POLYMORPHONUCLEAR LEUKOCYTE RHEOLOGY, CYTOSOLIC CA^{2+} CONTENT, $BETA_2$-INTEGRIN EXPRESSION AND OXIDATIVE STRESS IN HYPERTENSION

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Introduction

An elevated leukocyte count is associated to arterial hypertension, but in this brief review we focus our attention on some functional aspects of polymorphonuclear leukocytes (PMNs), considering in particular our data previously published on this topic. Leukocytes have a likely role in the pathogenesis of organ injury accompanying arterial hypertension. Monocytes and neutrophils can in fact participate in the progression of vascular lesions and in the pathophysiology of their ischaemic complications.

Leukocyte rheology

Hypertensives have a whole-blood hyperviscosity, due to increased leukocyte count, erythrocyte rigidity and hematocrit, and also to a raise of plasma viscosity, fibrinogen concentration, erythrocyte and platelet aggregation. As blood viscosity influences peripheral vascular resistances, changes of the haemorheological parameters may contribute to the development of hypertension and can facilitate myocardial and vascular remodelling[1].

In hypertensives the reduced membrane fluidity of blood cells affects carrier activity: in PMNs alterations of sodium and calcium plasma membrane pumps have been observed. Higher sodium cytosolic concentrations promote leukocyte activation, superoxide anion synthesis, adhesion to endothelium and progressive organ injury[2]. Several researches have previously demonstrated normal cytosolic Ca^{2+} concentrations in hypertensive monocytes and PMNs[3-5]. On the contrary, we observed increased cytosolic Ca^{2+} concentrations in PMNs[6], as well as in platelets and erythrocytes[7]. This datum was confirmed by more recent studies in insulin-resistant hypertensive men[8].

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In our research, at baseline, no correlation was present between PMN membrane fluidity and cytosolic Ca^{2+} content or between membrane fluidity and arterial pressure in hypertensives[7,9], whereas PMN membrane fluidity was decreased in hypertensives compared to normals[10]. After *in vitro* activation with PMA the initial relative flow rate (obtained by filtering unfractionated WBC and PMN suspension using a St. George's Filtrometer) was decreased in normals and hypertensives, but no other modifications were found. After activation with fMLP, Ca^{2+} content was furtherly increased only in hypertensives[11,12].

Leukocyte activation

In hypertensives, leukocytes have higher activation levels than in normal subjects[13]. Stimulated granulocytes release reactive oxygen species (ROS) which may inactivate nitric oxide (NO), arachidonic acid metabolites which induce vascular inflammation, and cathepsin G which stimulates the angiotensin system [14]. Primed PMNs may also initiate platelet aggregation[14] and provoke leukocyte adhesion to the endothelium promoting the hyperexpression of adhesion molecules[15], and the NO inactivation by superoxide anion. NO is in fact an endogenous inhibitor of leukocytes adhesion in postcapillary venules[16].

Subjects with essential hypertension have increased plasma levels of leukocyte elastase[17,18], that is considered a sensitive diagnostic marker of cardiovascular disease. Higher elastase levels are associated with the presence of atheromatous plaques[19] and are related to arterial stiffness[18]. In spontaneously hypertensive rats (SHRs) circulating PMNs have lower alkaline phosphatase content, while the plasma levels of this enzyme are elevated in these models[16,20]; this finding is another indicator of leukocyte activation and spontaneous degranulation and reflects the alteration of leukocyte function too.

Leukocytes and oxidative stress

Leukocytes activated by soluble or cellular stimuli start the respiratory burst, characterized by increased oxygen consumption and generation of superoxide (O_2^-), hydrogen peroxyde (H_2O_2) and other ROS. O_2^- synthesis by hypertensive PMNs is related to blood pressure[21]. ROS contribute to hypertension and its complications causing endothelial damage, oxidizing circulating low density lipoproteins and degrading NO. Serum NO levels and arterial pressure are negatively correlated[22]. The decreased availability of NO has negative effects on vascular tone and influences platelet aggregation, leukocyte adhesion and expression of adhesion molecules[23]. Oxidative stress in PMNs is related to arterial mean pressure, as well as oxidative stress in monocytes is related to C-reactive protein (CRP)[24].

