Acta Veterinaria (Beograd), Vol. 62, No. 5-6, 543-551, 2012.

DOI: 10.2298/AVB1206543S UDK 599.1.3./8.4.:579.24:616.94

BIOCHEMICAL PARAMETERS IN RATS WITH AN APPLYED MODEL OF SEPSIS (CECAL LIGATION AND PUNCTURE) WITH PURE AND MIXED BACTERIAL CULTURE

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(Received 29th February 2012)

The goal of this work was to induce clinical sepsis in rats in order to measure the following biochemical parameters (glucoses, triglycerides, cholesterol, total proteins, albumins and creatinine). The experiments were done on 104 male Wistar rats, body weight 190 to 240 g. The rats were divided in four groups. Treated groups consisted of 28 animals while in the control groups there were 20 animals. Animals were observed and sacrificed at 12, 24, 72 and 120 hours after surgery.

In this model of sepsis (cecal ligation and puncture – CLP) with pure and mixed bacterial cultures, significant changes were noticed in all biochemical parameters. Significant hypoglycemia, hypercholesterolemia, decreased concentration of urea and increased concentration of creatinine were found in the first half of the experiment in all groups of rats with sepsis (at 12 and 24 hours). Hyperproteinemia and hypotriglyceridemia reached statistically significant values at 24 h in the groups of rats in which sepsis was induced with pure bacteria culture. In the other half of the experiment significant hypoalbuminemia was found in all rats with sepsis (at 71 and 120 h).

Key words: biochemical parameters, Escherichia coli, rats, sepsis, Staphylococcus aureus

INTRODUCTION

Sepsis is by definition a state of metabolic shock with prominent changes in concentration of cytokines, hormones and other biochemical parameters in the blood. Pathogenic mechanisms of the metabolic changes are still unknown. Some authors emphasize the role of catabolic hormones i.e. chatecholamines, corticosteroids, glucagon and growth hormones (Desborough, 2000; Berghe 2002; Beishuizen and Thijs, 2003; Annane *et al*., 2004; Minneci *et al*., 2004), others explain the disproportion between the process of moderate anabolism and increased hyper catabolism by increasing the blood concentrations of catabolic

mediators such as tumor necrosis factor (TNF) and interleukin -1 IL-1 (Bone, 1996; Opal and DePalo, 2000; Cavaillon, 2003; Das, 2003; Johnston and Webster, 2009). The fate of the released mediators could be two-way. They could be inactivated or preserve their activity in circulating endothelial cells, providing at the same time, the conditions for hypotension and septic shock (Parrillo, 1993; Webb, 2002).

Although some pathogenic events during sepsis are well known, the prognosis is still uncertain (Vincent and Abraham, 2006). Even though terms like: infection, bacteriemia, septicemia, septic syndrome, septic shock, are often used as synonymous (Rixen *et al*., 1996; Levy *et al*., 2003), the terminology that is not precise, causes uncertainty when comparing clinical trials. It causes also uncertainty during selection of certain therapeutic procedures that are not adequate and are risky (Sibbald and Marshall, 1991; Eichacker *et al*., 2002). In addition, the term septic syndrome, creates further confusion in terms of the infectious state that is not induced by microorganisms and in terms of inflammation, as well.

Sepsis requires medical treatment that needs to change the occouring pathophysyological processes. During treatment of sepsis it is not understood what actually happens during tissue metabolism. Keeping this in mind including the fact that we do not know much about some phases in sepsis, for instance how microorganisms and other insults induce the release of cytokines and secondary mediators (Lin *et al*., 2000; Cohen, 2002), it is now clear that informations about the systemic inflammatory response can be found in studies related to animal models of sepsis (Fink and Heard, 1990; Deitch, 1998).

