VIII International Meeting on Cholinesterases

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EFFECTS OF THE ACETYLCHOLINESTERASE INHIBITOR, VELNACRINE MALEATE IN THE LEUKOCYTE-ENDOTHELIAL CELL INTERACTIONS

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ABSTRACT

It is known that velnacrine maleate (VM), an acetylcholinesterase (AChE) inhibitor, modulates the leukocyte activation. Previous studies have shown that in the presence of velnacrine there is an increase in the number of rolling leukocytes in post-capillary venules of Wistar rats' cremaster muscle. The aim of this work was to evaluate the effect of the intravenous (i.v.) injection of VM in absence and presence of a proinflammatory effector, the lipopolysaccharide from *E.coli* serotype 026:B6 (LPS). Using intravital microscopy the number of rolling and adherent leukocytes was monitorized. Blood samples were collected for leukocyte count and AChE activity determination.

Our results showed an increase in the number of rolling leukocytes by the action of LPS and VM, and a decrease in the AChE activity with both the used efectors. The administration of VM after LPS intensifies the increase of rolling leukocytes. Although the opposite, the administration of LPS after VM doesn't express an increase, but a lowering, in the number of rolling leukocytes. VM seems to have a protective effect in the endothelial or leukocyte responses to the LPS. The significance of these findings and the role of AChE are to be study more thoroughly.

AIMS

The presence of acetylcholinesterase in the sanguineous cells and in the endothelial cells has come to base the hypothesis of this protein to play, beyond the catalytic functions, functions of intercellular adhesion. It is known, by previous studies, that VM modulates the leukocyte activation. 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, in this work we intend to evaluate the effect of the i.v. administration of VM in the inflammatory response of LPS.

METHODS

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- ➔ 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 measure
- ➔ Cremaster preparation for intravital microscopy in an inverted microscope.
- ➔ 20 minutes of postsurgical equilibration period in NaCl 0,9% pH 7,4 perfusion

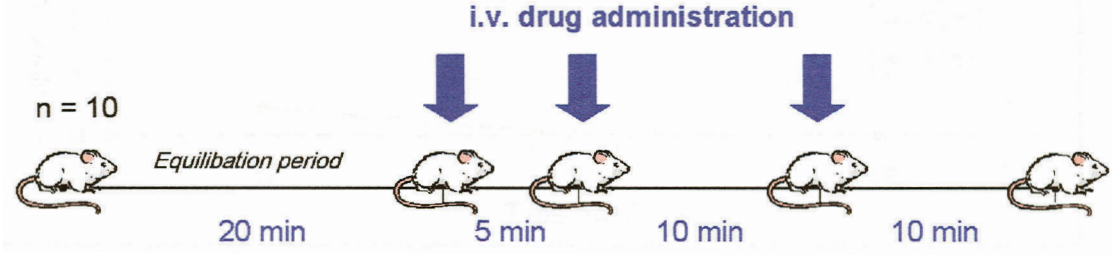

Figure 1 — Schematical representation of the experimental procedure

Four experimental groups were used:

1. LPS administration
2. VM administration
3. VM administration after 5 min of LPS induction
4. LPS administration after 5 min of VM induction

- ➔ Determination of the AChE activity in red blood rells (RBC) by Kaplan method.

