Advanced Glycation End-products (AGEs) mediate platelet hyperactivation through RAGE-dependent NFκB and p38 MAPK signalling

Poster

Fisiología Celular y Molecular

Daniela Recabarren-Leiva [1], Marcelo Alarcón Lozano [2, 3].

(1) Universidad de Talca, Department of Biomedical Science, Faculty of Health Sciences, 3460000, Talca, Chile.
(2) Universidad de Talca, Thrombosis Research Center, Faculty of Health Sciences, 3460000, Talca, Chile.
(3) Universidad de Talca, Department of Clinical Biochemistry and Immunohematology, Faculty of Health Sciences, 3460000, Talca, Chile.

drecabarrenleiva@gmail.com

Introduction: Receptor for Advanced Glycation End Products (RAGE) is a multi-ligand receptor implicated in atherothrombosis. However, its functional significance in platelets has not yet been characterized. Advanced glycation end products (AGEs) accumulate in diabetic and elderly patients, contributing to an increase in thrombotic risk.

Objective: Characterization of the AGE/RAGE axis and its molecular signalling pathways in the activation of human platelets.

Methods: We randomly selected 18 young volunteers, both male and female, aged between 18 and 35 years.The expression of RAGE on platelets was assessed by flow cytometry and western blot analysis. Platelet aggregation was measured using aggregometry after stimulation with AGE-BSA and TRAP-6. NFκB (p65) and p38 MAPK phosphorylation were analyzed by western blot. The specific RAGE inhibitor FPS-ZM1 and NFκB inhibitor BAY117082 was used to evaluate platelet aggregation. Finally, miRNA analysis identified potential regulatory targets. The statistical analysis T-Test and one-way ANOVA were performed with Prism 6.0c.

Results: The expression of RAGE was confirmed in platelets after stimulation with ADP or TRAP-6 by flow cytometry with up to 90% positive expression levels (Test T, $p < 0.001$).

AGE-BSA combined with TRAP-6 significantly improved platelet aggregation compared to the basal condition (T-Test, $p < 0.001$). FPS-ZM1 dose-dependently inhibited AGE-mediated platelet aggregation (one-way ANOVA, $p < 0.01$). Western blot analysis revealed an increase in NF-κB (p65) and p38 MAPK phosphorylation after AGE-RAGE activation, which was inhibited by both FPS-ZM1 and BAY 117082 (T-Test, $p < 0.01$). miRNA analysis showed potential regulatory networks, including miR-126-3p, miR-146a-5p, and miR-223-3p as therapeutic targets.

Conclusions: Human platelets express RAGE, which can be upregulated after stimulation with agonists such as ADP or TRAP-6. RAGE activation triggers intracellular signalling cascades, including activation of the NF-κB and p38 pathway in platelets. This study provides a novel mechanistic link between AGEs and platelet hyperactivation, identifying potential therapeutic targets for thrombotic disease.

Financiamiento:

This research was supported by the Centro de Investigación Trombosis y Envejecimiento Saludable (CITES), Universidad de Talca, and the doctoral scholarship from Universidad de Talca. We thank laboratory staff for technical assistance and participants for blood sample donation according to approved ethics protocols.

Declaración de certificación bioética:

FOLIO 01-2019, Comité Ético Científico (CEC) Universidad de Talca.