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Peptide VSAK Derived from the *C*-Terminal Region of CETPI Blocks LPS in an Animal Model of SIRS. Evidence Using PET-Imaging

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Introduction

Sepsis and septic shock are considered two of the most important life-threatening conditions worldwide. It is known that an uncontrolled inflammatory response is the main process responsible behind the harmful effects during the development of these conditions. This inflammatory response is well established and defined to be triggered by pathogen-derived molecules such as lipopolysaccharides (LPS) located at the surface of Gram-negative bacteria, known to be released in the early stages of sepsis.

Work carried out in our laboratory has demonstrated both *in vitro* and *in vivo*, that VSAK (VSAKPLSARSPGGRPLSP), a peptide derived from the last eighteen amino acids of the carboxy-end segment of CETPI, an isoform of the cholesteryl-ester transfer protein (CETP), identified by us several years ago [1,2], is able to bind LPS, and therefore also capable to prevent their deleterious effects [3].

The experimentation that is now presented employing Positron Emission Tomography (PET) using 2-[^{18}F] fluoro-2-deoxy-D-glucose (FDG) shows new evidence supporting the fact that the intravenous administration of peptide VSAK in a model of Systemic inflammatory response syndrome (SIRS) established in the rabbit, prevents these animals from developing septic shock after the administration of LPS [4].

Materials and Methods

During the experiment, twelve male Dutch dwarf rabbits were used randomly assigned to four experimental groups: 1) Control, 2) VSAK treatment, 3) LPS treatment, and 4) LPS+VSAK treatment. Rabbits from the different groups were administered FDG as radiotracer and monitored by PET for 90 minutes. Depending on the experimental group, LPS, VSAK, or LPS+VSAK were further administered. For the LPS+VSAK group, the intravenous administration was carried out with 2-minutes interval using different ears. PET data analysis was carried out using spherical areas to define Volume of Interest (VOI) in order to reduce slight variations associated with differences in animal size. In addition, specific tissue activity was measured using 1gm of tissue samples from each animal. Additionally, the level of several pro-inflammatory cytokines was analyzed in the plasma obtained from all experimental animals [5].

Results and Discussion

In this work, we studied the effects of VSAK as a potential peptide with the ability to hamper LPS effects *in vivo*. This study was focused on the metabolic dysfunction of glucose that occurs during the early stages of SIRS after an LPS intravenous challenge, studied by PET technology. Figure 1 shows representative images obtained from the PET data reconstruction analysis. The left panel corresponds to an animal from the control group, only administered with saline buffer, showing a normal distribution FDG pattern in organs such intestine and liver. The organs showing the highest signal correspond to the kidneys showing after 90 min an important rate of FDG elimination. The central panel shows a representative

image from the LPS-treated animals, showing the lack of glucose perfusion in most organs, mainly the liver. In contrast with this result, animals that were administered first with LPS and almost simultaneously with peptide VSAK (LPS+VSAK, right panel), the recovery of FDG uptake in peripheral tissues is observed, showing in the experimental animals from this group an uptake pattern similar to the one observed with the control group. Non-relevant changes in FDG uptake were observed in animals administered only with VSAK (data not shown). These findings suggest that an alteration in the uptake of glucose occurs when animals have LPS present in circulation. That effect is prevented when peptide VSAK is administered in a simultaneous way. Based on these results, the levels of circulating insulin and glucose were also measured in all experimental groups. It was found that an important increase in circulating glucose occurs in animals treated with LPS alone. That effect is prevented in animals also treated with peptide VSAK. Additionally, when insulin was analyzed, an increase was also found in circulation in LPS-treated animals. Circulating levels of insulin were found to be in the normal range when peptide VSAK was administered.

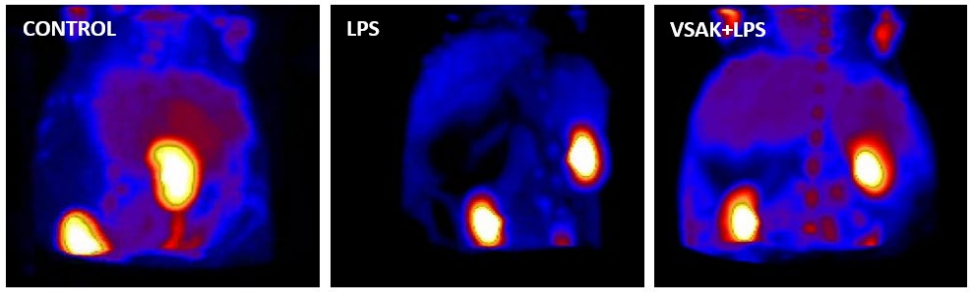

Fig. 1. Representative images obtained from animals of each experimental group. All images were rendered using data obtained from the 90-minute PET scans.

Altogether, the effects observed in FDG uptake, together with the alterations found in insulin and glucose levels in the LPS group, indicate that these experimental animals might be experiencing an insulin-resistant-like state (Figure 2). Since there is evidence that hyperglycemia observed during the course of sepsis and septic shock, can be due in part to the inability for glucose to enter the cell, energy deficiency has been proposed among the mechanisms responsible for cell dysfunction in septic shock, where low ATP levels and phosphocreatine/ATP ratios have been associated to non-surviving cases of septic shock.

