ACTION OF MODIFIED ERYTHROPOIETINS ON ENDOTHELIAL CELLS IN A PROINFLAMMATORY ENVIRONMENT

Chamorro ME, Schiavinatto D, Maltaneri R, Schiappacasse A , Nesse A, Vittori D.

Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto del Departamento de Química Biológica (IQUIBICEN) de la Facultad de Ciencias Exactas y Naturales. Buenos Aires, Argentina.

Erythropoietin (Epo), the erythropoiesis growth factor, is also known as an antiapoptotic agent. In this context, carbamylated Epo (cEpo) maintains the neuroprotective effect but fails to stimulate erythropoiesis. Despite the benefit of Epo treatment to overcome anemia associated to different pathologies, a significant number of patients, particularly those with cardiovascular or chronic renal diseases, fail to respond. This may be related to the simultaneous presence of other independent risk factors, such as hyperhomocysteinemia. This study was aimed at identifying Epo structural changes due to carbamylation and *N*-homocysteinylation, and investigating whether these changes could affect Epo activity on EA.hy926 endothelial cells in a proinflammatory environment. cEpo was prepared by reaction with potassium cyanate while homocysteine thiolactone was incubated with Epo to yield the *N*-homocysteinylated protein. Analysis by gel and capillary electrophoresis revealed structural changes with respect to the native protein. Wound healing assays showed a stimulatory effect of TNF-α on cell migration which was significantly increased by TNF-α+Epo combination (C 21±2%, *TNF-α 44±3%, Epo 30±2%, *EpoTNF-α 66±4%, *P<0.05, n=6),mediated by a mechanism involving EpoR induction. A similar effect of TNF-α+Epo was found on VCAM and ICAM mRNA expression (Real Time PCR, respect to C=1; VCAM: *TNF-α 22±3%, Epo 2±1%, *EpoTNF-α 36±2%, *P<0.01; ICAM: *TNF-α 76±9%, Epo 3±1%, *EpoTNF-α 123±5%, *P<0.05, n=3) as well as in monocytic cell adhesion assays (Fluorescence microscopy and fluorometric quantification with monocytic THP1 cells, n=6).The fact that no such activities were shown by the modified Epos suggests altered Epo function due to structural changes. In summary, the proangiogenic ability of Epo, enhanced in the presence of proinflammatory factors, might favor its action as a vascular protectant in ischemia and a mediator of lymphocyte migration dependent of adhesion molecules.

Keywords: Erythropoietin, TNF-alpha, V-CAM, cell adhesion, cell migration