

## THE USE OF A NEW MODEL FOR THE INVESTIGATION OF SEPSIS

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*The purpose of the investigation was to induce the clinical form of sepsis in rats by inoculation of pure cultures of certain species of microorganisms into the ligated caecum. The experiments were performed on 104 Wistar strain male rats of 190-240 g body weight. The rats were divided into four groups; three of which contained 28 animals each and one control group of 20 animals. In order to monitor the development of sepsis, rats were killed at: 12, 24, 72 and 120 h after the surgical intervention.*

*Clinically apparent sepsis in two groups of rats was induced in the following way: the previously emptied, tied off and washed out caecum was inoculated with pure cultures of *Escherichia coli* or *Staphylococcus aureus*. In the third group, sepsis was produced by the caecal content of the tied off and punctured caecum (a mixed culture of microorganisms). A false surgical intervention was performed in the control group by opening the abdomen.*

*Clinically manifest symptoms of sepsis, such as higher body temperature, diarrhoea, anorexia, tachycardia, tachypnoea, changes in appearance and behaviour, were observed in all the rats, from 24 to 120 h after surgery. Bacteriological findings in the blood and parenchymal organs of the investigated rats, in the model of sepsis induced by the gram-negative bacterium *E.coli*, showed the presence of *E.coli* at all times from 12-120 h after inoculation. In the model of sepsis caused by *Staphylococcus aureus* this gram-positive bacterium was detected in the blood and tissue samples from 12 to 72 h after inoculation. In the model of sepsis induced by mixed bacterial cultures (*E.coli*, *Proteus mirabilis*, *Enterococcus* spp.) only *E.coli* was detected in the blood and parenchymal organ samples at all the monitored times from 12 to 120 h, whereas *P.mirabilis* and *Enterococcus* spp. were detected only during the first 24 h of the experiment.*

*Key words: E.coli, rat, sepsis, Staph. aureus.*

## INTRODUCTION

As result of a systemic inflammatory response triggered by infection, sepsis represents a serious and topical problem both in everyday clinical work and in research laboratories (Bone, 1996; Rixen *et al.*, 1996; Sibbald & Marshall, 1991; Talan, 1993). Numerous endogenous and exogenous factors, including endotoxins of both gram-negative and gram-positive bacteria, are the basic elements that trigger the pathophysiological mechanisms of sepsis (Bone, 1991; Parrillo, 1993). Sepsis, in affected animals, requires suitable medical treatment to bring about changes in the pathophysiological processes, although it is difficult to discern what is going on at the level of tissue metabolism. Therefore, experimental studies on laboratory animals are of utmost importance for investigation of the pathogenesis and therapy of sepsis (Piper *et al.*, 1996).

A good model of sepsis should contain the following: a) the blood culture must be positive throughout the experiment; whereas organ cultures should contain some of the causal agents identified during the infectious stage; b) animals should exhibit clinical signs of sepsis (tachycardia, tachypnoea, hypo- or hyperthermia, unawareness of the appearance etc); c) the model should be reproducible, inexpensive and easy to prepare; d) the duration of septic insults should be long enough to secure an appropriate response on the part of the animal; e) the model must be obvious, with a possibility of determining biochemical parameters in the sera (Wichterman *et al.*, 1980).

Sepsis and septic conditions in experimental animals are brought about by an infusion of live microorganisms, an inoculation of faecal content or "pure" bacteria into the abdominal cavity, by intramuscular abscesses, endotoxin administration, tying off and puncture of a part of the caecum just below the ileocaecal valve (Fink and Heard, 1990). Among the mentioned models, tying off of the caecum with puncture is the most appropriate one, being reproducible, allowing sepsis to develop gradually, and being economical due to its standardization on rats. The disadvantages of this model lie in the fact that sepsis is caused by a mixed culture of microorganisms, and by agents already present in the caecum on which the person performing the experiment can have no influence whatsoever. It may be presumed, this being the working hypothesis of our experiment, that the problem can be overcome if sepsis could be produced by inoculation of "pure" cultures of microorganisms, inside the ligated caecum, from which the content had previously been squeezed out.

## MATERIALS AND METHODS

The experiments were conducted on 104 male white Wistar strain rats, of  $215 \pm 25$  g body weight. Reference strain *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 5923) were used, as well as a prepared culture medium (0.5 ml 1% agar and 1 ml of saline solution) into which two similar colonies were transferred by streak-plate technique from a nutrient culture medium inoculated with *E.coli* while one colony was transferred from a nutrient culture medium inoculated with *S.aureus*.

The model of sepsis with caecal ligation and puncture (CLP) was reproduced using the explained technique with improvements made by

Wichterman (1980). The surgical intervention was performed on rats anaesthetized with thiopentobarbitol (50 mg/kg) intraperitoneally. The abdominal incision was made along the median line, 2cm below the level of the caecum, and two punctures were made in the caecum with needles of diameter 18.