A research regarding Sabra hypertension-resistant rats (SBN) and hypertension-prone rats (SBH) has shown that in pre-hypertensive SBH oxidative stress and primed PMNs are present. After salt-loading for four weeks, SBH rats develope hypertension and their PMNs pro-

duce elevated levels of ROS[25]. A long-term antioxidant treatment with Tempol (a membrane-permeable SOD mimetic), initiated in pre-hypertensive SHR, can attenuate vascular oxidative stress and prevent the age-related elevation of blood pressure in this animal model of genetic hypertension[26].

A recent study revealed a spontaneous up-regulation of CD11a and CD18 in circulating PMNs and an increased ROS generation by T-lymphocytes and monocytes in the peripheral blood of obese Zucker rats, before the development of hypertension[27]. This data suggest that primed PMNs, oxidative stress and inflammation anticipate and possibly contribute to the development of hypertension and its complications.

In Sabra rats salt-loaded for sixty days, the increase of blood pressure can be inhibited by phenylarsine oxide, an NADPH oxidase inhibitor. This finding suggests that primed PMNs play a role in the development of hypertension via activation of NADPH oxidase and production of ROS[28]. Because the leukocyte NADPH oxidase is localized in the plasma membrane, the alterations of its activity may be related to the membrane abnormalities observed in hypertension[29].

Leukocyte integrins

In hypertensives, the increased expression of adhesion molecules on endothelial cells and leukocytes promotes leukocyte adhesion to the endothelium[30]. A research on SHR has demonstrated a decreased endothelial expression of P-selectin[31]. On the contrary, another study showed a venular P-selectin hyperexpression, that is considered responsible for an increased rolling of PMNs[32]. The increased level of soluble E– and P-selectin in malignant and renovascular hypertension is due to platelet activation[33]. E-selectin, an indicator of organ injury, is related to blood pressure even in hypertensives without complications[34]. Benidipine, a calcium channel blocker, decreases arterial pressure and the concentrations of E– and P-selectin[35]. In coronary endothelial cells derived from human heart, angiotensin II induces a concentration-dependent increase in E-selectin expression via activation of AT1 receptors. So, the E-selectin-mediated leukocyte adhesion to the endothelium can be blocked using AT1 receptor antagonists[36]. The infusion of angiotensin II *in vivo* induces high levels of soluble ICAM-1 (Intracellular Adhesion Molecules-1) in hypertensives and normals[37]. Hypertension increases cytokine expression and concentrations of ICAM-1 and MCP-1 (monocyte chemoattractant peptide-1), responsible for the inflammatory cell infiltration of the subendothelium[38]: exposing mouse carotid arteries at high intraluminal pressure, after 24 hours an increased mRNA expression of MCP-1, IL-6, chemokines and VCAM-1 (Vascular Cell Adhesion Molecule-1) is detectable in monocytes[39]. VCAM-1 is positively related to systolic blood pressure[40]. The induction of hypertension in genetically normal rats can lead to an overexpression of the CD18 integrin on circulating helper T-cells[41]. In obese hypertensives an increased expression of CD11b on circulating monocytes and of CD68

on macrophages in the adipose tissue was found[42]. Studies evaluating PMN beta$_2$-integrins showed at baseline a phenotypical hyperexpression of CD11a, CD11b and CD18, but not of CD11c. After *in vitro* activation an increase of CD11b, CD11c and CD18 has been demonstrated, but also a decrease of CD11a, especially with PMA[43]. The integrin overexpression on PMNs at baseline can be due to the spontaneous granulocyte activation. The fall in CD11a expression after activation might be due to different mechanisms: because CD11a is constitutively expressed on PMN surface and it is not internalized during activation, its decrease may be related to an altered cleavage or to a dysregulated phosphorylation/dephosphorylation process[44]. However, up to now the mechanisms that explain the integrin pattem are only partially known.

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