MITOCHONDRIAL DYSFUNCTION IS ASSOCIATED WITH LONG-TERM COGNITIVE IMPAIRMENT IN AN ANIMAL SEPSIS MODEL

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ABSTRACT

Background: Several different mechanisms have been proposed to explain long-term cognitive impairment in sepsis survivors. The role of persisting mitochondrial dysfunction is not known. We thus sought to determine whether stimulation of mitochondrial dynamics improves mitochondrial function and long-term cognitive impairment in an experimental model of sepsis.

Methods: Sepsis was induced in adult Wistar rats by caecal ligation and perforation. Animals received intracerebroventricular injections of either rosiglitazone (biogenesis activator), rilmenidine, rapamycin (autophagy activators), or n-saline (sham control) once a day on Days 7-9 after the septic insult. Cognitive impairment was assessed by inhibitory avoidance and object recognition tests. Animals were sacrificed 24 hours, 3 and 10 days after sepsis with the hippocampus and prefrontal cortex removed to determine mitochondrial function**.**

Results: Sepsis was associated with both acute (24 hours) and late (10 days) brain mitochondrial dysfunction. Markers of mitochondrial biogenesis, autophagy and mitophagy were not upregulated during these time points. Activation of biogenesis (rosiglitazone) or autophagy (rapamycin and rilmenidine) improved brain ATP levels and *ex vivo* oxygen consumption and the long-term cognitive impairment observed in sepsis survivors.

Conclusion: Long-term impairment of brain function is temporally related to mitochondrial dysfunction. Activators of autophagy and mitochondrial biogenesis could rescue animals from cognitive impairment.

Key Words: autophagy; biogenesis; brain dysfunction; mitochondrial dysfunction; sepsis.

ABBREVIATIONS LIST

ADP, adenosine 5′-diphosphate;

ATG, autophagy-related proteins;

COX, cytochrome C oxidase;

DNA, deoxyribonucleic acid;

GAPD, glyceraldehyde 3-phosphate dehydrogenase;

MOF, multiple organ failure;

NRF-1, nuclear respiratory factor;

PGC-1α, peroxisome proliferator-activated receptor gamma co-activator-1 alpha;

PINK-1, PTEN-induced kinase-1;

ROS, reactive oxygen species;

RT- PCR, reverse transcriptase - polymerase chain reaction;

SOD2, superoxide dismutase-2;

TBARS, thiobarbituric acid reactive substance assay;

TEM, transmission electron microscopy;

TFAM, transcription factor A mitochondrial.

Perspectives

I. Long-term cognitive impairment in sepsis survivors is related to brain mitochondrial dysfunction

II. Activators of autophagy, mitophagy and mitochondrial biogenesis could rescue septic animals from cognitive impairment

INTRODUCTION

Sepsis represents organ dysfunction resulting from a dysregulated host response to infection. This is associated with a complex interaction between cytokines, mediators, and cell surface receptors (1). Multiple other pathways are also involved including up- and down-regulation of gene transcription, epigenetic modifications, hormonal-metabolic interactions, altered immune function, bioenergetic changes and perturbations of both macro- and micro-circulation. The net effect is cellular and organ dysfunction (2).

Neurons are highly oxygen-dependent. Metabolic cooperation between glial cells and neurons, e.g. for neurotransmitter reuptake, oxidative stress defence and energy substrate delivery, critically depend on energy availability (3). In this context, normal mitochondrial function is vital for maintenance of brain function (3). Damage to the electron transport chain is an important factor in the pathogenesis of many neurodegenerative diseases (3) and is observed in the early stages of sepsis (4).

Mitochondrial homeostasis is regulated by mitochondrial dynamics including the processes of auto/mitophagy and mitochondrial biogenesis (5). Autophagy recycles redundant, non-essential or damaged organelles, and macromolecular components (5). Proteins that regulate mitochondrial dynamics are closely involved in autophagy (6). Two proteins, PTEN-induced kinase-1 (PINK-1) and Parkin, play important roles in mitophagy and mitochondrial quality control (7). Mitophagy is a process by which mitochondria are targeted for degradation via the autophagy pathway (8). Of note, stimulation of autophagy with rapamycin protected renal function in an animal model of critical illness (9).