MATERIALS AND METHODS

The experiment was carried out on 104 male Wistar rats. Body weight was 190 to 240 g. The rats were divided into four groups: three consisting of 28 animals each and one control group consisting of 20 animals. In order to monitor the development of sepsis, the animals were killed 12, 24, 72 or 120 hours after surgery. Clinically visible sepsis in two groups of rats was provoked by inoculation of pure culture of *Escherichia coli* (EC) or *Staphylococcus aureus* (SA) into the previously emptied, ligated and perfused caecum (Stojanović *et al*., 2002). In the third group of experimental animals sepsis was provoked using the content of the ligated and punctured caecum (mixed culture of microorganisms – MC) (Wichterman *et al*., 1980). The control group of animals was sham operated on by opening the abdominal wall.

Blood was withdrown from the abdominal aorta and used to measured concentrations of the following biochemical parameters employing an automatic analyzer (H-1 Technicon). Data were expressed as the mean±standard deviation (SD). Statistical analysis was performed using a statistical software program (Statistic 5.0 for Windows). Groups were compared by Student's t-test and two-way analysis of variance. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

In the present work statistically significant differences in biochemical parameters in the septic rat sera were found. Septic conditions are closely related to metabolic parameters including the increased utilization of glucose. Hypoglycemia was primarily found (Goto *et al*., 1993; Maitra, 1993) although it did not last for a long time. Soon after that long lasting hypoglycemia occurs and it could not be corrected by glucose infusion (Yelich, 1990). During the experiment a significant hypoglycemia was noticed in MK group of rats (except at 120h) as well as in groups EC and SA at 12 and 24 h (Figure 1). This is in accordance with other authors who used the model of sepsis in animals. From literature data such hypoglycemia appears because glycogen reserves decrease, as bacterial toxins inhibit gluconeogensis or its mediators and because of enhanced entrance of glucose into the tissue especially into skeletal muscles (Titheradge *et al*., 1995). Experimental data suggest that endotoxins have an insulin-like effect or potentiate tissue sensibility to insulin, most likely modifying the glucose membrane transport. Gamelli *et al*. (1996) have found that in mice, easy entrance of glucose into the macrophages is due to increased glucose-transporter 1 (GLUT-1) iRNA. In *in vitro* experiments Cepp *et al*. (1996) have found that the treatment of hepatocite cells with cytokines (combination of TNF-alfa, INF-gama, IL-1 beta) and LPS prevents glyconeogenesis and decreases glycogen depending on the time increase for NO synthesis. Similar results were published by Casada *et al*. (1996) *in vitro* and *in vivo* when the model of toxic shock was used. This author reported that the glyconeogenetic flux is changed if a decrease of glucose transporter 2 (GLUT-2) iRNA and phosphoenolpiruvate carboxykinases occurs at the time when the level of iRNA inducible NO synthetase increases.

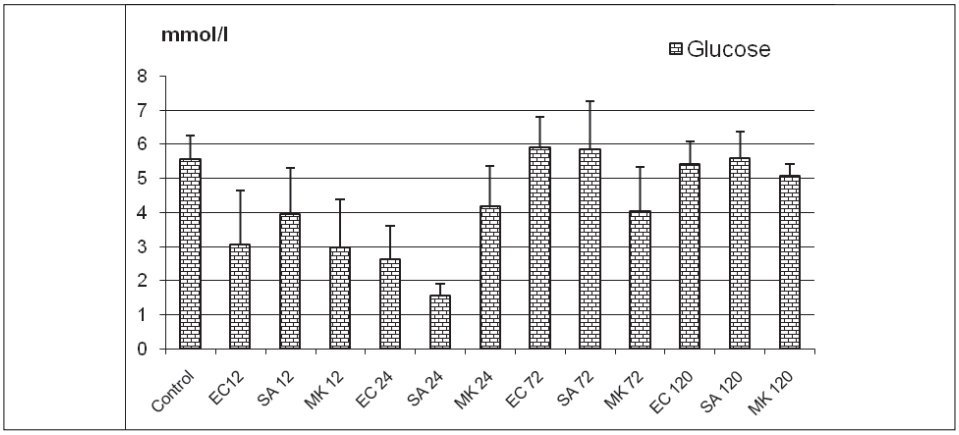

Figure 1. Results of concentration of glucose in blood sera of experimental rats
MK – Mixed sepsis; EC - Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; 12, 24, 72, 120 – Time after surgery (h)* $p<0.05$ compared with control

