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LEUKOCYTE-ENDOTHELIAL CELL INTERACTIONS IN PRESENCE OF ACETHYLCHOLINESTERASE EFFECTORS AND LIPOPOLYSACCHARIDE

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ABSTRACT

There is evidence of elevated levels of acetylcholine (ACh) in response to hypoxia and an anti-inflammatory function of this molecule is also known. Acetylcholinesterase (AChE) is found both on neuronal and non-neuronal membrane cells and has acetylcholine as its natural substract; velnacrine maleate (VLN) has been used as an AChE inhibitor. The aim of this work was to evaluate the effect of intravenous injections of VLN and ACh, in absence and presence of a *pro*inflammatory effector – lipopolysaccharide (LPS). Using an intravital microscopy technique the number of rolling and adherent leukocytes was monitorized and blood samples were collected for cytokines (IL-1β and TNF-α) plasma concentrations determination. Our results show that in presence of an inflammatory state, induced by LPS, ACh has an anti-inflammatory effect, showed by a decrease in TNF-α levels and without alterations in the number of rolling and adherent leukocytes. The administration of LPS after ACh doesn't seem to have any protective effect on inflammation because increased levels of cytokines and leukocytes interactions are obtained. At variance the administration of VLN after LPS intensifies the increase of rolling leukocytes which is accompanied by augmented levels of IL-1β and TNF-α, although the opposite, the administration of LPS after VLN shows a lowering in the number of rolling leukocytes without significant alterations in the normal levels of cytokines. Thereby VLN seems to have a protective effect in the endothelial responses to an inflammatory state and ACh an anti-inflammatory role.

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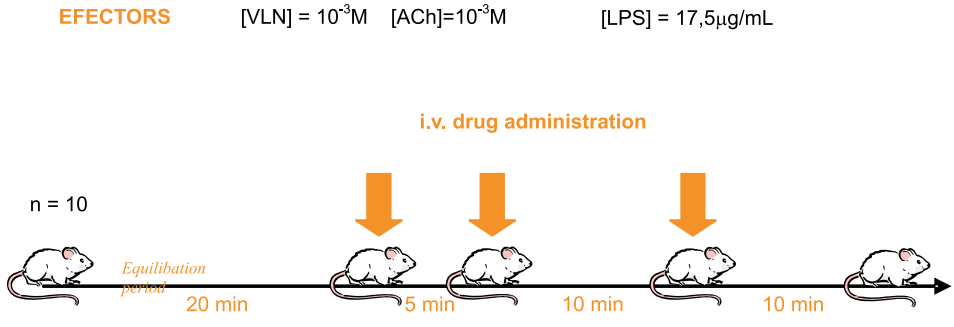
AIMS

The presence of acetylcholinesterase in sanguineous and endothelial cells has come to base the hypothesis of this protein to play, beyond the catalytic functions, functions of intercellular adhesion. It is already known that VLN modulates the leukocyte activation and it is also known that LPS, a component of the outer wall of most Gram-negative bacteria, is a potent inflammatory agent and plays a primary role in bacteria-induced leukocyte recruitment. In this basis, this work intends to study the effect of acetylcholinesterase effectors, VLN and ACh, before and after a LPS induced inflammatory state.

METHODS

- Anaesthesia – i.p with 1,5g/Kg body weight and i.m. 50mg/Kg body weight with ketamine;
- Tracheotomy – to maintain the animal in spontaneous breath;
- Catheterization – of right jugular for drug administration and left carotid artery for arterial pressure and cardiac frequency control;
- Cremaster preparation for intravital microscopy in an inverted microscope; 20 minutes of *post-s*urgical equilibration period in NaCl 0.9% pH 7.4.



RESULTS

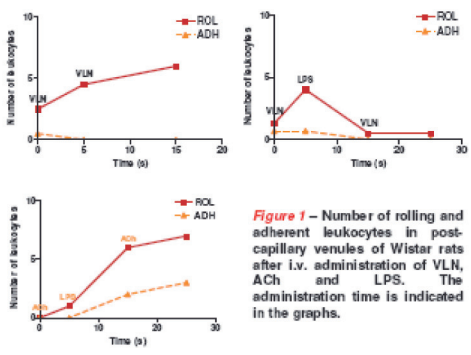


Figure 1 – Number of rolling and adherent leukocytes in post-capillary venules of Wistar rats after i.v. administration of VLN, ACh and LPS. The administration time is indicated in the graphs.