Mitochondrial biogenesis involves coordination of expression, import and assembly of mitochondrial proteins from nuclear and mitochondrial genomes and regulation of mitochondrial content and morphology (10). Biogenesis represents an important mechanism through which regulation of mitochondrial capacity can occur during multiple organ failure (MOF) and its recovery phase (11). In a long-term murine peritonitis model, activation of mitochondrial biogenesis preceded recovery of mitochondrial function, metabolism, and organ function (12).

Long-lasting consequences following recovery from sepsis often include significant brain disorders (13). Even after full recovery, animals subjected to septic insults demonstrated significant impairment in behavioural tasks, indicating cognitive deficits (14,15). In recovered patients, persistent deficits in quality of life are observed (16), which may be associated with long-lasting impairment in cognitive capacities (17). Various mechanisms have been proposed to explain long-term cognitive impairment in sepsis survivors (18,19). However, the role of persisting mitochondrial dysfunction is not known.

We thus hypothesized that mitochondrial dysfunction persists in sepsis survivors due to ongoing impairment of mitochondrial dynamics, and contributes to long-term cognitive dysfunction.

MATERIALS AND METHODS

Ethics approval

The experimental procedures involving animals were approved by the Ethics Committee of the Universidade do Extremo Sul Catarinense (approval ID: 012/2016-2). Animals were handled in accordance with Brazilian legislation on animal welfare and ARRIVE guidelines were followed.

Animals

A total of 450 male Wistar rats (60-70 days old, weighing 250-300 g) were used in this study. Animals were housed in groups of five per cage with food and water available *ad libitum* and maintained on a 12 h light/dark cycle.

Experimental design

Sepsis induction – cecal ligation and perforation (CLP) model

Rats were subjected to cecal ligation and perforation (CLP) as previously described (20). In brief, animals were anesthetized using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. Under aseptic conditions, a 3 cm midline laparotomy was performed to expose cecum and adjoining intestine. The cecum was ligated with a 3.0 silk suture at its base, below the ileocecal valve, and

perforated once with a 14-gauge needle. The cecum was then squeezed gently to extrude a small amount of feces through the perforation site. The cecum was then returned to the peritoneal cavity, and the laparotomy closed with 4.0 silk sutures. Animals were immediately resuscitated with normal saline (30 mL/kg) subcutaneously (s.c.) and at 12 h. All animals received ceftriaxone antibiotic at a dose of 30 mg/kg given subcutaneously every six hours for a maximum of three days. To minimize variability between experiments, the CLP procedure was always performed by the same investigator. The mortality rate of this model is around 40%, which is consistent with sepsis outcomes in patients. We have extensively characterized long-term cognitive impairment using this model (14,15).

Animals were killed 24 hours, 3 and 10 days after sepsis and the hippocampus and prefrontal cortex removed. In a separate cohort of animals, inhibitory avoidance and object recognition tests were performed at 10 days' post-sepsis. In some experiments animals were treated either with activators of autophagy: rapamycin (10 mg/kg, 5µl) or rilmenidine (5mg/kg, 5µl), or activators of biogenesis: rosiglitazone (10/kg mg,5µl), once daily via intracerebroventricular injection on days 7 to 9 after sepsis. DID YOU NOT HAVE A CONTROL GROUP??

The effectiveness of intracerebroventricular injection is based on stereotactic coordinates, determined according to the Paxinos and Watson Atlas, to access the lateral ventricle. A cannula for performing the intracerebroventricular procedures (C311 / G, 20G, Plastics One Inc., VA, USA) and a suitable stylet (C311 / DC, Plastics One Inc., VA, USA) to prevent tissue ingress and/or foreign bodies into the cannula were sited. Placement of the cannula in the lateral ventricle was confirmed by CSF drainage through the cannula. These procedures were performed under sterile conditions.

Assays

All analyses were made by investigators blinded to the grouping.

