Cathelicidin Protects the Brain from Mitochondrial DNA Damage in Health, but not Following Septic Shock

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Short Report

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Abstract

Recent discoveries have demonstrated that mitochondria play a critical role in innate immune signaling. By the other hand, immune responses may lead to mitochondrial deregulation.

Cathelicidins play a critical role in innate immunity, promoting poorly understood cellular responses that may enhance or inhibit several signaling pathways, depending on the health conditions and subjacent microenvironment.

Here, we investigated the role of CRAMP, the murine cathelicidin, in healthy mice and following experimental sepsis. We found that sepsis induces significant mitochondrial DNA damage in the prefrontal cortex and that cathelicidin protects the brain from this kind of damage in healthy animals, but not following septic shock.

Introduction

On one hand, we know that DNA is highly susceptible to chemical damage. The DNA replication and repair machinery, moreover, make mistakes. On the other hand, cells possess a sophisticated DNA repair system [1].

Mitochondria are particularly susceptible to DNA damage, since they act as the cellular powerhouses and have to deal with a permanent production of reactive oxygen species (ROS). It is true that an imbalance between ROS generation and cellular system's ability for clearance, promotes damage to lipids, proteins and nucleic acids throughout the cell [2]. Mitochondrial DNA, however, besides its close contact with the respiratory chain, is not protected by histones or a nuclear envelope, becoming an easy target to oxidative lesions. Finally, it is important to cite that besides small, the mitochondrial genome encodes 13 proteins that take part in the oxidative phosphorylation complex [3] and mutations in such genes can also serve to increase ROS cellular levels. Such factors contribute to a high mutagenesis rate [3].

ROS accumulation can lead to DNA base modifications, deletions, strand breaks and crosslinks. Oxidative stress and DNA damage have been linked with multiple chronic conditions, such as cancer [4], neurodegenerative processes [5], diabetes [6], cardiovascular diseases [7, 8], chronic inflammatory diseases [9] and aging [10]. Here, we hypothesize that DNA damage may also be an important phenomenon to the pathophysiology of sepsis, an acute condition characterized by deregulation of the immune response and intense systemic inflammation. Since the brain is particularly susceptible in sepsis and mitochondrial and immune functions are tightly linked [11, 12], we decided to investigate DNA damage in the prefrontal cortex of wild-type and CRAMP-deficient mice, submitted or not to experimental sepsis.

Cathelin-derived antimicrobial peptide (CRAMP) is an antimicrobial peptide that modulates several aspects of the immune response [13]. It is the only cathelicidin in rodents and its counterpart in humans is named LL-37. Cathelicidins are a family of antimicrobial peptides able to directly kill a range of

pathogens, including bacteria, protozoa and virus. Despite that, cathelicidins also play a dual role in the immune-inflammatory response through intriguing and poorly understood mechanisms. Indeed, depending on the disease and cellular context, cathelicidins can stimulate or inhibit the immune-inflammatory system [14].

Materials And Methods

Cecal ligation and puncture

Young (8 weeks-old) and aged (18 months-old) male CRAMP−/− mice on a C57BL/6 genetic background and their matched WT controls were purchased from The Jackson Laboratory (ME, USA).

We induced peritonitis using the model of cecal ligation and puncture (CLP), as previously described [15]. Briefly, animals were anesthetized and the cecum ligated and punctured twice with a 21G needle, allowing fecal material to be released into the peritoneal cavity. Animals were sacrificed 24 hours after the surgery and plasma and tissue samples of the brain (prefrontal cortex) were collected for further analyses.

DNA extraction

The samples were prepared according to the instructions in the Qiagen DNeasy Blood and Tissue kit (#69506 Qiagen). DNA was eluted in 100 µl of elution buffer. The concentration of genomic DNA was determined using Nanoview (GE). Samples were diluted in elution buffer for the PCR assays (6 ng/µL).