In the sepsis model lipid metabolism depends on the level and duration of cytokine stimulus. It also depends on body condition and cytokines interactions (Hardardottir *et al*., 1994). This explains some variations in triglyceride concentrations in rats that experience septic condition versus control values. The statistically significant differences were in the EC and SA groups of rats at 24h (Figure 2). It is known that in the models of sepsis in animals the level of triglycerides is increased due to TNF, IL-1, IL-2 and INF-alfa. The lower doses induce production of hepatic triglycerides and secretion of VLDL (very low density lipoprotein). High doses inhibit the activity of lipoprotein lipase (LPL) and the clearance of lipoproteins enriched with triglycerides (Samra et al., 1996). Statistically significant increase of cholesterol in the sera of septic rats in the first half of the experimental protocol (Figure 2) could be attributed, according to literature (Abarca and Garcia, 1993), to increased LDL (low density lipoprotein) cholesterol. These mediators and modulators increase *de novo* synthesis of cholesterol in the endoplasmatic reticulum in the liver accompanied with increased activity of beta-hydroxy-beta-methyl-glutaryl coenzyme A reductase (HMGR), so that increased LDL cholesterol results in enhanced synthesis, but not in low clearance (Liscum and Faust, 1994). Pretreatment of septic rats with the TNF antibodies will weaken the effects of endotoxins moderating the level of serum cholesterol, synthesis of hepatic cholesterol and lowering activity of hepatic HMGR. This implies the significance of this mediator in the metabolism of cholesterol (Dinarello, 1994a).

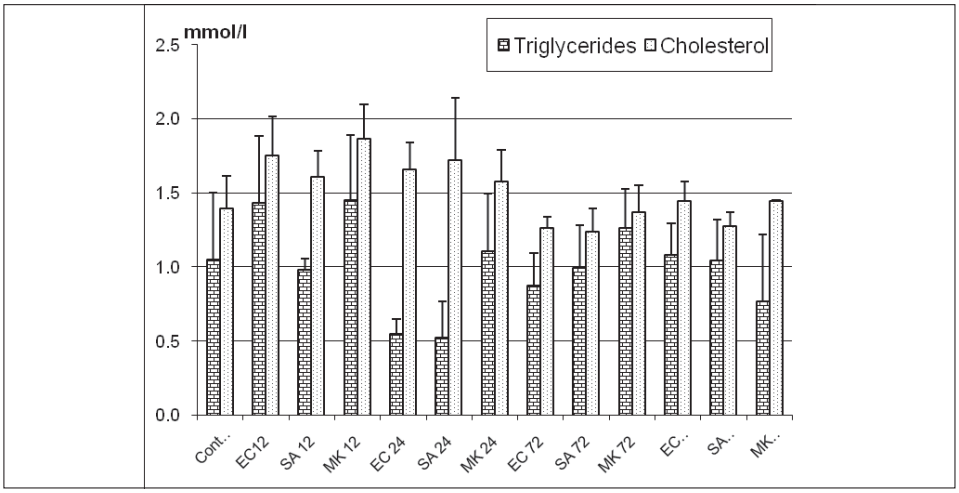

Figure 2. Results of concentration of triglycerides and cholesterol in the blood sera of experimental rats
MK – Mixed sepsis; EC – Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; 12, 24, 72, 120 – Time after surgery (h)* $p<0.05$ compared with control