Ex vivo oxygen consumption

The hippocampus and pre-frontal cortex were obtained from anaesthetized animals, and permeabilized with saponin. Oxygen consumption was assessed in small bundles of tissue by high-resolution respirometry (2k-Oxygraph; Oroboros Instruments,

Innsbruck, Austria) using glutamate/malate, α-ketoglutarate and succinate as substrates. State 3 mitochondrial respiration was initiated by addition of ADP. Results are expressed as pmol O_2/min/mg protein.

ATP levels

Intracellular ATP levels were determined in hippocampus and prefrontal cortex using a fluorometric kit (ATP assay, Abcam 83355) according to the manufacturer's instructions. The ATP assay protocol relies on phosphorylation of glycerol to generate a product quantified by fluorometry (Ex/Em = 535/587 nm). Data are expressed as nmol/mg protein.

Determination of markers of mitochondrial biogenesis.

Gene expression analysis by quantitative real time RT-PCR (RT-qPCR) was used to determine markers of mitochondrial biogenesis. Details are presented in the Supplemental Digital Content. *PGC-1α, TFAM* and *NRF-1* gene expression were amplified, and GAPD used as a reference gene for normalization.

Determination of markers of autophagy and mitophagy

Protein content of markers of autophagy (beclin-1, LC3A/B, Atg5, Atg12, Atg16L1 Atg7 and Atg3) and mitophagy (parkin and PINK-1) in samples of brain were measured by Western blot. Details are provided in the Supplemental Online Content. All results are expressed as a relative ratio between the target and β-actin.

Determination of mitochondrial protein content

Protein content of cytochrome C oxidase (COX) and superoxide dismutase (SOD)-2 in samples of brain were measured by Western blot. Details are provided in the Supplemental Online Content. All results are expressed as a relative ratio between the target and β-actin.

Mitochondrial Respiratory Chain Activities

Complex I activity was determined as the rate of NADH-dependent ferricyanide reduction at 420 nm (21). Complex II activity was determined as the decrease in absorbance of 2,6-DCIP at 600 nm (22). Complex II-III activity was determined by measuring cytochrome c reduction to succinate at 550 nm (22). Complex IV activity was determined based on the decrease in absorbance of cytochrome c oxidation at 550 nm (23). Activity of these complexes is expressed as nmol/min/mg protein.

Krebs' cycle enzyme activities

Citrate synthase activity was determined using acetyl CoA, 5,5′-di-thiobis-(2-nitrobenzoic acid), and oxaloacetate, and monitoring the reaction at 412 nm (24). Succinate dehydrogenase activity was determined by the change of 2,6-DCIP absorbance at 600 nm (22).

Genomic: mitochondrial DNA ratio

Genomic (g) DNA was determined using a genomic DNA Mini Kit (Pure Link™) performed according to the manufacturer's recommendations. This test is based on selective binding of DNA to a silica-based membrane in the presence of chaotropic salts. Mitochondrial (mt) DNA was determined using an isolation kit (Abcam) and performed as recommended by the manufacturer. Data are expressed as g/mt DNA µg/ml.

Transmission electron microscopy

For observation under transmission electron microscopy (TEM), brain samples were fixed overnight with 2.5% glutaraldehyde, 4% paraformaldehyde in 0.1M sodium cacodylate buffer (pH 7.2) plus 0.2M sucrose. Material was post-fixed with 1% osmium tetroxide for 2 h, dehydrated in an acetone gradient series, and embedded in Spurr's resin. Thin sections were stained with aqueous uranyl acetate (1%), followed by lead citrate (1%). Four replicates were made for each experimental group; two samples per replication were then examined under TEM JEM 1011 (JEOL Ltd., Tokyo, Japan) at 80 kV. Specimens were examined over a minimum of 8–10 random fields (to minimize unintended sampling bias) for a qualitative analysis of mitochondrial morphology. Representative images from the hippocampus are shown.