PCR reaction

Amplification of a 16540 bp segment of mitochondrial DNA was performed using Accu Prime Taq DNA Polymerase High Fidelity (#12346-086 Invitrogen) with forward and reverse primers (10µM); total DNA (30ng); Buffer II 10X (5µL); Taq DNA Polymerase (0,2 µL) and H_2O to complete 50 µL. Primers sequences were: forward, 5‘-TGAGGCCAAATATCATTCTGAGGGGC-3’ and reverse, 5‘-TTTCATCATGCGGGAGATGTTGGATGG-3’. PCR conditions were (1) 94°C for 30 sec; (2) 60°C for 30 sec, and (3) 68°C for 18 min (26 cycles).

Amplification of a 140 bp segment of mitochondrial DNA was performed using Taq DNA Polymerase (#10342-053 Invitrogen) with dNTP (10mM); MgCl2 (50mM); forward and reverse primers (10µM); total DNA (3 ng); Buffer 10X (5µL); Taq DNA Polymerase (0,2 µL) and H_2O to complete 50 µL. Primers sequences were: forward, 5‘-ACTTACGCAAAGGCCCCAACG-3’ and reverse, 5‘-GAGCTAAGGTCGGGGCGGTG-3’. PCR conditions were 94°C for 3 min; (1) 94°C for 45 sec; (2) 56°C for 30 sec and (3) 72°C for 1 min (22 cycles).

Statistical Analysis

Results were analyzed using Kruskal-Wallis test, followed by Mann-Whitney U test with Bonferroni adjustment. Results are shown in boxplots. All analyses were performed using R statistical software (www.r-project.org). A p-value < 0.05 was considered significant.

Results

CRAMP protects the brain from mtDNA damage under normal conditions, but not following experimental sepsis.

Both young wild-type mice and young CRAMP-deficient submitted to experimental sepsis showed significant mtDNA damage in the brain, when compared to the control groups ($p < 0.001$ and 0.003, respectively). Secondly, the presence of CRAMP protected the brain of wild-type mice from further mtDNA damage under normal conditions, but not following sepsis ($p = 0.011$). Aged mice were used as positive controls. As expected, aged mice exhibited more DNA damage in the brain than young mice.

Discussion

It is widely accepted that oxidative stress has a crucial role in sepsis evolution [16, 17]. Mitochondrial dysfunction and several ultrastructural changes have been reported in many organs during sepsis [18–22]. It has even been postulated that mitochondrial dysfunction plays a central role in the pathogenesis of the multiple organ dysfunction syndrome (MODS) that frequently follows the course of septic shock and many other inflammatory catastrophes [23, 24]. The topic, however, remains controversial. Some authors argue that the studies are still very heterogeneous and inconsistent [25]. DNA damage, for example, as far as we know, had never been investigated in sepsis. Here, we show that mitochondrial DNA damage is aggravated in the brain of wild-type and CRAMP-deficient mice, 24 hours after the induction of experimental sepsis, when compared to the control groups, putting in evidence that sepsis induces significant mtDNA damage (Fig. 1). Mitochondrial DNA damage, moreover, is more severe in the brain of healthy CRAMP KO mice, when compared to healthy wild-type mice, showing that CRAMP protects from mitochondrial DNA damage under normal conditions (Fig. 1)

We believe that the increase in mtDNA damage in CRAMP-deficient mice, detected only under normal conditions, but not following sepsis, occurred because sepsis induces such a robust inflammatory response that the protective effects of CRAMP became subtle in this situation. Septic encephalopathy patients, thus, may benefit from a targeted therapy directed to restore mitochondrial integrity [26].

Declarations

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Conflicts of interest/Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and material

The detailed results of our experiments are available upon request.

Code availability

Not applicable.

Authors' contributions

SKA performed the *in vivo* experiments. DFB and HVB performed the *in vitro* experiments. FPS and NCSP conceived the project. FPS analyzed the data and wrote the first draft. FPS and NCSP wrote the final manuscript.

Ethics approval

Protocols were in accordance with the University of São Paulo Faculty of Medicine Ethical Committee (project number 953/2017).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Not applicable.