The rats were divided into four groups, three of which contained 28 animals each, and one control (K) with 20 animals. All four groups underwent the surgical intervention of opening the abdomen, while the caecum was tied off only in rats from the three experimental groups. Namely, in the control group K only an abdominal incision was made (false surgery). In the second group (MK) the caecum was pulled through the incision, and then, by pushing the faecal content, filled and tied off tightly with nylon (3,0) 1/3 lower than the ileocecal valve with preserved gastrointestinal tract continuity. Two punctures (openings) were made with sterile needles on the side of the caecum opposite to the mesenterium. After the ligature and puncture, the caecum was pushed back into the peritoneal cavity, and the incision closed at two levels (muscles and skin). In the third group (EC) the caecum was emptied by pushing out the faeces, and then tied off and washed out with physiological saline (the first puncture) and filled with the prepared inoculum (1ml) containing pure cultures of gram-negative bacteria *E. coli*. ( $10^9$ ) (the second puncture). In the fourth group of animals (SA) same procedure was followed but the second puncture contained an inoculum (1 ml) of gram-positive bacteria *Staphylococcus aureus* (SA) at ( $10^9$ ).

Microorganisms in the blood and parenchymal organs (liver, spleen, lungs, kidneys) were isolated and identified using standard microbiological methods in addition to the API 20E system for the identification of enterobacteria and the API *Staph.* system for the identification of *Staphylococcae*. Sepsis development was monitored by sacrificing the rats at 12, 24, 72 and 120 h after surgical intervention.

## RESULTS AND DISCUSSION

The control, sham operated animals (K), were clinically healthy throughout the experiment. The inoculation of pure cultures of bacteria into the emptied and tied off caecum very rapidly brought about changes in the general condition of the experimental animals. In addition to an increase in body temperature and anorexia, the general clinical findings, included persistent diarrhoea, dry and inelastic skin, somnolence, rapid respiration and abnormally rapid heart rate, inflamed conjunctivae, particularly during the period from 24 and 120 h. These findings are generally in agreement with data in the literature, and the observed changes can be ascribed to toxic effects of microorganisms and sepsis mediators (Schletter et al., 1995; Shapiro and Gelfand, 1993). A central role has been ascribed to tumour necrosis factor (TNF) and interleukin-1 (IL-1) (Beutler, 1993; Remick and Kunkel, 1993). The drop in body temperature in our experimental animals, observed during the first 6 hours after surgical intervention, can be attributed to the known microcirculatory changes, resulting from anaesthesia. A significant increase in the body temperature in septic rats was, more or less, noticeable throughout the experimental period, particularly at 24 h (Figure 1). Our findings are consistent with the results of other authors, obtained both in clinical practice (Casey et al., 1993), and in various models of sepsis in animals (Lang et al., 1987; Waymack et al., 1990). Namely, it is considered that fever is mediated by

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## UPOTREBA NOVOG MODELA ZA IZUČVANJE SEPSE

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### SADRŽAJ

Cilj istraživanja je bio da se, inokulacijom čiste kulture jedne vrste mikroorganizama u ligirani cekum, izazove klinička forma sepsa kod pacova. Ogledi su izvedeni kod 104 mužjaka pacova, Wistar soja, telesne mase od 190 do 240 grama. Pacovi su podeljeni u četiri grupe od kojih tri sa po 28 životinja, i jedna kontrolna od 20 životinja. U cilju praćenja razvoja sepsa termini posmatranja i žrtvovanja životinja su bili: 12, 24, 72 i 120 časova posle hirurške intervencije.

Klinički vidljiva sepsa u dve grupe pacova izazvana je tako što su u prethodno ispražnjen, podvezan i ispran cekum inokulisane čiste kulture bakterija *Escherichia coli* i *Staphylococcus aureus*. Kod treće grupe životinja sepsa je izazvana cecalnim sadržajem podvezanog i punktiranog cekuma (mešana kultura mikroorganizama). Grupa kontrolnih životinja je lažno operisana otvaranjem abdomena.

Klinički manifestni znaci sepsa kao što su povišena telesna temperatura, dijareja, anoreksija, promene u izgledu i ponašanju pacova, registrovani su kod svih modela sepsa počev od 24 do 120 časova eksperimenta. Bakteriološkim pregledom krvi i parenhimatoznih organa ispitivanih pacova kod modela sepsa izazvane Gram-negativnom bakterijom *E. coli*, ustanovljeno je prisustvo *E. coli* u svim terminima od 12 do 120 časova eksperimenta. Prisustvo *Staphylococcus aureus*-a kod modela sepsa izazvane ovom Gram-pozitivnom bakterijom ustanovljeno je u uzorcima krvi i parenhimatoznih organa od 12. do 72. časa eksperimenta. Kod modela sepsa izazvane mešanim kulturama bakterija (*E. coli*, *P. mirabilis*, *Enterococcus*) samo je *E. coli* ustanovljena u uzorcima krvi i parenhimatoznih organa u svim ispitivanim terminima od 12 do 120 časova, dok je prisustvo *P. mirabilis*-a i *Enterococcus*-a ustanovljeno samo u prvih 24 časa eksperimenta.