Behaviour tests

Animals underwent behavioural tasks 10 days after surgery. Different animals were submitted to different tasks. For inhibitory avoidance, animals received a foot shock (0.4 mA), as described (25). Test sessions were performed 24 hours after training with step-down latency used as a measure of retention (25).

For object recognition animals were allowed to explore an open field. Training was conducted by placing rats in the field in which two identical cubes (objects A1 and A2) were positioned. At 24 hours' post-training animals were allowed to explore the field in the presence of the familiar object A but a novel object C (a sphere with a square-shaped base). A recognition index was calculated and reported as the ratio TB/ (TA + TB)

(TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B) (26).

Statistical analysis

All data, except for those from behavioural experiments, were expressed as mean ± SD and analyzed by Student's t-test for unpaired samples or factorial-ANOVA. Data from the inhibitory avoidance task and the recognition index were reported as median and interquartile ranges with comparisons among groups performed using the Mann–Whitney U test. For behavioural analyses individual groups were compared by Wilcoxon tests. Differences were considered significant when $p < 0.05$. All tests were performed with SPSS version 20 and/or GraphPad Prism 4.0.

RESULTS

Mitochondrial function is compromised in the central nervous system during sepsis

ATP levels in hippocampus and prefrontal cortex were significantly decreased at both 24 hours and 10 days after sepsis when compared to sham animals (Fig.1A). *Ex vivo* mitochondrial oxygen consumption was decreased only at 10 days following CLP (Fig. 1B).

Since these alterations could be related to a decrease in mitochondrial protein content, the content of specific mitochondrial proteins (superoxide dismutase-2 (SOD2) and cytochrome C oxidase (COX)) were measured. Compared to sham animals, neither changed with sepsis at either timepoint analyzed (Supplemental Digital Content Fig. 1).

Mitochondrial dynamics is not upregulated during sepsis

Alterations in mitochondrial dynamics is associated with worse outcomes in both septic patients (11) and animal models of critical illness (12). We thus evaluated markers of mitochondrial biogenesis (*PGC-1α, TFAM* and *NRF-1* gene expression), autophagy (beclin-1, LC3A/B, Atg5, Atg12, Atg16L1 Atg7 and Atg3) and mitophagy (Parkin and PINK-1). No significant differences were noted between sham and septic animals at any timepoint (data not shown).

Activators of autophagy or mitochondrial biogenesis improve mitochondrial function after sepsis

Inducers of autophagy or mitochondrial biogenesis were administered from the Days 7-9 after induction of sepsis to assess their effects on mitochondrial and cognitive function. Activators of both biogenesis (rosiglitazone) and autophagy (rilmenidine and rapamycin) significantly increased brain ATP levels (Figs. 2A, 2C and 2E) and *ex vivo* oxygen consumption (Figs. 2B, 2D and 2F).

To further understand the effects of these treatment on mitochondrial function the activities of mitochondrial respiratory chain and Krebs' cycle enzyme were measured. Activators of autophagy, but not mitochondrial biogenesis, increased mitochondrial respiratory chain (Fig. 3A-D) and Krebs' cycle enzyme activities (Supplemental Digital Content Fig. 2). In addition, only rapamycin was able to increase the mtDNA: gDNA ratio (Supplemental Digital Content Fig 3).

To better characterize mitochondrial morphology mitochondrial ultrastructure was analyzed qualitatively by TEM (Supplemental Digital Content Fig 4). Comparing to normal mitochondria from sham animals (Supplemental Digital Content Fig 4A) mitochondrial swelling and reduction of mitochondrial cristae were observed in CLP animals (Supplemental Digital Content Fig 4B). These alterations were minimized mainly by rapamycin treatment (Supplemental Digital Content Fig 4C), and to a minor extent by rilmenidine (Supplemental Digital Content Fig. 4D). Rosiglitazone did not have any effect on mitochondrial ultrastructure (Supplemental Digital Content Fig 4E).

Effects of rapamycin, rilmenidine and rosiglitazone on long-term cognitive deficits in sepsis survivor animals

Enhancing mitochondrial function has been postulated to rescue animals from cognitive deficits in different brain injury models (10). We thus evaluated the effect of inducers of autophagy or mitochondrial biogenesis on the performance of septic animals of two different cognitive tasks (Fig. 4). All treatments improved inhibitory avoidance performance and recognition index (Fig. 4).