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Figures

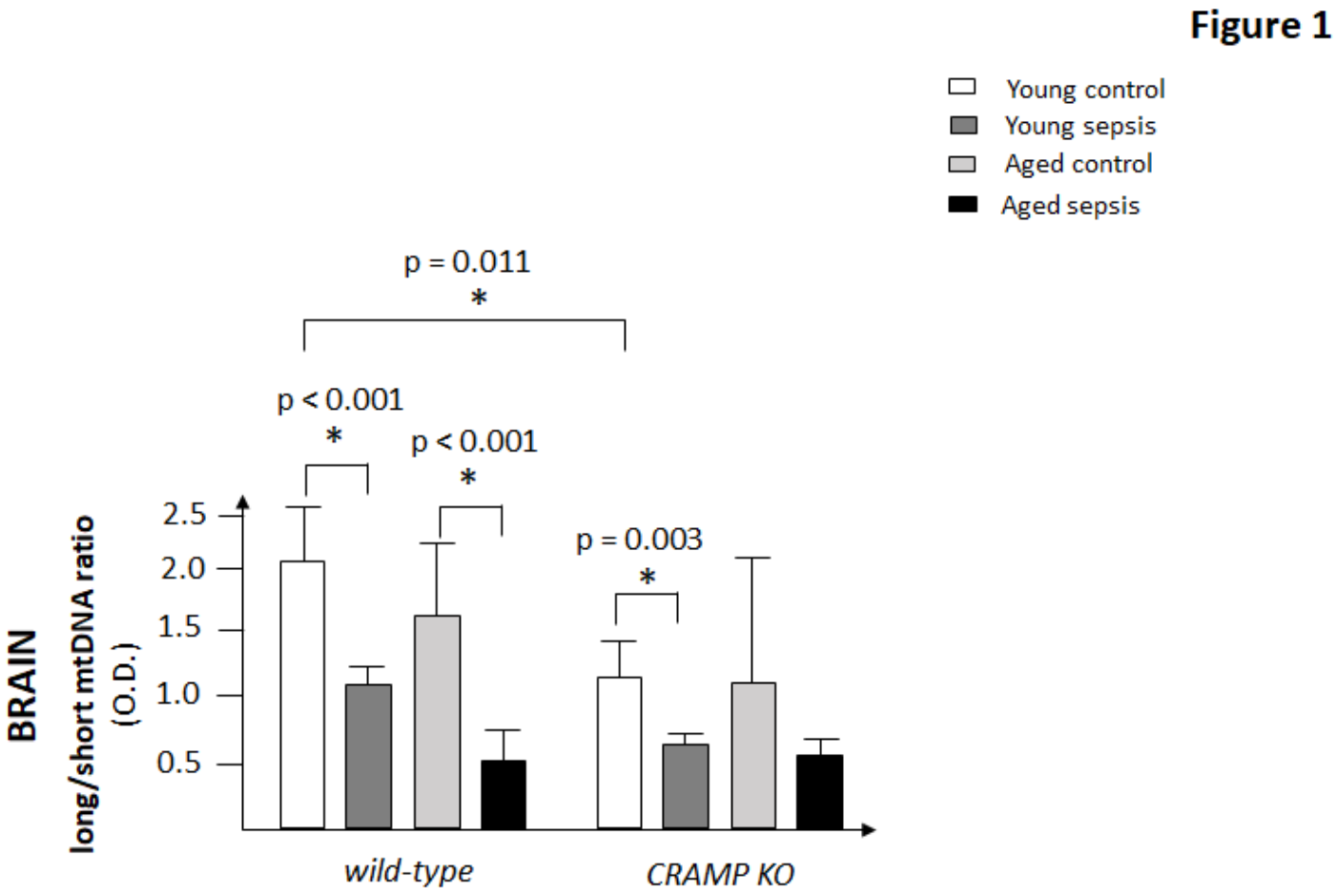

Figure 1

Ratio of mtDNA damage in the brain (prefrontal cortex) of wild-type and CRAMP-deficient mice under normal conditions and following experimental sepsis (n = 5-9 animals per group).