ISSN: 1220-3173; E-ISSN: 2457-7618

# CONTROLLED HYPOTHERMIA SIGNIFICANTLY INCREASES THE SURVIVAL RATE IN A RAT EXPERIMENTAL MODEL OF POLYMICROBIAL SEPSIS

## HIPOTERMIA CONTROLATĂ CREȘTE TIMPUL DE SUPRAVIEȚUIRE LA ȘOBOLANII CU SEPSIS POLIMICROBIAN INDUS EXPERIMENTAL

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**ABSTRACT | REZUMAT**

Therapeutic management of sepsis is complex, evolution is fast and the complications that arise are fatal. An important role in the evolution of sepsis is the immune system; it can fight the pathogen factor, but it can also cause damage to the organism with an aberrant inflammatory reaction. Induced hypothermia is known for the protective effect in local inflammation. Our aim was to evaluate the survival rate in rats with polymicrobial sepsis induced by cecal ligation and puncture (CLP) maintained in a normal range of temperature (35.9-37.5°C) versus CLP and induced hypothermia (30-32°C). 20 Wistar male rats were anesthetized and aseptically prepared for the abdominal surgical CLP technique. After anesthetic recovery, the rats were randomized in to 2 groups (n=10), group A (normothermic) and group B (hypothermic). Normothermia was maintained with in a physiological temperature range of 35.9-37.5°C and compared to the second group where hypothermia was induced resulting in a core temperature of 30-32°C. All subjects where monitored until death occurred. The average survival time in the normothermic group, was 299.1 minutes with a SD of 69.8 minutes compared with the hypothermic group where the average survival rate was 2246.1 minutes with a SD of 1020.9 minutes. Controlled hypothermia significantly increases the survival time when compared to the normothermia in CLP induced septicemia individuals.

**Keywords**: sepsis, cecal ligation and puncture, moderate hypothermia, survival time

Managementul terapeutic al sepsisului este complex, evoluția sa este rapidă iar complicațiile asociate sunt fatale. Un rol important în evoluția sepsisului îl reprezintă sistemul imunitar; poate lupta împotriva factorului patogen, dar poate de asemenea provoca afecțiuni organismului printr-o reacție inflamatorie aberantă. Hipotermia indusă este cunoscută pentru efectul protector în inflamația locală. Scopul studiului a fost de a evalua rata de supraviețuire la șobolanii cu sepsis polimicrobian indus prin puncția și ligatura cecumului (CLP) menținuți într-un interval normal de temperatură (35,9-37,5°C) comparativ cu șobolani cu CLP cărora li s-a indus o hipotermie moderată spre severă (30-32°C). 20 șobolani masculi Wistar au fost anesteziați și pregătiți abdominal aseptic pentru tehnica de CLP. După recuperarea din anestezie, șobolanii au fost randomizați în două loturi (n=10), lot A (normotermic) și lotul B (hipotermic). Normotermia a fost menținută într-un interval de temperatură fiziologică de 35,9-37,5°C, iar al doilea grup cu hipotermie indusă a fost menținut într-un interval de temperatură de 30-32°C. Toți șobolanii au fost monitorizați până la moarte. Timpul mediu de supraviețuire în grupul normotermic a fost de 299,1 minute cu un SD de 69,8 minute comparativ cu grupul hipotermic unde rata medie de supraviețuire a fost de 2246,1 minute cu un SD de 1020,9 minute. Hipotermia controlată crește semnificativ tim-pul de supraviețuire în comparație cu normotermia la indivizii cu septicemie indusă prin puncție și ligatura cecală.

**Cuvinte cheie**: sepsis, puncție și ligatură cecală, hipotermie moderată, timp de supraviețuire

Sepsis represents the systemic inflammatory response to infection (SIRS) and can be clinically identified by hyperthermia, tachypnea, tachycardia and leukocytosis/leukopenia. The most common source of sepsis in animals is gram-negative enteric bacteria. It can contaminate the abdominal cavity and induce peritonitis in case of impairment of the enteric wall (trauma, surgery, enteritis) but also can develop bacteriemia and endotoxemia by translocation across the gastrointestinal tract. The host's immune system plays an important role in evolution of sepsis and SIRS. In its fight with the pathogen, excess of proinflammatory mediators are produced or the immune system fails to modulate the inflammatory response which leads to SIRS or even multiple organ dysfunction syndrome (MODS). Sepsis is considered a challenge for the clini-

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cians in veterinary and human medicine. When the immune response is falling, classic treatment it's not enough and supplemental therapy is necessary for increasing the survival in this disease (1, 3, 5, 7, 18).

