Chronic subdural hematoma is highly prevalent in the elderly and represents one of the most frequent lesions encountered in neurosurgical practice. Although surgical treatment of CSDH is relatively simple, recurrence is not uncommon and is associated with increased adverse outcomes. Chronic subdural hematoma typically manifests following head trauma, and current hypotheses on its pathogenesis include the progression from an acute oligosymptomatic or asymptomatic hematoma and/or subdural hygroma. Morphologically, CSDH is characterized by the presence of an inner membrane of collagen-rich avascular tissue and an external neomembrane containing fragile and leaky capillaries, smooth muscle, and inflammatory cells. As a corollary, pathophysiological processes, including local inflammation and angiogenesis, several studies have previously investigated the contribution of VEGF, a key inducer of angiogenesis and vascular permeability. Thus, VEGF is highly upregulated in hematoma fluid, whereas both VEGF and VEGFR-1 have been previously shown to be enhanced in neomembrane.
brane cells. Furthermore, the level of VEGF immunopositivity in the neomembrane was previously shown to correlate with CSDH recurrence. Placental growth factor is a member of the VEGF family of cytokines. Unlike VEGF, abundant physiological expression of PlGF is limited to the placenta, where PlGF actions have been previously associated with placental trophoblast growth and differentiation. Nevertheless, previous clinical findings suggest that under various pathological conditions associated with excessive and aberrant angiogenesis, including proliferative diabetic retinopathy, arthritis, and atherosclerosis, enhanced PlGF expression contributes to inflammatory and angiogenic processes. The results of several experimental studies support a similar role for PlGF in promoting inflammation, angiogenesis, and plasma extravasation under pathological conditions alone or in synergism with VEGF and via actions mediated at least partly through VEGFR-1 receptors. The actions of both VEGF and PlGF are antagonized by a high-affinity receptor, namely sVEGFR-1. As a consequence, conditions that favor an imbalance between sVEGFR-1 and proangiogenic factors have been hypothesized to contribute to the induction of pathological angiogenesis.

Thus, given: 1) previous evidence implicating PlGF and its soluble receptor sVEGFR-1 in pathological angiogenesis and inflammation, and 2) considerable evidence implicating these 2 processes in CSDH development and progression, we sought to establish the levels of PlGF and sVEGFR-1 in both serum and hematoma fluid obtained in CSDH patients. Furthermore, we tested the hypothesis that the enhanced angiogenic capacity of CSDH is reflected by significant changes in the ratio of sVEGFR-1 and PlGF in hematoma fluid compared with serum.

Methods

The present study included all patients (n = 16) in whom a CSDH was surgically treated in our clinic between December 2010 and March 2011. Informed consent was obtained. There were 12 male and 4 female patients whose mean age (± SD) was 79.6 ± 7 years. All patients had a unilateral CSDH confirmed by CT or MRI; in 2 patients a layered CSDH was documented.

One hour prior to bur hole surgery, venous blood samples were collected, allowed to clot, and centrifuged at 2000g for 10 minutes prior to division of serum samples into aliquots and storage at −80°C. During surgery, 7–10 ml of hematoma fluid was collected and processed in a manner similar to blood samples. PlGF and sVEGFR-1 levels in serum and hematoma were quantified simultaneously in duplicate samples, using an automated electrochemiluminescence assay (Elecsys sFlt-1 [for sVEGFR-1] and Elecsys PlGF; Roche Diagnostics [Hellas]) according to the manufacturer’s instructions. Paired t-tests were used for the comparison of mean values. Statistical significance was set at p < 0.05, and statistical analysis was conducted using SPSS version 17.

Results

The mean levels of both PlGF and sVEGFR-1 as well as the mean sVEGFR-1/PlGF ratios in serum and hematoma fluid acquired in the 16 CSDH patients are presented in Table 1. Statistical analysis indicated that PlGF and sVEGFR-1 levels were significantly higher in hematoma fluid than in serum (p < 0.0001). In serum, levels of sVEGFR-1 were higher than those of PlGF (p < 0.0001), whereas in hematoma fluid this difference was not apparent. Furthermore, the sVEGFR-1/PlGF ratio was significantly lower in hematoma fluid than in serum (p < 0.0001).

Discussion

The present findings indicate that levels of PlGF and sVEGFR-1 were markedly higher in hematoma fluid compared with serum obtained in CSDH patients. Significantly, the present findings also indicate a lower sVEGFR-1/PlGF ratio in hematoma fluid compared with serum, a result consistent with the aberrant angiogenic capacity of CSDH.