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RESULTS

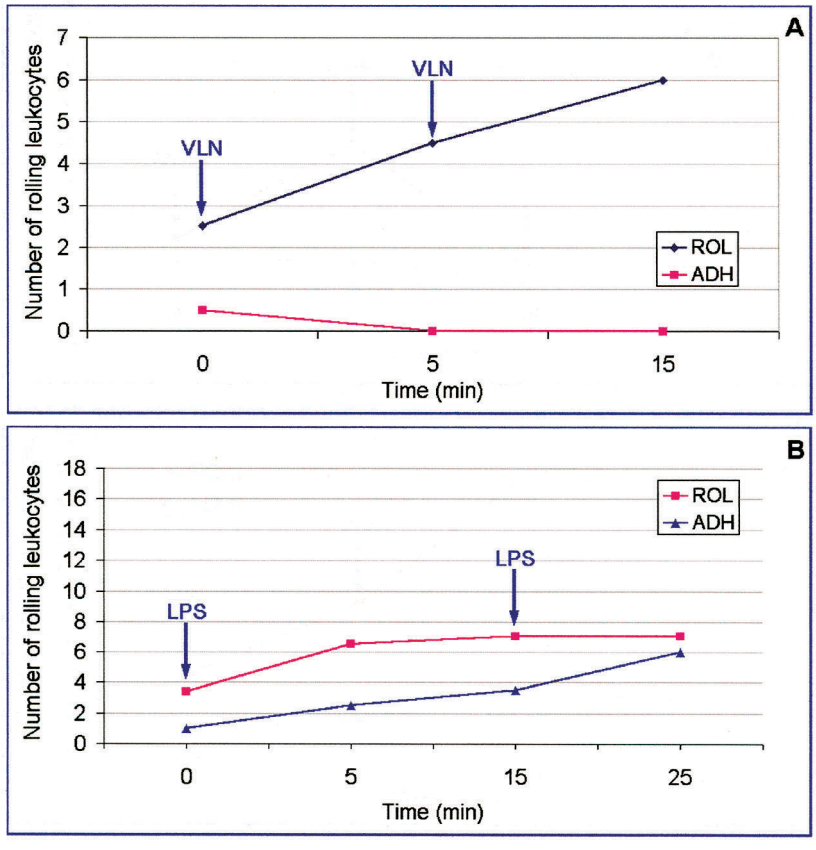

Figure 2 — Number of rolling (ROL) and adherent (ADR) leukocytes in the endothelium of post-capillary venules of Wistar rats cremaster muscle in presence of VLN (A) or LPS (B).

Time (min)	AChE (U/m/mg Hb)	
0	29,7	
5	LPS 21,5	VLN 20,0

Figure 3 — Determination of AChE activity in RBC after VLN and LPS administration.

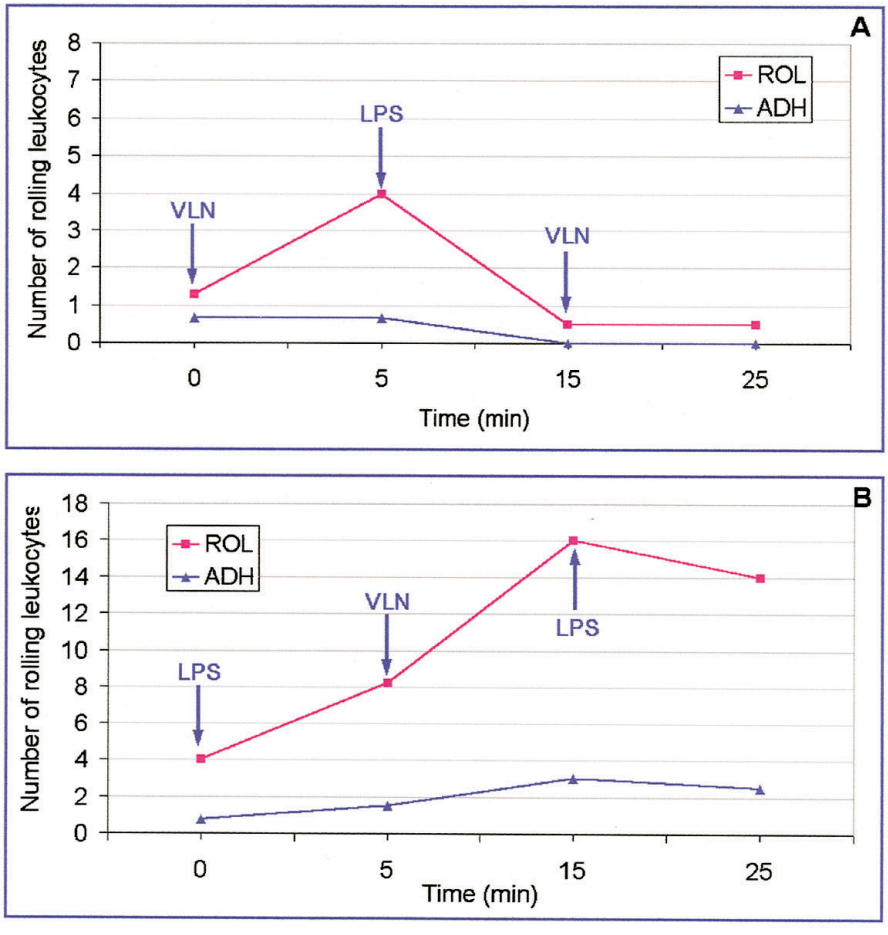

Figure 4 — Number of rolling and adherent white blood cells in the vascular wall of postcapillary venules of Wistar rats cremaster muscle, after the administration of LPS with VLN induction (A) and after VLN administration with LPS induction (B).

- in presence of LPS the number of rolling and adherent leukocytes increases
- with VM only the rolling leukocytes show significant increase
- both efectors lead to a decrease in the AChE activity
- the use of VM after the LPS induction intensifies the increase in the rolling leukocytes due to the LPS action
- although the rolling leukocytes decrease in presence of LPS after VM administration
- the AChE activity decreases after 5 min of VLN or LPS administration.