Hypothermia as a therapeutic agent is used since antiquity. Because of empiric application and severe side effects further studies were performed to understand the mechanism of action and its positive effects on different pathologies. Today, application of controlled hypothermia is advanced, the techniques are sophisticated and its application improves classic medicine. It is used as a protective treatment on tissue, in pathologies associated to ischemia, hypoxia and inflammation. Hypothermia is applied in surgery and trauma, especially for tissue protection in vital organs like heart and central nervous system. By controlled reducing temperature, the metabolism is slowed down, oxygen consume is reduced and also local inflammatory response is minimized and aggression to the tissue is diminished (6, 9, 10, 11, 14).

Recent studies performed on laboratory animals tested the protective effect of controlled hypothermia in sepsis by reducing liver and lungs injury. Other studies showed a significant improvement in the survival rate of rats with endotoxin shock, if treated with induced hypothermia (12, 14).

None of the studies used as a model for sepsis induction the polymicrobial contamination of the abdominal cavity (PLC). Different protocols to induce SIRS and maybe secondary sepsis were used. Our experimental model based on PLC induction of sepsis has the ability to imitate the evolution of clinical sepsis and SIRS (8, 13, 16).

The aim of the current study is to induce a polymicrobial sepsis based on PLC, to monitor the clinical evolution and the time of survival in normal conditions, with body temperature 35.9-37.5°C. Additionally, the research investigates the potential effect of moderate induced hypothermia (30-32°C) and its potential to reduce the evolution, improve the clinical signs and increase the survival rate in rats with sepsis.

## MATERIALS AND METHODS

The research has been carried out on 20 healthy adult male Wistar rats. The procedures involved in the present project were conducted under the guidelines of Directive 2010/63/EU and the national law 43/2015. The rats were housed under standard conditions: temperature 23ºC, humidity 55%, light/dark cycle 12/12. The animals were fed on standard rodent granular food and they had unrestricted access to water. The study was done under the approval of the Bioethics Comity of the University of Agricultural Sciences and Veterinary Medicine of Cluj Napoca and they were authorised by the State Veterinary Authority (aut. No. 116 from 11.05.2018). All rats included in the study were clinically examined by inspection, auscultation, and thermometry, also blood sample was collected from suborbital sinus for hematologic exam. Only clinical healthy subjects with values within the reference limits were included in the study.

Each rat was weighed and anesthetized and marked on the tail. Anesthesia was performed with a mix of Xilazine (5mg/kg) and Ketamin (75mg/kg), intra-muscular according to literature (4). Rats were positioned in dorsal recumbence and limbs were fixed on the table with adhesive tape. The abdominal region was prepared for surgery, the hair was trimmed and antisepsis was performed with gauze immersed in betadine and isopropyl alcohol. Folidrape was fixed and surgical material was prepared for the caecum ligation and puncture procedure. A 2 cm skin incision was performed on the middle line, between the navel and pubis. The muscles were punctured in the middle line, carefully not to damage the organs and the muscle lairs were sectioned on the white line with the Metzenbaum scissor. The caecum was identified, gently isolated outside the abdominal cavity and easily massaged to mobilize the content to the tip of the caecum. A ligature was applied distally to the ileocecal valve at 1 cm from the tip of the caecum with a 5.0 polyglycolic acid suture material. This ligature will not produce intestinal obstruction and will not interfere with the normal intestinal transit. The place of the ligature will influence the grade of sepsis induced. The technique used was described in literature and applied in many studies (Fig.1) (8, 13).

