Journal of Human Hypertension (2008) 22, 223–225

www.nature.com/jhh

RESEARCH LETTER

Linking inflammation and hypertension in humans: studies in Bartter's/Gitelman's syndrome patients

Journal of Human Hypertension (2008) **22,** 223–225;
doi:10.1038/sj.jhh.1002309; published online 29 November 2007

Inflammation and the relationship between inflammation, angiotensin II (Ang II) and its signalling have been recognized as playing a critical role in hypertension and atherosclerosis, but few data are available in humans. We explored in Bartter's/Gitelman's syndrome (BS/GS) patients, who do not develop hypertension and related cardiovascular remodelling and atherosclerosis despite high Ang II levels, the inflammation status through the evaluation of factors included in Ang II signalling involved in inflammation, and found unchanged phosphorylated extracellular signal-regulated kinase (pERK)/ERK ratio and p66^{shc} compared to controls while IκB, the inhibitory subunit of nuclear factor-κB, was increased. This, together with the unchanged level of inflammatory markers that we previously reported in BS/GS, depicts a picture opposite to that of hypertension and indirectly supports a link between inflammation and hypertension.

Hypertension and atherosclerosis are thought as diseases in which inflammation plays a central role.[1,2] The development and progression of these diseases are in fact associated with increased expression and production of pro-inflammatory mediators, including cytokines, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, monocyte chemotactic protein-1, nuclear factor-κB (NF-κB) and growth factors.[1,2] Both inflammation and Ang II are involved in the pathogenesis of cardiovascular and renal diseases, and Ang II itself is a powerful pro-inflammatory cytokine and growth factor.[3,4] In addition, Ang II activates NF-κB, a key nuclear transcription factor in inflammation and fibrosis, and this activation leads to the release of downstream inflammatory signalling molecules and induction of oxidative stress, tightly linked with inflammation.[3,4] The possibility of confirming this mechanism in humans could come from the study of a human model for vascular tone and structure regulation, which has biochemical and hormonal characteristics typical of hypertension, yet shows hyporesponsiveness to pressors, reduced vascular resistance and normo/hypotension such as BS/GS.[5,6] Our extensive series of studies have, in fact, provided mechanistic explanations for the vascular hyporeactivity typical of these patients and also led us to propose that BS/GS is a good human model to explore the mechanisms responsible for Ang II signalling.[5,6] In BS/GS, we have demonstrated that the Ang II signalling pathway is blunted as documented by the increased regulator of G-protein signalling-2 gene and protein expression, reduced gene and protein expression of the α-subunit of the Gq-binding protein, which transduces Ang II signal, and reduced related downstream cellular events such as intracellular Ca^{2+} and IP_3 release and PKC activity.[5,6] This abnormal G-protein-mediated signalling of Ang II shown in BS/GS patients, combined with downregulation of RhoA/Rho-kinase pathway[7] and upregulation of NO system,[5,6] reduced peripheral resistance, vascular hyporeactivity, normo/hypotension typical of these patients and their collection of biochemical characteristics, presents a mirror image of that found in hypertension. In addition, BS/GS patients have unchanged levels of C-reactive protein, serum amyloid A, vascular cell adhesion molecule, intercellular adhesion molecule, interleukin-6 and tumor necrosis factor-α.[8] This in the presence of high levels of Ang II and upregulated activity of the NO system again suggests that BS/GS is the mirror image of hypertension, and further stresses the critical role of Ang II in inflammation-related processes. The current study extends our findings, determining in our cohort of extensively characterized 12 BS/GS patients, 2 BS and 10 GS,[5–8] the levels of another set of pathways involved in Ang II signalling in inflammation such as ERK, IκB, the inhibitory subunit of NF-κB, and p66^{shc}, adaptor protein involved in the response to oxidative stress. Twelve sex- and age-matched normotensive healthy subjects from the staff of the Department of Clinical and Experimental Medicine at the University of Padova were used as controls. None of the patients or controls took drugs for at least 2 weeks prior to the study and all abstained from food, alcohol and caffeine-containing drinks for at least 12 h prior to sample collection. Mononuclear cell ERK-1 and ERK-2 phosphorylation and IκB protein content were evaluated using western blot analysis, while p66^{shc} gene expression was evaluated using reverse transcription-PCR. Their quantification was done using NIH image software through the ratio between phosphorylated ERK-1/2 and ERK-1/2 as indexes of