In our experiments significant changes in nitrogen from protein and non protein sources in the sera of septic rats related to controls were found meaning that intensive metabolism of proteins occur. This is in accordance to the data published by other authors. The statistically significant increase of total protein concentration was notified in EC and SA groups at 24 h ($p<0.01$) and 120h ($p<0.05$) while a decrease in the MK group of rats ($p<0.05$) was found at 12 and 72h (Figure 3). A statistically significant decrease in albumin concentration in septic rats was found in the second half of the experiment at 72 and 120h (Figure 3). The increased concentration of proteins could be attributed to the enhanced synthesis of proteins in the liver. Namely, it is known that during sepsis there is an increase of IL-1 cytokine. This cytokine induces increased catabolism of proteins in skeletal muscles and induces increased synthesis of proteins in the liver (liver enzymes, structural proteins, plasma proteins and acute phase proteins) (Dinarello, 1994b). The enhanced efficiency of amino acids for protein synthesis could be higher than for amino acids whose carbon skeleton is used for glucose production or for oxidative energy. Pittiruti *et al*. (1989) have found out that patients with sepsis after starvation release three times more amino acids in the muscle than normally. At the same time the total loss of nitrite is increased only to 30%, meaning that a large part of these amino acids is used for protein synthesis. This data implies the need for competition for amino acids between oxidative catabolism and protein synthesis. This could be answered by hepatocellular production of acute phase proteins. Sganga *et al*. (1985) have analyzed the protein fraction of sera from people with sepsis. They have realized that the level of C reactive protein, fibrinogen, ceruloplasmin and alfa-1 antitrypsin is growing continuously while the level of transferin, albumin and alfa-2 macroglobulin decreases after few days. The lower concentration of albumin in our experiments could be explained by increased synthesis of acute phase proteins on behalf of albumins. The lower concentration of proteins in the MK group of rats points on enhanced oxidative catabolism of amino acids in skeletal muscles.

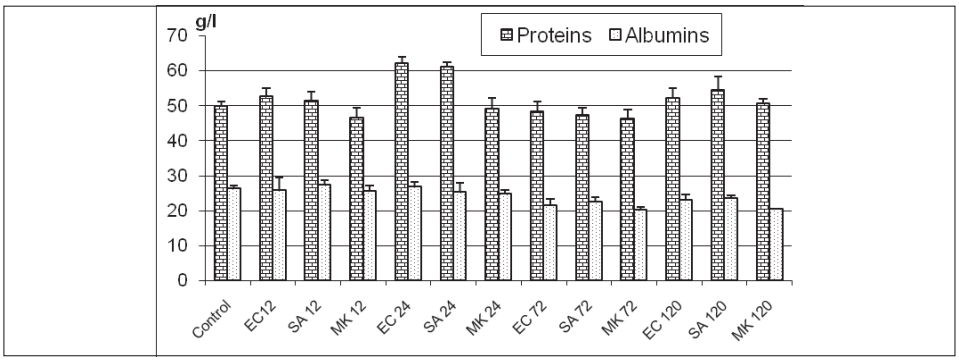

Figure 3. Results of concentration of total proteins in blood sera of experimental rats
MK – Mixed sepsis; EC – Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; 12, 24, 72, 120 – Time after surgery (h)* $p<0.05$ compared with control

In our experiments a lower synthesis of urea was noticed in all groups except in the SA group at 72h (when it was significantly increased) (Figure 4). Ureagenesis can occur after direct hormonal stimulation of gluconeogenesis and/or ureagenesis and this explains why increased urea in our experiments was not significant. It is known that a high level of alanine in the plasma can stimulate secretion of glucagon, a gluconeogenetic hormone (Berczi, 1993). On the other hand the significant decrease in urea could be explained by the competitive relationship for energy between the process of ureagenesis, glucogenesis and protein synthesis during the acute phase (Pittiruti, 1989). It is known that if septic conditions are getting more difficult (Zamir *et al*., 1994) and depending on clinical conditions (Casey *et al*., 1993) relative disjunction of the liver occurs that could be followed by lower clearance of amino acids and urea synthesis.