- **The previous results seem to describe two different mechanisms of action.**

When a LPS induction is made before VLN administration, the LPS response is intensified. In this case, when VLN is added the LPS signal transduction chain is already established and can not be inhibit by the formation of the VLN-AChE complex.

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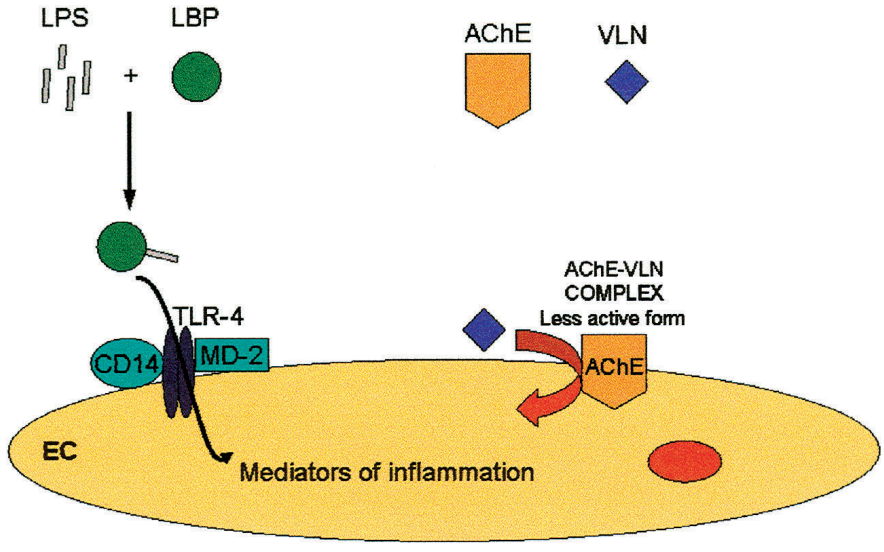

Figure 5 — Mechanism of action when the LPS induction is made after the VLN administration. LPS – lipopolysaccharide; LBP – LPS binding protein; AChE – Acetylcholinesterase; VLN – Velnacrine maleate

When the EC is first exposed to VLN the LPS induction doesn't lead to an increase in the rolling leukocytes, as seen in presence of LPS alone. This suggest that the formation of the VLN-AChE complex, witch is a less active form, inhibits the signaling pathways characteristic of LPS responses.

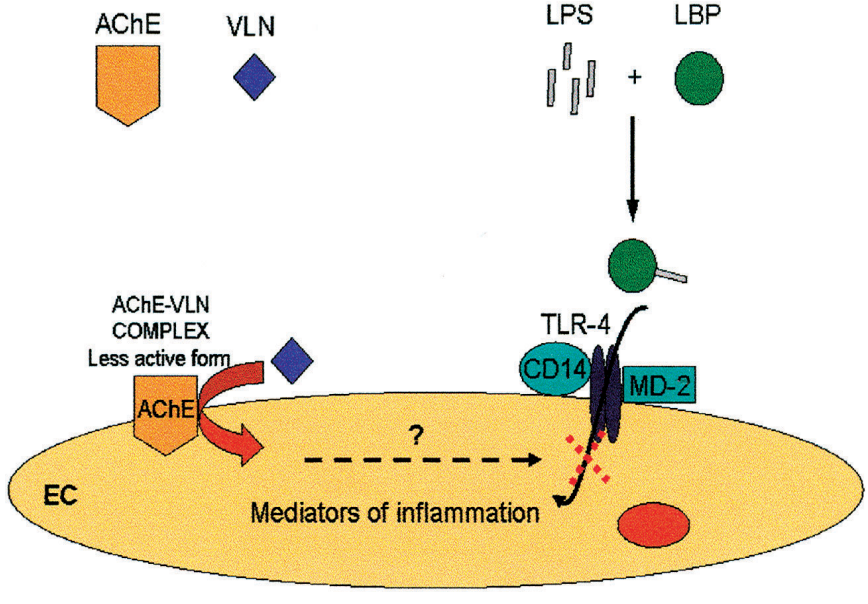

Figure 6 — Mechanism of action when the VLN is administrated after the LPS. LPS – lipopolysaccharide; LBP – LPS binding protein; AChE – Acetylcholinesterase; VLN – Velnacrine maleate

CONCLUSIONS

Velnacrine maleate, an acetylcholinesterase inhibitor, seems to have a protective effect in the endothelial or leukocyte responses to the LPS. The different results obtained in presence of both the effectors suggest that the less active form of AChE interacts in some way with the inflammatory signaling pathways of LPS. This interaction is to be study more thoroughly.