DISCUSSION

In this long-term animal model of sepsis there was impaired mitochondrial functionality and no net temporal activation of key processes of recovery, namely autophagy, mitophagy or mitochondrial biogenesis. Sustained mitochondrial dysfunction was temporally related to cognitive impairment. Pharmacological activators of autophagy, mitophagy and mitochondrial biogenesis were able to increase brain ATP levels and oxygen consumption, and improve long-term cognitive impairment.

The concept of mitochondrial dysfunction and bioenergetic failure during sepsis is not new (27,28). Patient and animal studies have reported a clear association between the degree of mitochondrial dysfunction, severity of organ dysfunction and mortality (4,19,29). Brain mitochondrial dysfunction occurs early after sepsis (30,31), with an increase in state 4 respiration, and decreases in respiratory control (31), and respiratory complex activities (30). In a long-term animal model of critical illness global oxygen consumption and respiratory exchange ratio were both reduced (32). In muscle satellite cells isolated from an animal model of sepsis (33), there were sustained energetic abnormalities similar to those reported here. Thus, it is plausible that persisting bioenergetic failure could partially explain the long-term cognitive impairment observed in sepsis.

Long-term bioenergetic adaptations could be achieved by modifying the number or activity of functional mitochondria through changes in autophagy, mitophagy and mitochondrial biogenesis. Deficient repair or failure to up-regulate these mechanisms in situations of mitochondrial damage lead to accumulation of damaged mitochondria, a compromise of energy substrate provision and, ultimately, cellular dysfunction (34). Biogenesis improves the capacity for energy substrate production if energy demands increase over time (5,6). On the other hand, autophagy is the process of bulk protein degradation through an autophagosomal pathway (6). It is important for cell differentiation, survival during nutrient deprivation, and normal growth control (6). Cross-talk between inflammation and these processes has recently attracted attention (35). The biogenesis transcription factor, *PGC-1a* was persistently decreased in muscle satellite cells after sepsis (32). In a model of critical illness, the phenotype of insufficient autophagy was more pronounced in non-surviving animals with impaired mitochondrial

function and more severe organ damage (36). Critical illness-induced bone loss has also been related to deficient autophagy (37). Incomplete clearance of damaged cellular components due to insufficient autophagy is also reported in critically ill patients (38). Specifically, in the brain, autophagy is depressed in the hippocampus soon after the onset of sepsis and this is related to brain inflammation (39). We could not find any previous literature regarding deficient mitochondrial dynamics in the brain late after sepsis. We here demonstrate a temporal association between bioenergetic dysfunction and a lack of upregulation of mitochondrial dynamics. This is pertinent as mitochondrial dynamics play a role in numerous neurodegenerative diseases (40) that show some similarities to long-term brain dysfunction in survivors of sepsis (41).

Since inadequate mitochondrial control is proposed to be one of the mechanisms explaining the lack of recovery from organ failure in critically ill patients (38), we examined whether pharmacological upregulation of mitochondrial dynamics could impact upon sepsis-induced brain dysfunction. Rosiglitazone, an agonist of PPARγ, induced neuronal mitochondrial biogenesis and improved glucose utilization and cognition in Alzheimer's disease (42). The efficacy of rosiglitazone has also been demonstrated in animal models of focal ischaemia, spinal cord injury, and traumatic brain injury (43). Autophagy is regulated by both mTOR-dependent and independent pathways. Rapamycin is widely used as a canonical inhibitor of mTORC1 (44) while rilmenidine, a rapamycin analogue, is an mTOR-independent autophagy inducer (45).