From a technical perspective, the automated methods used to assess levels of PlGF and sVEGFR-1 have been readily validated as tools for assessing changes in circulating levels of the 2 factors associated with pre-eclampsia. Schiettecatte et al. reported a particularly good correlation between the results obtained with the automated assays used herein and manual enzyme-linked immunosorbent assays; the latter have been used to assess PI GF and sVEGFR-1 protein levels in a variety of biological fluids. Nevertheless, a significant advantage of the automated platform used in the present study is that, as preestablished by the manufacturers, analyte concentration measurement are not affected by hemolysis or lipemia, a significant consideration when analyzing CSDH samples. Moreover, the relatively high sensitivity, precision, and repeatability of the automated platform used in the present study allows for routine clinical applications. Finally, levels of PlGF and sVEGFR-1 that were quantified fell well within the defined limits of both electrochemiluminescence assays. An increased level of PlGF in the hematoma fluid of our CSDH patients is consistent with a large body of clinical and experimental evidence indicating enhanced expression of this factor under conditions that favor pathological angiogenesis. Expression of PI GF under such conditions has been previously detected in various cell types, including inflammatory cells, vascular endothelial cells, fibroblasts, and smooth muscle cells. The potential of PI GF in promoting angiogenesis and inflammation is supported by numerous observations. Thus, in vitro PI GF has been previously shown to promote the growth, migration, and survival of endothelial cells, while either the exogenous application of PI GF or its endogenous overexpression potently stimulates angiogenesis in vivo. Additionally, PI GF has been shown to induce the proliferation of vascular smooth muscle cells and fibroblasts, to promote the recruitment of angiogenesis-competent myeloid progenitor cells, and to attract and activate inflammatory cells in releasing proinflammatory and angiogenic cytokines, including VEGF. Mice lacking PI GF show not only impaired angiogenesis but also reduced plasma extravasation dur-
and smooth muscle cells in vitro. Enhance PlGF expression in human vascular endothelial cells as another stimulus, as it has been shown to participate in an experimental atherosclerotic plaque model. In the context of CSDH angiogenesis, several additional findings regarding the cellular and molecular modes of PlGF action seem pertinent. Thus, in addition to a direct effect of PlGF on inducing angiogenic gene expression in endothelial cells via VEGFR-1 receptors, previous findings have revealed that PlGF can potentiate VEGF-driven angiogenesis via mechanisms that involve amplification of VEGF signaling.

Considering the possible mechanisms that underlie increased PlGF levels in CSDH, the following points can be made. Among the diverse stimuli previously shown to enhance PlGF expression under pathological conditions, those implicated in the mechanisms of CSDH include local elevation of inflammatory cytokines and VEGF overexpression. Moreover, enhanced expression of angiotensin 2 in the neomembrane of CSDH may represent yet another stimulus, as it has been shown to enhance PlGF expression in human vascular endothelial and smooth muscle cells in vitro. In this context, it is noteworthy that prior use of angiotensin-converting enzyme inhibitors for the control of arterial hypertension in CSDH patients was shown to correlate not only with reduced VEGF hematoma fluid levels but also with a reduced risk of lesion recurrence.

The results of the present study also indicated a higher level of sVEGFR-1 in subdural fluid than serum. Nevertheless, compared with serum, the ratio of sVEGFR-1 to PlGF in hematoma fluid was reduced approximately threefold. On the basis of previous findings, potential sources of sVEGFR-1 in CSDH may include vascular endothelial cells. By virtue of its capacity to inhibit the biological actions of VEGF and PlGF, sVEGFR-1 has been previously implicated in the control of angiogenesis, vascular permeability, and inflammation. Furthermore, the efficiency of sVEGFR-1 in suppressing abnormal angiogenesis and inflammation has been shown in experimental models of retinal neovascularization, arthritis, and tumor growth. Thus, a reduced sVEGFR-1/PlGF ratio in hematoma fluid of CSDH versus serum of CSDH, as demonstrated in the present study, is consistent with the aberrant inflammatory and angiogenic characteristics of CSDH. A limitation of the present study is that it is observational and thus provides no direct evidence for a causal link between the overt changes in PlGF and/or sVEGFR-1 levels and the formation/development of CSDH. In this context, future studies are warranted to dissect the role of PlGF and sVEGFR-1 in CSDH.

Conclusions

The present findings indicating markedly higher levels of PlGF in hematoma fluid suggest the involvement of this factor in the mechanisms of local inflammatory angiogenesis in CSDH. Consistent with such a role for PlGF in CSDH are previous findings implicating PlGF in the mechanisms of angiogenesis, inflammatory cell chemotaxis, and stimulation, as well as amplification of VEGF-driven signaling under various conditions characterized by excessive and aberrant angiogenesis. Furthermore, given that sVEGFR-1 has been previously implicated in the control of angiogenesis, vascular permeability, and inflammation, the reduced ratio of sVEGFR-1 to PlGF in hematoma fluid, as demonstrated by the present findings, is consistent with the inflammatory and proangiogenic characteristics of CSDH. Future studies are warranted to investigate the role of PlGF and sVEGFR-1 in CSDH.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Kalamianos. Acquisition of data: Kalamianos, Stavrinou, Koutsarnakis, Psachoulia. Analysis and interpretation of data: Kalamianos, Psachoulia. Drafting the article: Kalamianos. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Kalamatianos. Administrative/technical/material support: Stavrinou, Koutsarnakis, Psachoulia. Study supervision: Kalamianos, Psachoulia, Sakas, Stranjalis.

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PlGF and sVEGFR-1 in chronic subdural hematoma


Manuscript submitted February 10, 2012. Accepted October 8, 2012. Please include this information when citing this paper: published online November 9, 2012; DOI: 10.3171/2012.10.JNS12327. Address correspondence to: Theodosis Kalamatianos, Ph.D., Hellenic Centre of Neurosurgical Research “Professor Petros S. Kokkalis,” Poutarchou 3, Athens 106 75, Greece. email: theodosis.kalamatianos@kcl.ac.uk.