsis in human hands since it is a heterogeneous, dependent on process of the genetic susceptibility of each individual and is accompanied by the significant comorbidities and drug consumption.[14] Research lines are currently focused on the use of "humanized" mice. These are mice that are transplanted with human hematopoietic stem cells they carry out the inflammatory response.[16,17] The main disadvantage is to obtain these mice the procedure it is slow and expensive. Another way to improve models would be to get older mice with/without comorbidity associated data.

The model described here establishes a lethal dose of *E. coli*10 10 CFU diluted in 16 ml sterile water. After an injection, intraperitoneal generates an effective, controlled, and easy infection reproducible set that could serve as the basis portion of future lines of research.

Conclusion

Most models infect healthy rats without comorbidities unlike humans where the most vulnerable populations are the extremes of life with comorbidities and where the treatment of sepsis is multidisciplinary including nutrition strategies, ventilation separatory, and hemodynamic support. So far, they have described many models of sepsis in animal experimentation, but none can fully reproduce sepsis in human hands since it is a heterogeneous, dependent on process of the genetic susceptibility of each individual and is accompanied by the significant comorbidities and drug consumption.

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