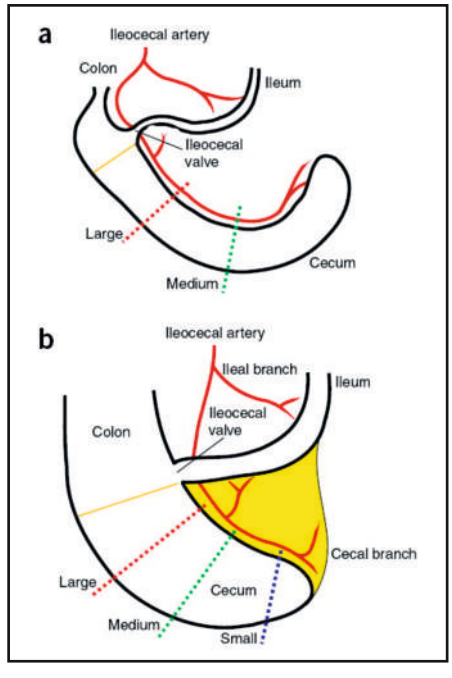


**Fig. 1**. Anatomy of the caecum and the degree of sepsis according to the position of the ligature (13)

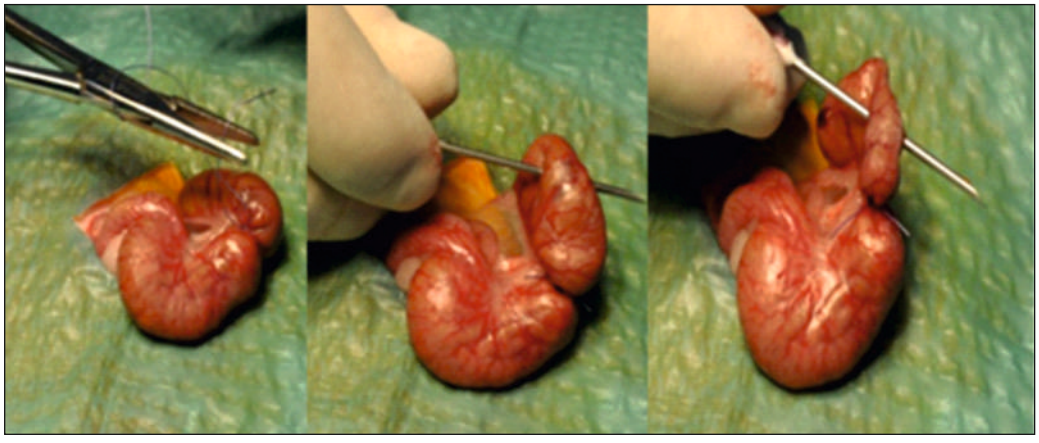

**Fig. 2**. Placing the cecal ligature and two punctures creating four holes in the caecum

The ligated part of the caecum was then punctured with an 18 G needle, twice, with care not to damage the vessels. The needle penetrated the caecum on both sides and 4 holes were performed in the organ. Light compression was performed to expose the content trough the holes and the caecum was gently repositioned in the abdominal cavity without contaminating the incision line. Abdominal layers were closed with the same suture material used for cecal ligation. Firstly, the muscles were sutured in continuous pattern and then the skin in simple points. Then, the rats were placed in ventral recumbence on clean, warm surface until they recovered from anaesthesia and received Tramadol 10mg/kg, intra muscular for further analgesia. Temperature was registered every 5 minutes and kept in normal values until the surgical procedure was finished. From this point forward, the rats were divided in two groups of 10 rats. Group A, the norm-thermic group, were maintained in physiological values between 35.9-37.5 °C. Due to the hypothermic effect of the anaesthetic products, the temperature values were kept in present interval with electric pillow and infrared lamp (Fig. 3).

**Fig. 3**. Normothermic group A, rats marked on the tail

Group B, the hypothermic group, the body temperature was slowly decreased to the values of 30.0-32.0 °C. Hypothermia was induced by increasing the loss of heat through conduction and convection. Rats are small animals and only extracorporeal techniques can be applied to induce hypothermia. Rats hair was trimmed, they were kept in ambient temperature of 17-19 °C, skin was sprayed with alcohol and Cool Brick were placed under the cages to increase the heat loose (Fig. 4). Temperature was recorded every 10 minutes with Dräger Cato® temperature probe and clinical signs were evaluated continuously until death occurred.

**Fig. 4**. Hypothermic group B, marked and trimmed in dorsal region to promote heat loos

The values recorded were statistically processed and interpreted per groups, by calculating the standard deviations (SD), the confidence intervals of the averages and the variance coefficients for the risk level p=0.05. The comparative analysis between the results of the experimental groups was done by the Student application, the interpretation being made by comparison with the theoretical "t" values depending on the degrees of freedom (df) and the risk levels p = 0.05 - 0.001.