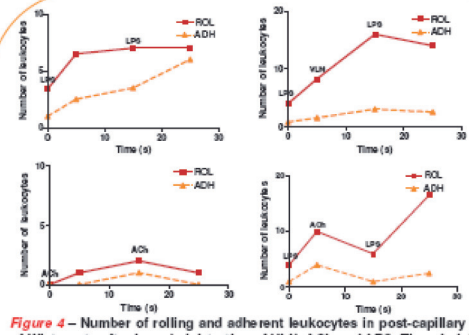
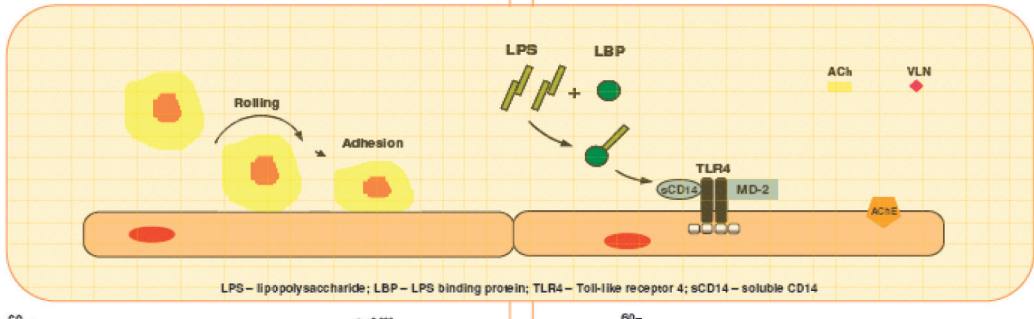


Figure 4 – Number of rolling and adherent leukocytes in post-capillary venules of Wistar rats after i.v. administration of VLN, ACh and LPS. The administration time is indicated in the graphs.



LPS – lipopolysaccharide; LBP – LPS binding protein; TLR4 – Toll-like receptor 4; sCD14 – soluble CD14

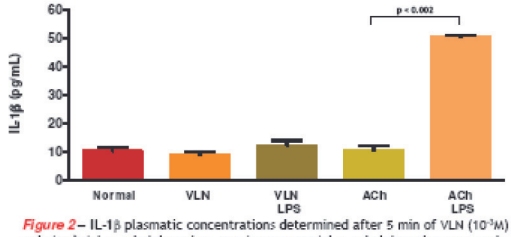


Figure 2 – IL-1β plasmatic concentrations determined after 5 min of VLN (10^{-3}M) or ACh (10^{-3}M) i.v. administration. LPS (17,5mg/mL) i.v. administration was made 10 min after the addition of the acetylcholinesterase effectors VLN and ACh.

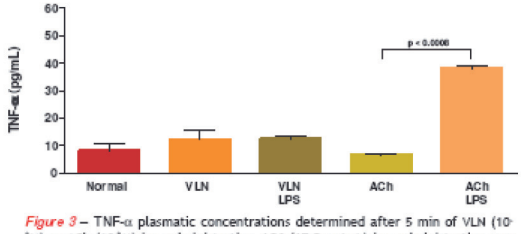


Figure 3 – TNF-α plasmatic concentrations determined after 5 min of VLN (10^{-3}M) or ACh (10^{-3}M) i.v. administration. LPS (17,5mg/mL) i.v. administration was made 10 min after the addition of the acetylcholinesterase effectors VLN and ACh.

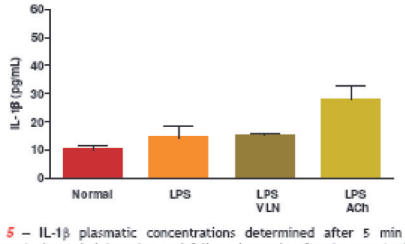


Figure 5 – IL-1β plasmatic concentrations determined after 5 min LPS (17,5μg/mL) i.v. administration and followed 10 min after by VLN (10^{-3}M) or ACh (10^{-3}M) i.v. administration.

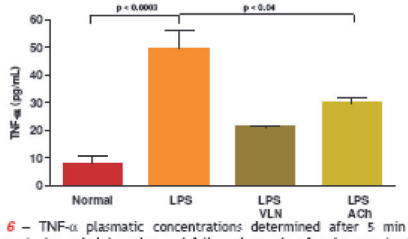


Figure 6 – TNF-α plasmatic concentrations determined after 5 min LPS (17,5μg/mL) i.v. administration and followed 10 min after by VLN (10^{-3}M) or ACh (10^{-3}M) i.v. administration.

CONCLUSIONS

Our *in vivo* experimental model of inflammation, induce:

- ⇧ rolling leukocytes, ⇧ TNF-α plasma levels
- After ACh administration: ⇩ rolling leukocytes, ⇩ TNF-α plasma levels
- After VLN administration: ⇧ rolling leukocytes, ⇩ TNF-α plasma levels

The same model with a "preconditioning" of the inflammation state, induce:

- With ACh: ⇧ rolling leukocytes, ⇧ TNF-α and IL-1β plasma levels
- With VLN: ⇧ rolling leukocytes

In inflammatory stimuli:

VLN shows a protective effect
ACh behaves as an anti-inflammatory

Ongoing experiences

sCD14, ACh and LBP determinations in order to evaluate
ACh and VLN effects on LPS signal transduction mechanism

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