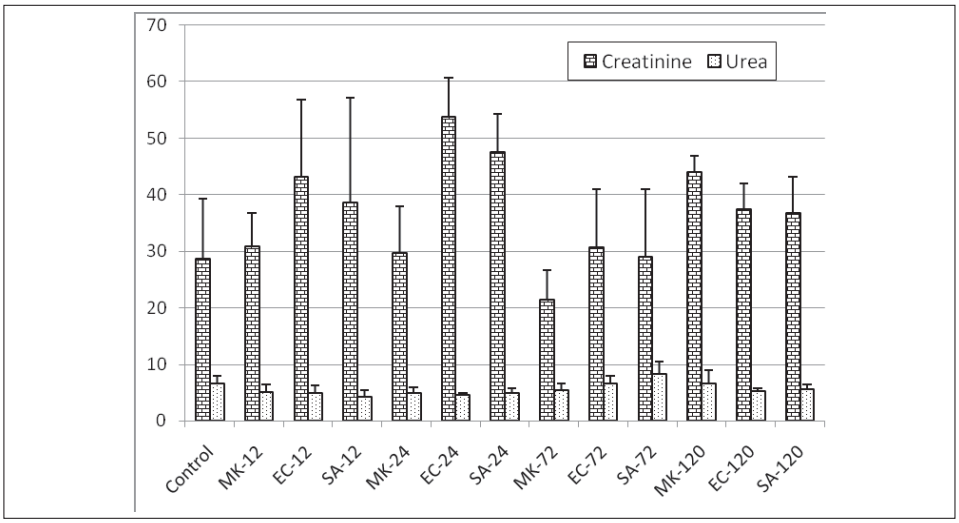

Figure 4. Results of concentration of creatinine and urea in blood sera of experimental rats MK – Mixed sepsis; EC – Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; 12, 24, 72, 120 – Time after surgery (h)* $p<0.05$ compared with control

The concentration of creatinine in the septic rat sera was more or less above the level in the control group. They were statistically significant at 12h (EC-$p<0.05$) and 24h (EC, SA-$p<0.001$) during the experiment (Figure 4). This implies that energy was intensively used and that transient acute renal weakness develops during the first half of the experiment. Lambalgen *et al*. (1993) tested the hypothesis that early changes in renal metabolism of rats treated with endotoxins (8 mg/kg) contribute in the development of acute renal weakness. Authors noted that renal functional clearance (the renal plasma flow and the volume of glomerular filtration) 30 to 90 minutes after inoculation of endotoxins is lower and that the concentration of creatinine in plasma is higher by 193% related to basal

values. In similar experiments on sheep Weber *et al*. (1994) have found that lower renal blood flow (40%) and creatinine clearance (75%), as well as sodium bicarbonate at 12h after the treatment with endotoxin (20 ng/kg, 3 days) is accompanied with the increase of renin concentration and 6-keto prostaglandine F1-alfa in the plasma of sheep. Froon *et al*. (1994) emphasize that an increase in plasma soluble TNF receptor in patients with sepsis syndrome correlated well with the consequences of renal weakness and to plasma of creatinine concentration and death rate.

ACKNOWLEDGMENT:
This work was supported by a grant from the Ministry of Education and Science, Republic of Serbia, number TR 31071.

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ANALIZA VREDNOSTI BIOHEMIJSKIH PARAMETARA U KRVI KOD PACOVA SA SEPSOM IZAZVANOM ČISTIM I MEŠOVITIM BAKTERIJSKIM KULTURAMA POSLE PODVEZIVANJA I PUNKCIJE CEKUMA

STOJANOVIĆ DRAGICA, VELHNER MAJA, STOJANOV I, PETROVIĆ JELENA,
AŠANIN JELENA i KOVAČEVIĆ ZORANA

SADRŽAJ

Cilj ovog rada je bio da se indukuje sepsa i u krvi prate promene u koncentracijii sledećih biohemijskih parametara: glukoze, triglicerida, holesteriola, ukupnih proteina, albumina i kreatinina. Ogled je izveden na 104 Wistar pacova muškog pola, težine 190 – 240 g. Pacovi su bili podeljeni u četiri ogledne grupe. Kod jedinki prve tri grupe (po 28 pacova) izazvana je sepsa dok je 20 pacova sačinjavalo kontrolnu grupu.

U prvoj polovini ogleda, registrovane su značajna hipoglikemija, hiperholesterolemija, smanjenje koncentracije uree i povećanje koncentracije kreatinina (12 i 24 h po operativnom zahvatu). Kod pacova sa sepsom, indukovanom čistom bakterijskom kulturom, registrovana je posle 24h značajna hiperproteinemija uz smanjenje koncentracije triglicerida. U drugoj polovini ogleda (72 i 120 h po operativnom zahvatu), registrovana je kod svih jedinki sa indukovanom sepsom značajna hipoalbuminemija.