These therapeutic approaches have also been demonstrated in animal models of other critical illnesses. PGC-1α accelerated renal recovery after ischaemia by regulating nicotinamide adenine dinucleotide biosynthesis (46). Activation of autophagy attenuated liver injury after ischaemia-reperfusion by restoring mitochondrial mass and membrane potential (47). Rosiglitazone was protective in a model of traumatic brain injury (48) and improved mitochondrial and cardiac dysfunction in a model of endotoxaemia (49). Early after sepsis the activation of autophagy improved survival and decreased acute lung injury (50). In accord with our findings, rapamycin rescued cognitive dysfunction induced by sepsis (51). Of note, in that study rapamycin was administered early after sepsis induction thus the observed effects could be secondary to a decrease in the early systemic inflammatory response rather than a brain-specific effect. Taken together, the results presented in these two studies are complementary, and suggest that activation of

autophagy could be an interesting approach to improve long-term cognitive deficits in sepsis survivors. Two recent trials failed to demonstrate improved long-term recovery from critical illness using hospital- or primary care-based non-pharmacological rehabilitation strategies (16,52). Failure to recover from critical illness could result from persistent inflammation (53) bioenergetic failure (54), and/or abnormal repair and remodeling (55). Our study reinforces this hypothesis. Our findings that late pharmacological interventions, commencing a week after sepsis, could still restore brain function during the recovery phase may be clinically relevant.

As activation of either autophagy or biogenesis could both improve mitochondrial function and cognitive impairment, this reinforces the notion that bioenergetic failure is central to late brain dysfunction. However, these activators may have worked through different mechanisms. In our study, both rapamycin and rilmenidine increased the activities of different electron transfer chain proteins but this was not observed with rosiglitazone. Additionally, only rapamycin was able to increase the mtDNA:gDNA ratio and have a major effect on mitochondrial ultrastructure. Activation of PPARγ can impact upon cell function by altering lipid metabolism, inflammation and metabolic homeostasis as well as mitochondrial biogenesis. Rosiglitazone has been previously shown to improve sepsis-associated cerebral microcirculatory alterations (56). These pleotropic effects of PPARγ activation reinforce the view that activation of different mechanisms could partially explain the demonstration of brain protection after sepsis. Similarly, activation of autophagy, but not rosiglitazone, may accelerate removal of toxic aggregate-prone proteins from neurons (45), in agreement with the discrepant effects observed here. A more in-depth look at the differential effects of the pharmacological tools used in this study may provide a better understanding of their effects upon brain function following sepsis.

Some limitations of our study must be noted. Firstly, activators of autophagy and biogenesis were administered via intracerebroventricular injection. While these limit the clinical translatability of our results, all these drugs can be administered systemically to man. However, from a mechanistic point of view local injection of these substances decreases the probability of systemic, non-specific effects. When administered systemically these drugs have proven effective in different animal models, although the optimal time and duration of administration is poorly understood. Additionally, despite the fact that blood brain barrier (BBB) dysfunction occurs with sepsis (57), we cannot

ascertain whether drugs can cross BBB when administered systemically. Additional studies are needed to define the breadth of the therapeutic window when systemically administered. Secondly, none of the drugs used in our study has specific effects upon mitochondrial dynamics, and this may account for some of the variable effects observed between the different drugs. For example, rosiglitazone plays an anti-inflammatory role, through agonism of PPAR-γ (**Ref???).** Nevertheless, the improvement in mitochondrial function does implicate sustained mitochondrial dysfunction as a central player in long-term cognitive dysfunction.

CONCLUSIONS

Long-term impairment of brain function is temporally related to mitochondrial dysfunction. Pharmacological improvement of mitochondrial function by activators of autophagy and mitochondrial biogenesis could rescue animals from cognitive impairment and suggest a potential therapeutic use in patients.

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Figure legends:

Figure 1 – The evolution of mitochondrial function in the brain during sepsis development. Adult Wistar rats were submitted to cecal ligation and perforation (or sham operated) and 24 hours, 3 and 10 days after the hippocampus and prefrontal cortex were removed to the determination of (**A**) ATP concentration, (**B**) *ex vivo* oxygen consumption. Data are expressed as mean ± standard deviation * $p<0.05$ compared to sham group at same time point. n = 7 animals per group.