## RESULTS AND DISCUSSIONS

The survival time of the normothermic tested rats - group A, ranged from 180 to 406 minutes (Table 1).

The survival time of the hypothermic rats (group B) is much higher compared to the first batch, ranging from 522 to 3497 minutes.

**Table 1**

**Survival rate in normotermic septic group A**

| Subject number | Weight (grams) | Survival rate (minutes) |
|---|---|---|
| 1 | 296 | 337 |
| 2 | 342 | 202 |
| 3 | 270 | 306 |
| 4 | 338 | 180 |
| 5 | 370 | 279 |
| 6 | 350 | 308 |
| 7 | 360 | 370 |
| 8 | 298 | 406 |
| 9 | 390 | 328 |
| 10 | 330 | 275 |

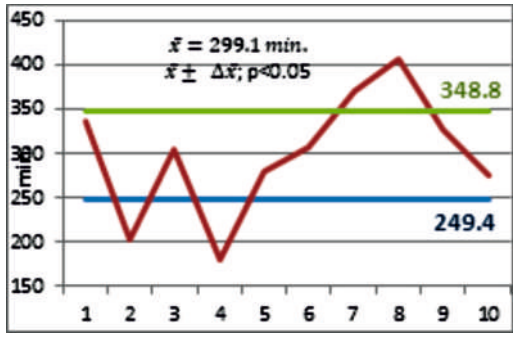


**Fig. 5**. The confidence interval of the average survival time characteristic for group A

The mean survival time for rats in group A was 299.1 min, with a standard deviation (SD) of 69.8 min. Average survival interval calculated based on average error Δx was 49.7 minutes and the statistical risk level $p < 0.05$, is between 249.4 and 348.8 minutes (Fig. 7).

Survival time was between 4 and 6 hours. The mean survival time for rats in group B was 2246.1 minutes (Table 2), with a standard deviation (SD) of 1020.9 minutes. Average survival interval calculated based on average error Δx was 730.3 minutes and between 1515.8 and 2976.4 minutes for the statistical risk level $p < 0.05$ (Fig. 8). The survival time is greatly increased, compared to the normotherm group, between 25 and 50 hours.

The comparison of the two batches of rats in terms of the survival period is presented in Fig. 9. The shortest survival time in the hypothermic group (522 min) exceeds the longest survival time, recorded in the normothermic group (406 min) by 116 minutes (~2 h).

**Table 2**

**Survival rate in hypothermic septic group B**

| Subject number | Weight (grams) | Survival rate (minutes) |
|---|---|---|
| 1 | 280 | 3497 |
| 2 | 280 | 3073 |
| 3 | 280 | 2509 |
| 4 | 298 | 1610 |
| 5 | 322 | 875 |
| 6 | 260 | 522 |
| 7 | 355 | 2758 |
| 8 | 290 | 1820 |
| 9 | 300 | 2393 |
| 10 | 292 | 3404 |