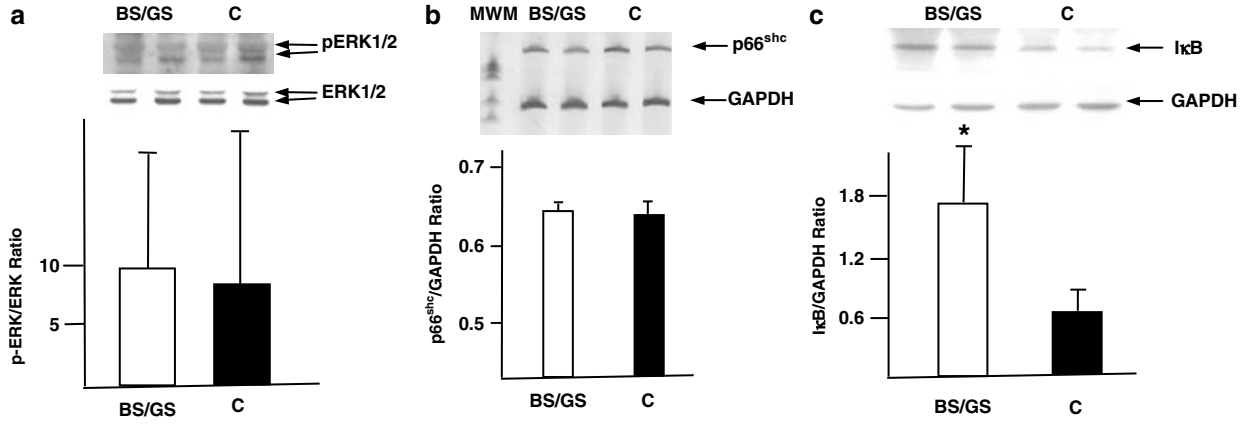

Figure 1 Densitometric analysis of the ratio of phosphorylated ERK to ERK (**a**), and $p66^{shc}$ to GAPDH (**b**) and IκB to GAPDH (**c**) in mononuclear cells of patients with BS/GS and healthy controls (C). The top of the figure shows a representative phosphorylated ERK1/2 and ERK 1/2 (**a**) and IκB and GAPDH (**c**) immunoblot products from two BS/GS patients and two controls. (**b**) Polyacrylamide silver-stained gel of $p66^{shc}$ and GAPDH PCR products of two representative BS/GS patients and two controls. BS/GS, Bartter's and Gitelman's syndrome; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MWM, molecular weight marker. $*P<0.02$.

ERK-1/2 activation, the ratio between IκB-α and glyceraldehyde-3-phosphate dehydrogenase as an index of IκB-α protein content and the ratio between $p66^{shc}$ and glyceraldehyde-3-phosphate dehydrogenase PCR products as an index of $p66^{shc}$ gene expression.

Phosphorylated ERK to ERK ratio was not different between BS/GS patients and controls: 9.02 densitometric units (d.u.) ± 5.8 versus 8.04 d.u. ± 4.45, $P=0.89$, pointing toward an unchanged activation of ERK in BS/GS patients. $p66^{shc}$ gene expression was also unchanged in patients with BS/GS: 0.65 d.u. ± 0.08 versus 0.64 d.u. ± 0.09, $P=0.84$, confirming the normal oxidative status of BS/GS patients. On the contrary, the protein level of the inhibitory subunit of NF-κB, IκB, was significantly increased in patients with BS/GS: 1.75 d.u. ± 0.69 versus 0.67 d.u. ± 0.31, $P<0.02$, pointing toward a reduced activity of NFκB and therefore toward a reduced transcription of genes involved in local and systemic inflammation, which is mediated by NFκB activation (Figure 1).

Phosphorylated ERK1/2 is a member of the mitogen-activated protein kinases, which controls cellular protein synthesis and growth. Ang II activates ERK1/2, which has been implicated in cardiovascular disease by affecting vascular cell adhesion molecule differentiation, proliferation, migration and fibrosis.[9] Increased responsiveness of ERK to Ang II in hypertensive patients has been shown as a result of increased levels of oxidative stress and this was later shown to be correlated to the levels of regulator of G-protein signalling-2, the major regulator of Ang II signalling.[10] The increased expression of regulator of G-protein signalling-2 and the reduced expression of oxidative stress-related proteins, such as $p22^{phox}$ and PAI-1, and the reduced oxidative status that we have shown in BS/GS patients[5,6] fit with the results of the unaltered ERK signalling in BS/GS patients compared with healthy controls despite their high levels of Ang II.

$p66^{Shc}$ is an adaptor protein involved in the response to oxidative stress. When phosphorylated in response to oxidative stress, it sensitizes cells to apoptosis. A link between Ang II and $p66^{shc}$ has been demonstrated through the protective effect of $p66^{shc}$ genetic deletion on the Ang II-induced myocardial damage in rats, which implies a close relationship between the profibrotic action of Ang II and the Ang II-induced activation of $p66^{shc}$.[11] $p66^{shc}$ signalling was unaltered in BS/GS patients despite their high levels of Ang II, which fits with the low level of oxidative stress and other inflammatory markers that we have demonstrated in these patients.[5,6]

NFκB is another mediator involved in inflammatory and acute stress responses. It activates the transcription of many genes responsible for the production of factors involved in local and systemic inflammation playing a central role in cardiovascular disease and remodelling.[12] The activation of NFκB follows the induced degradation of IκB. IκB levels in BS/GS patients were increased compared to controls. This increase may be attributed to the reduced level of the RhoA–Rho kinase system that we reported in BS/GS[7] as this system induces the phosphorylation of IκB leading to its inactivation.[12] Thus, the combination of reduced RhoA–Rho kinase system[7] with increased IκB levels points toward a reduced NFκB activity.

In conclusion, the findings of the current study together with those derived from our earlier studies on BS/GS[8] contribute to give further insights into the pathophysiological mechanisms linking inflammation, hypertension and long-term complications such as cardiovascular remodelling and atherogenesis in humans.

What is known about this topic

- Hypertension and atherosclerosis are diseases in which inflammation plays a central role.
- Inflammation and Ang II play a role in the pathogenesis of cardiovascular and renal diseases, and Ang II itself is a powerful proinflammatory cytokine and growth factor.

What this study adds

- The present study demonstrates a decreased inflammatory status in a human clinical condition characterized by hormonal and biochemical abnormalities typical of hypertension, such as activation of RAAS, high Ang II level yet normo/hypotension, blunted Ang II cell signalling and upregulation of NO.
- This observation indirectly confirms the involvement and the critical roles played by both inflammation and Ang II in the pathogenesis of cardiovascular and renal diseases and their long-term complications such as atherogenesis and remodelling.
- The findings of this study may further highlight the utility of this human model in investigating pathways involved in cardiovascular pathophysiology.

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Published online 29 November 2007

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