



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

Denise Frediani Barbeiro¹, Suely Kubo Ariga¹, Hermes Vieira Barbeiro¹, Nadja C. Souza-Pinto², Fabiano Pinheiro da Silva^{1*}

¹Department of Clinical Emergencies, University of Sao Paulo, Brazil

²Department of Biochemistry, University of Sao Paulo, Brazil

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.

Keywords: Cathelicidins; Innate immunity; Brain; DNA damage; Sepsis; Inflammation

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 and mutations in such genes can also serve to increase ROS cellular levels [3]. 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, neurodegenerative processes, diabetes, cardiovascular diseases, chronic inflammatory diseases and aging [4-10]. 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. Cathe-

Received:	31-January-2023	Manuscript No:	IPJICC-23-15590
Editor assigned:	02-February-2023	PreQC No:	IPJICC-23-15590 (PQ)
Reviewed:	16-February-2023	QC No:	IPJICC-23-15590
Revised:	21-February-2023	Manuscript No:	IPJICC-23-15590 (R)
Published:	28-February-2023	DOI:	10.35248/2471-8505.23.9.011

Corresponding author Fabiano Pinheiro da Silva, Department of Clinical Emergencies, University of Sao Paulo, Brazil, E-mail: pinheirofabiano@hotmail.com

Citation Barbeiro DF, Ariga SK, Barbeiro HV, Pinto NCS, Pinheiro da Silva F (2023) Cathelicidin Protects the Brain from Mitochondrial DNA Damage in Health, but not Following Septic Shock. J Intensive Crit Care. 9:011.

Copyright © 2023 Barbeiro DF, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

lin-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 C57 BL/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 21 G 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 μ l 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/ μ L).

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 (10 μ M); Total DNA (30 ng); Buffer II 10X (5 μ L); Taq DNA Polymerase (0,2 μ L) and H₂O to complete 50 μ L. Primers sequences were: Forward, 5'-TGAGGCCAAATATCATTCTGAGGGGC-3' and reverse, 5'-TTTCATCATGCGGGAGATGTTGGATGG-3'. PCR conditions were (1) 94 °C for 30 seconds; (2) 60 °C for 30 seconds, and (3) 68 °C for 18 minutes (26 cycles). Amplification of a 140 bp segment of mitochondrial DNA was performed using Taq DNA Polymerase (#10342-053 Invitrogen) with dNTP (10 mM); MgCl₂ (50 mM); Forward and reverse primers (10 μ M); Total DNA (3 ng); Buffer 10X (5 μ L); Taq DNA Polymerase (0,2 μ L) and H₂O to complete 50 μ L. Primers sequences were: Forward, 5'-ACTTACGCAAAG-GCCCAACG-3' and reverse, 5'-GAGCTAAGGTCTGGGCGGTG-3'. PCR conditions were 94 °C for 3 minutes; (1) 94 °C for 45 seconds; (2) 56 °C for 30 seconds and (3) 72 °C for 1 minute (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 [26]. 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 (Figure 1).

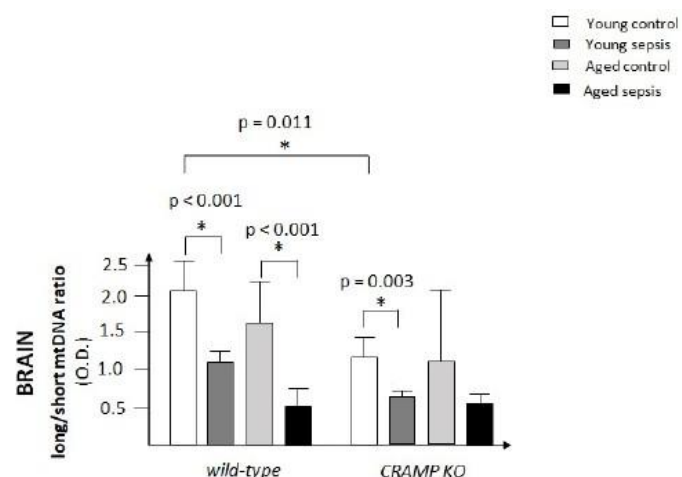


Figure 1: Ratio of mtDNA damage in the brain (prefrontal cortex) of wild-type and CRAMP-deficient mice under normal conditions and following experimental sepsis (n=5-9 animals per group).

CONCLUSION

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.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

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

FUNDING

FPS is supported by FAPESP, the Sao Paulo Research Foundation (grant #2020/03905-2020/03908), and by CNPq, the National Council for Scientific and Technological Development (grant #303924/2018-303924/2017).

AVAILABILITY OF DATA AND MATERIAL

The detailed results of our experiments are available upon request.

AUTHOR'S 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 Sao Paulo Faculty of Medicine Ethical Committee (project number 953/2017).