Figure 2 – The effect of activators of autophagy or mitochondrial biogenesis on brain mitochondrial function. Adult Wistar rats underwent cecal ligation and perforation (or sham operation). From days 7-9 after sepsis inducers of autophagy (rilmenidine or rapamycin) or mitochondrial biogenesis (rosiglitazone) were administered intracerebroventricularly. At Day 10 the hippocampus and prefrontal cortex were removed to determine ATP content (A, C and E) and *ex vivo* oxygen consumption (B, D and F). Data are expressed as mean ± standard deviation * $p<0.05$ compared to sham group at same timepoint. n = 7 animals per group.

Figure 3 – The effect of activators of autophagy or mitochondrial biogenesis on brain Krebs cycle enzymes activities. Adult Wistar rats underwent cecal ligation and perforation (or sham operation). From days 7-9 after sepsis inducers of autophagy (rilmenidine or rapamycin) or mitochondrial biogenesis (rosiglitazone) were administered intracerebroventricularly. At Day 10 the hippocampus and prefrontal cortex were removed to determine Krebs cycle enzymes activities. Data are expressed as mean ± standard deviation * $p<0.05$ compared to sham group at same time point. n = 7 animals per group.

Figure 4 – The effect of activators of autophagy or mitochondrial biogenesis on long-term cognitive deficits in sepsis surviors. Adult Wistar rats underwent cecal ligation and perforation (or sham operation). From days 7-9 after sepsis inducers of autophagy (rilmenidine or rapamycin) or mitochondrial biogenesis (rosiglitazone) were administered intracerebroventricularly. At Day 10 animals were submitted to (**A**) inhibitory avoidance and (**B**) object recognition tasks. Data are expressed as median and interquartile range * $p<0.05$ compared to training section, same group. n = at least 10 animals per group.

Supplemental Content 1 - Brain superoxide dismutase-2 (SOD2) and cytochrome C oxidase (COX) protein content during sepsis evolution. Adult Wistar rats underwent caecal ligation and perforation (or sham operation). At 24 hours, 3 and 10 days later the hippocampus and prefrontal cortex were removed to enable determination of SOD2 and COX. Data are expressed as mean ± standard deviation. n = 7 animals per group.

Supplemental Content 2 – Effect of activators of autophagy or mitochondrial biogenesis on brain citrate synthase and succinate dehydrogenase activities. Adult Wistar rats underwent caecal ligation and perforation (or sham operation). From days 7-9 after sepsis inducers of autophagy (rilmenidine or rapamycin) or mitochondrial biogenesis (rosiglitazone) were administered intracerebroventricularly. At Day 10 the hippocampus and prefrontal cortex were removed to determine citrate synthase and succinate dehydrogenase activities. Data are expressed as mean ± standard deviation * $p<0.05$ compared to sham group; # compared to CLP group. n = 7 animals per group.

Supplemental Content 3 – Effect of activators of autophagy or mitochondrial biogenesis on brain genomic:mitochondrial DNA ratio. Adult Wistar rats underwent caecal ligation and perforation (or sham operation). From days 7-9 after sepsis inducers of autophagy (rilmenidine or rapamycin) or mitochondrial biogenesis (rosiglitazone) were administered intracerebroventricularly. At Day 10 the hippocampus and prefrontal cortex were removed to determine genomic:mitochondrial DNA ratio. Data are expressed as mean ± standard deviation * $p<0.05$ compared to sham group; # compared to CLP group. n = 7 animals per group.

Supplemental Content 4 - Effect of activators of autophagy or mitochondrial biogenesis on brain mitochondrial ultrastructure. Adult Wistar rats underwent caecal ligation and perforation (or sham operation). From days 7-9 after sepsis inducers of autophagy (rilmenidine or rapamycin) or mitochondrial biogenesis (rosiglitazone) were administered intracerebroventricularly. At Day 10 the hippocampus and prefrontal cortex were removed to determine mitochondrial ultrastructure. Sham (A); CLP (B); CLP+ rapamycin treatment (C); CLP+ rilmenidine treatment (D); CLP+ Rosiglitazone treatment (E).