Polymorphonuclear leukocyte function in patients with carotid plaque undergoing endarterectomy

F. Marino, L. Schembri, M. Tozzi, S. Ferraro, P. Castelli, M. Cosentino

Center of Research in Medical Pharmacology, University of Insubria, Italy

Background: Atherosclerosis is a disease in which local and systemic low-grade inflammation plays a pivotal role (1); polymorphonuclear leukocytes (PMNs) are present in atherosclerotic plaques and contribute to plaque progression (2,3), possibly representing biological markers of disease progression as well as potential therapeutic target (4). PMNs favor neoangiogenesis e.g. in cancer through the *in loco* production of proangiogenic factors such as interleukin (IL)-8 (5) and vascular endothelial growth factor (VEGF) (6), however no information is available regarding their role in neovessel formation in atherosclerotic plaques, which is critical for plaque destabilization and rupture.
We investigated the functional response of PMNs obtained from venous blood and their presence in carotid plaque specimens of patients (Pts) undergoing carotid endarterectomy (CEA) according to the American Heart Association guidelines (7).
Methods: The study included Pts suitable for CEA and healthy subjects (HS, according to the 'low cardiovascular risk' classification). Pts enrolled were divided in two groups according to instrumental assessment of plaques, namely with soft plaques (SP) and mixed plaques (MP). The day of surgery, venous blood for PMN isolation was taken before the administration of preoperatory drugs. After surgery, hematoxylin and eosin-stained sections were used for the histopatological examination and for histological/immunohistochemical studies. Isolated PMNs were cultured for 24 h under standard conditions, alone or in the presence of 0.1 μM fMLP, and at the end of culture supernatants and cells were harvested and stored for real time PCR and ELISA assay of IL-8, elastase and VEGF.
Results: We studied so far 50 Pts (39 M, 11 F; 22 SP, 28 MP). In comparison to cells from HS, circulating PMNs of Pts showed a significantly different profile of production of IL-8, elastase and VEGF: in particular mRNA expression for IL-8, VEGF and elastase were always higher than values measured in cells of HS; resting protein levels were lower (IL-8 and elastase) or similar (VEGF), while stimulated protein levels were always lower in cells of Pts with respect to values measured in cells of HS.
Among Pts, no significant difference in PMN mRNA levels of IL-8, elastase and VEGF was found between SP and MP subjects. IL-8 and VEGF (but not elastase) production was however higher both in resting and in fMLP-stimulated cells in Pts with MP in comparison to Pts with SP.
Immunohistochemical analysis of 8 soft and 5 mixed plaques showed that PMN (defined as CD66b+ cells) were preferentially distributed in in proximity of the areas of maximal stenosis while regions corresponding to common carotid artery and to internal carotid artery were negative for CD66b+ cells. No differences were observed in the pattern of distribution of CD66b+ cells between soft and mixed plaques.
Conclusions: Results support a role for PMNs in the pathogenesis and progression of carotid plaques. Additional studies are needed to establish their contribution to plaque neoangiogenesis, which is nonetheless suggested by the apparent relationship between plaque characteristics and PMN production of proangiogenic factors such as IL-8 and VEGF. PMNs could represent a suitable marker for the classification of Pts grade of risk as well as a promising targets for pharmacological approaches aimed at plaque stabilization and prevention of rupture.

References

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