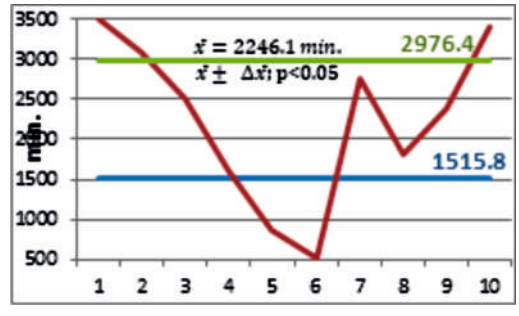


**Fig. 6**. The confidence interval of the average survival time characteristic for goup B

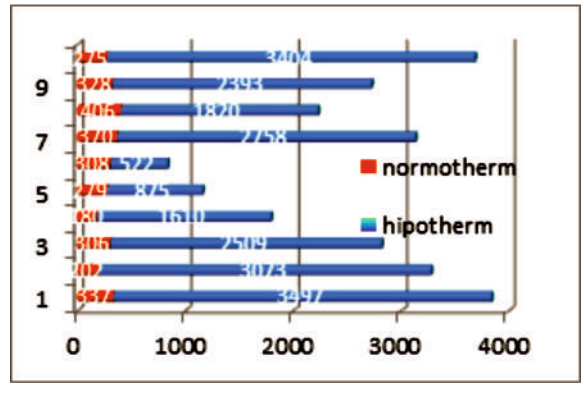


**Fig. 7**. Comparative analysis of the two batches of rats on postoperative survival time

The coefficient of variation (CV%) calculated for group A - analysed under normothermic conditions is 23.2%, which reveals a great variability of individual postoperative resistance, and in group B analysed under hypothermia, the coefficient of variation determined (CV%) exceeds 45%, demonstrating a very high variability, which is explained by the individual reaction of each individual to the physical stimulus in-

duced by lowering the body temperature.

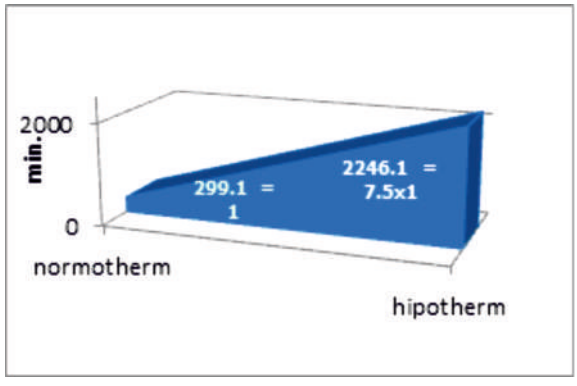


**Fig. 8**. The average survival time of the two comparative groups

The mean survival time in rats with induced hypothermia group was increases with 7.5 times compared to the average survival time of the rats in the normothermic group (Fig. 8). Statistical verification of the differences obtained between the two groups, by the "t" test (Student) demonstrates highly statistically significant differences: t (18df) = 6.017.

The death of individuals, both of the normotherm group and those in the hypothermic group, occurred shortly after the occurrence of increased dyspnoea with oral respiration (nonphisiological in rats), mucosal cyanosis and extremities, all as a result of septic shock. In the case of the hypothermic group, these manifestations were delayed.

The PLC procedure is a safe and easy method to perform and does not encounter major difficulties. The induction of controlled hypothermia is challenging in laboratory animals. Due to the small size of the animal, only extracorporeal procedures can be applied. From the experience of this study we can affirm that it was more difficult to maintain the rats in constant temperature, normothermic (37-39°C).

A similar study, conducted by L'Her et al. gives similar results. They observed an increase in survival time in the hypothermic group (32°C) compared to normothermic (37°C). The mean difference being 244 minutes, but they also observed an increase in lactate production (marker of sepsis and trauma). As mentioned previously, the technique of inducing polymicrobial septicaemia by PLC is variable, and the survival time much shorter than in our study, which could be explained by the degree of induced septicaemia (2, 7).

A randomized human clinical trial of patients with respiratory distress, a pathology frequently complicated with septicaemia and death, tracked the effects of hypothermia in the evolution of this pathology and the survival rate. Patients suffering from septic acute respiratory distress syndrome, ventilated mechanically, received conventional treatment. Additionally, in a number of randomized patients, hypothermia was used as adjunctive therapy at 32-35 °C as the last solution. There was an improvement in oxygenation and a decrease in the mortality rate to 67%, in conditions where it usually was 100% (17).

Other researchers compared survival time in rats with induced endotoxic shock, maintained in normothermia and 2 group with different degree of hypothermia: mild (35-34 °C) and mean (30-31 °C). There was a decrease in mortality from 75% (normothermic group) to 16% and 8% for the two mild and medium hypothermic groups, in this order. A significant reduction in TNF-α and IL-6 plasma cytokines, inflammation makers, have also been observed. This study demonstrates that hypothermia reduces inflammatory response and increases survival rate (14).

The disadvantage of this protocol is that it does not mimic the clinical development of septicaemia and systemic inflammatory response in comparison to our study.

All these studies prove that controlled hypothermia has a protective effect over the body and slows the evolution of sepsis. What is the physiopathology and how accurately hypothermia influences or interfere in the evolution of sepsis is still unclear, more studies being needed.