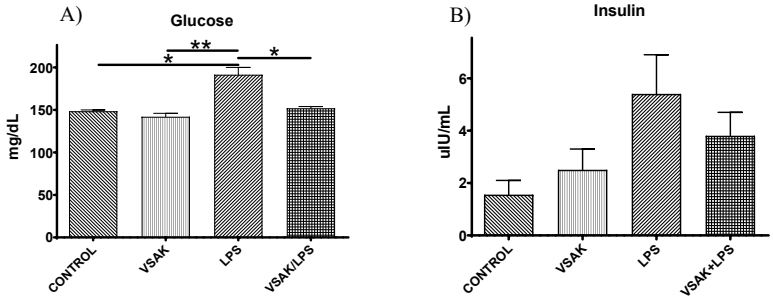

Fig. 2. Circulating levels of Glucose and Insulin. The graph A shows the levels of Glucose observed in the experimental animals. In the panel B the measured levels of Insulin are shown.

It has been previously reported that several cytokines involved in the pro-inflammatory response could cause alterations in the normal glucose uptake of insulin-dependent tissues.

This event does not occur exclusively during infectious processes since it can also be observed in other inflammation-related diseases. In this regard, an analysis of circulating pro-inflammatory cytokines was also performed in all the experimental groups studied (Figure 3). It is shown that not only circulating levels of TNFα, a highly related cytokine with a LPS challenge, but also IL-8 and MIP-1β, two cytokines with chemoattractant properties known to be released during the early stages of LPS-related sepsis, are importantly increased in the LPS treated group. Interestingly, TNFα that presents a central role in the response that leads to sepsis and septic shock, has been directly involved in the host's response mediated by the TLR4 receptor, and also associated to the development of insulin resistance.

Together with IL-1α, and IL-1β (data not shown), TNFα, IL-8, and MIP-1β show an important increase in their plasma level after the infusion of LPS, response shown to be avoided if peptide VSAK is concomitantly administered. Our results support the view that attenuation of the various pro-inflammatory molecules carried out by CETPI itself, or through peptides derived from this protein such as peptide VSAK, might be occurring during the early stages of SIRS.

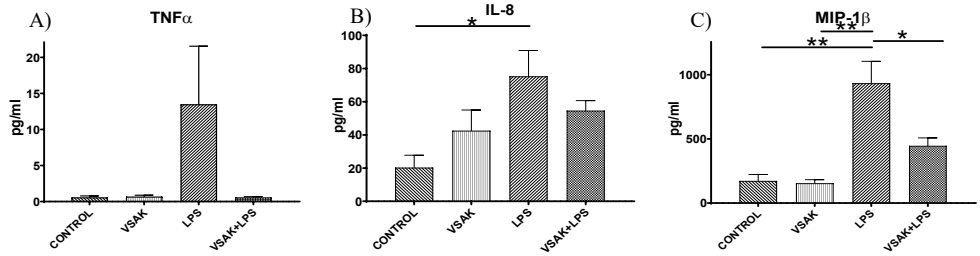

Fig. 3. Circulating pro-inflammatory cytokine levels observed in all experimental groups.

In order to find out and understand the molecular mechanisms that would explain the *in vitro* and *in vivo* association effect observed between LPS and peptide VSAK, it is important to understand the type of interactions that may occur between these two molecules. Therefore, we have employed coarse grain molecular dynamics of peptide VSAK peptide studied in association with LPS/DOPC composite systems (Figure 4). Our results support the possibility that independently of a direct interaction between peptide VSAK and LPS in plasma, an interaction between peptide VSAK and LPS already located in the plasma membrane of cells, might be able to decrease membrane fluidity that in turn interfere with the harmful cascade of cellular events known to be carried out by LPS.

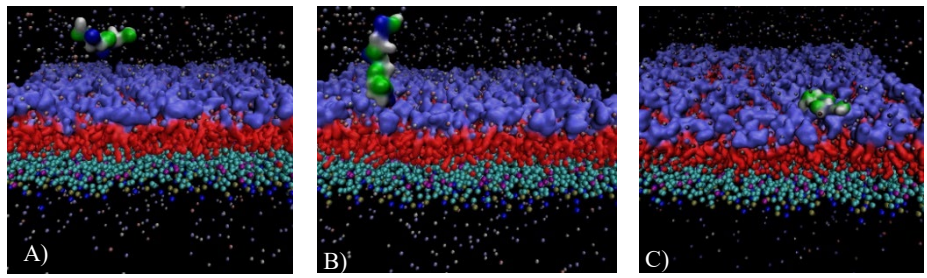

Fig. 4. Molecular Dynamics simulation of peptide VSAK and a LPS membrane. Panel A, the peptide is located in the solute interface. Panel B, the peptide shows a change in the orientation towards the LPS-POPC bilayer. Panel C, the peptide is incorporated into the lipid bilayer. LPS-core (purple), LPS-antigen A (red), POPC (cyan), VSAK hydrophobic aa (white), VSAK hydrophilic aa (green) and VSAK positive aa (blue).

Taken together, our results support that CETPI and peptides derived from its carboxy-end segment, such as peptide VSAK, present important LPS-binding properties associated with the regulation of key regulatory pathways linked to glucose metabolism and inflammation. These results reveal the physiological function of CETPI and support the role peptide VSAK might have in the clinic as a therapeutic LPS-binding peptide during sepsis and septic shock.

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