REFERENCES

- Chatterjee N, Walker GC (2017) Mechanisms of DNA damage, repair and mutagenesis. *Environ Mol Mutagen* 58(5): 235-263.
- Valera AM, Canto C (2018) Mitochondrial stress management: A dynamic journey. *Cell Stress* 2(10): 253-274.
- Sharma P, Sampath H (2019) Mitochondrial DNA integrity: Role in health and disease. *Cells* 8(2): 100.
- Sosa V, Teresa M, Rosa S, Rosanna P, Hiroshi K, et al. (2013) Oxidative stress and cancer: An overview. *Ageing Res Rev* 12(1): 376-90.
- Salim S (2017) Oxidative stress and the central nervous system. *J Pharmacol Exp Ther* 360(1): 201-205.
- Burgos ME, Zaida AJ, Aranzazu MM, Francesca I, Irene E, et al. (2019) Relationship between oxidative stress, ER stress, and inflammation in type 2 diabetes: The battle continues. *J Clin Med* 8(9): 1385.
- Peng W, Guoding C, Yiping X, Jinna C, Peng W, et al. (2019) Mitochondrial dysfunction in atherosclerosis. *DNA Cell Biol* 38(7): 597-606.
- Potz BA, Sellke FW, Abid MR (2016) Endothelial ROS and impaired myocardial oxygen consumption in sepsis-induced cardiac dysfunction. *J Intensive Crit Care* 2(1): 20.
- Kirkham PA, Barnes PJ (2013) Oxidative stress in COPD. *Chest* 144(1): 266-273.
- Belenguer VA, Francisco JTS, Juan AAZ, Marta MR (2019) Oxidative stress and exceptional human longevity: Systematic review. *Free Radic Biol Med* 149: 51-63.
- Chen Y, Zhou Z, Min W (2018) Mitochondria, oxidative stress and innate immunity. *Front Physiol* 9: 1487.
- Park DW, Zmijewski JW (2017) Mitochondrial dysfunction and immune cell metabolism in sepsis. *Infect Chemother* 49(1): 10-21.
- Pinheiro da Silva F, Marcel CCM (2012) Antimicrobial peptides: Clinical relevance and therapeutic implications. *Peptides* 36(2): 308-314.
- Pinheiro da Silva F, Machado MC (2017) The dual role of cathelicidins in systemic inflammation. *Immunol Lett.* 182: 57-60.
- Wichterman KA, Baue AE, Chaudry IH (1980) Sepsis and septic shock--a review of laboratory models and a proposal. *J Surg Res* 29(2): 189-201.
- Mantzaris K, Tsolaki V, Zakynthinos E (2017) Role of oxidative stress and mitochondrial dysfunction in sepsis and potential therapies. *Oxid Med Cell Longev* 2017: 5985209.
- Nagar H, Piao S, Kim CS (2018) Role of mitochondrial oxidative stress in sepsis. *Acute Crit Care* 33(2): 65-72.
- Aki T, Unuma K, Uemura K (2017) Emerging roles of mitochondria and autophagy in liver injury during sepsis. *Cell Stress* 1(2): 79-89.
- Hu Q, Jianan R, Huajian R, Jie W, Xiuwen W, et al. (2018) Urinary mitochondrial DNA identifies renal dysfunction and mitochondrial damage in sepsis-induced acute kidney injury. *Oxid Med Cell Longev* 2018: 8074936.
- Pan P, Wang X, Liu D (2018) The potential mechanism of mitochondrial dysfunction in septic cardiomyopathy. *J Int Med Res* 46(6): 2157-2169.
- Gonzalez AS, Elguero ME, Finocchietto P, Holod S, Romorini L, et al. (2014) Abnormal mitochondrial fusion-fission balance contributes to the progression of experimental sepsis. *Free Radic Res* 48(7): 769-783.
- Parikh SM, Yuan Y, Liyu H, Chengyuan T, Ming Z, et al. (2015) Mitochondrial function and disturbances in the septic kidney. *Semin Nephrol* 35(1): 108-119.
- Pool R, Gomez H, Kellum JA (2018) Mechanisms of organ dysfunction in sepsis. *Crit Care Clin* 34(1): 63-80.

24. Wu Y, Yao YM, Lu ZQ (2019) Mitochondrial quality control mechanisms as potential therapeutic targets in sepsis-induced multiple organ failure. *J Mol Med (Berl)* 97(4): 451-462.
25. Jeger V, Siamak D, Stephan MJ, Jukka T (2013) Mitochondrial function in sepsis. *Eur J Clin Invest* 43(5): 532-542.
26. Pinheiro da Silva F, Marcel CCM (2015) Personalized Medicine for Sepsis. *Am J Med Sci* 350(5): 409-413.