## CONCLUSION

Cecal ligation and puncture is an efficient and controllable technique to induce sepsis in rats. It was proved efficient in order to observe the effects of controlled hypothermia in sepsis. Hypothermia can be induced in rats only by noninvasive extracorporeal techniques but it should be taken in consideration the time taken for induction. The clinic evolution of sepsis was faster in the normothermic group comparing to the hypothermic group and the controlled hypothermia significantly increased the survival time of the rats with sepsis. This approach can improve the therapeutic management of this disease, this research being the first to demonstrate the mechanism of action of controlled hypothermia and also the evolution of sepsis for future application on clinical patients. Further studies to establish the level of hypothermia and time of exposure are needed.

## REFERENCES

1. *Angus D.C., Van der Poll T.,* (2013), Severe sepsis and septic shock. N Engl J Med, 369(9):840-851
2. *Blomkalns A.L.,*(2007), Lactate –A marker for sepsis and trauma. EMCREG-International, 43 -49
3. *Bone R.C.,* (1991), The pathogenesis of sepsis. Ann Intern Med, 115(6):457-469
4. *Carpenter J.W.,* (2012), Exotic animal formulary.

4th Edition, (Ed.) Saunders, Elsevier Health Sciences, St Louis, Missouri, USA, 1-744
5. *Ettinger S.J., Feldman E.C., Cote E.*, (2017), Textbook of Veterinary Internal Medicine-eBook. (Ed.) Saunders, Elsevier Health Sciences, St Louis, Missouri, USA, 1-2424
6. *Lampe J.W., Becker L.B.,* (2011), State of the art in therapeutic hypothermia. Annu Rev Med, 62:79-93
7. *L'Her E., Amerand A., Vettier A., Sebert P.,* (2006), Effects of mild induced hypothermia during experimental sepsis. Crit Care Med, 34(10):2621-2623
8. *Menezes G.B., Amaral S.S., Alvarenga D.M., Cara D.C.*, (2008), Surgical procedures to an experimental polymicrobial sepsis: cecal ligation and puncture. Braz J Vet Pathol, 1(2):77-80
9. *Mayer S.A., Sessler D.I.,* (2004), Therapeutic hypothermia, 1st Edition, (Ed.) CRC Press, Boca Raton, FL, USA, 1-648
10. *Oddo M., Schaller M.D., Feihl F., Ribordy V., Liaudet L.*, (2006), From evidence to clinical practice: effective implementation of therapeutic hypothermia to improve patient outcome after cardiac arrest. Crit Care Med, 34(7):1865-1873
11. *Polderman K.H., Herold I.*, (2009), Therapeutic hypothermia and controlled normothermia in the intensive care unit: practical considerations, side effects, and cooling methods.Crit Care Med, 37(3): 1101-1120
12. *Rim K.P., Kim K., Jo Y.H., Lee J.H., Rhee J.E., Kang K.W., Suh G.J., Kwon W.Y., Lee M.J., Lee H.S.*, (2012), Effect of therapeutic hypothermia according to severity of sepsis in a septic rat model. Cytokine, 60(3):755-761
13. *Rittirsch D., Huber-Lang M.S., Flierl M.A., Ward P.A.*, (2008), Immunodesign of experimental sepsis by cecal ligation and puncture.Nat Protoc, 4(1): 31-36
14. *Taniguchi T., Kanakura H., Takemoto Y., Yamamoto K.,* (2003), Effects of hypothermia on mortality and inflammatory responses to endotoxin-induced shock in rats. Clin Diagn Lab Immunol, 10(5):940-943
15. *Todd M.M., Hindman B.J., Clarke W.R., Torner J.C.*, (2005), Mild intraoperative hypothermia during surgery for intracranial aneurysm. N Engl J Med, 352(2):135-145
16. *Toscano M.G., Ganea D., Gamero A.M.*, (2011), Cecal ligation puncture procedure. J Vis Exp, (51) pp:2860
17. *Villar J., Slutsky A.S.,* (1993), Effects of induced hypothermia in patients with septic adult respiratory distress syndrome. Resuscitation, 26(2):183-192
18. *Wingfield W.E., Raffe M.R.,* (2002), The veterinary ICU Book, (Ed.) Teton NewMedia, Jackson, WY, USA, pp. 685-710.