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Deregulation of levels of angiopoietin-1 and angiopoietin-2 is associated with severe courses of hantavirus infection

Christian Nusshag[a], Anja Osberghaus[a], Alexandra Baumann[a], Paul Schnitzler[b], Martin Zeier[a], Ellen Krautkrämer[a,*]

[a] Department of Nephrology, University of Heidelberg, Heidelberg, Germany
[b] Center for Infectious Diseases, Virology, University of Heidelberg, Heidelberg, Germany

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ABSTRACT

Background: Hantavirus disease is characterized by endothelial dysfunction. Angiopoietin-1 (Ang-1) and its antagonist angiopoietin-2 (Ang-2) play a key role in the control of capillary permeability. Ang-1 is responsible for maintenance of cell-to-cell contacts whereas Ang-2 destabilizes monolayers. An imbalance of Ang-1 and Ang-2 levels results in enhanced permeability and capillary leakage.

Objectives: To analyze the involvement of angiopoietins in hantavirus-induced disruption of endothelia, we measured the levels of Ang-1 and Ang-2 in hantavirus infection.

Study design: Levels of angiopoietins of 31 patients with acute Puumala virus (PUUV) infection and a patient infected with Dobrava-Belgrade virus genotype Sochi (DOBV-Sochi) were analyzed. An age-matched group of 16 healthy volunteers served as control. The ratios of Ang-2 to Ang-1 levels were calculated and correlated with laboratory parameters.

Results: Patients with PUUV and DOBV-Sochi infection exhibited elevated ratios of Ang-2/Ang-1 compared to the control group. The imbalance of Ang-2 to Ang-1 levels was observed early after onset of symptoms and lasted for the acute phase of infection. The deregulation in DOBV-Sochi infection was more prominent than in PUUV infection. Analysis of Ang-2/Ang-1 ratio and laboratory parameters in the PUUV cohort revealed a positive correlation with serum creatinine and a negative correlation with serum albumin and thrombocyte levels.

Conclusions: We observed an imbalance between levels of Ang-1 and Ang-2 in patients infected with PUUV and DOBV-Sochi. Elevated Ang-2/Ang-1 ratios correlate with disease severity. The virus-induced deregulation of angiopoietin levels may enhance capillary permeability and contribute to the pathogenesis of hantavirus disease.

1. Background

Infectious diseases are often associated with an impairment of epithelial and endothelial barrier function. Direct and indirect effects during the life cycle of the pathogen are responsible for cellular damage. Hantaviruses belong to the viral hemorrhagic fever viruses and interfere with the barrier function of epithelia and endothelia leading to organ failure [1–3]. The up- and down-regulation of inflammatory cytokines and angiogenic factors during infections substantially contribute to the disturbance of endothelia and may influence the clinical course of the disease [4–6]. Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) in the Old World and hantavirus cardiopulmonary syndrome (HCPS) in the New World [7]. HFRS is characterized by sudden onset with fever, thrombocytopenia, hematuria, elevated levels of serum creatinine, and decreased serum albumin levels. The infection often results in acute renal failure with massive proteinuria. The severity of HFRS depends on the virus species and may even differ between hantavirus genotypes. In contrast to PUUV infections that induce a milder form of HFRS, the pathogenicity of DOBV genotypes differs enormously and the infection with DOBV-Sochi is characterized by severe courses and a high case fatality rate of 14.5% [8].

Angiopoietins have been described to play a crucial role in angiogenesis and maintenance of vascular integrity [9,10]. Ang-1 is

Abbreviations: ANDV, Andes virus; Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; CI, confidence interval; CRP, C-reactive protein; DOBV, Dobrava-Belgrade virus; dpo, days post onset; HTNV, Hantaan virus; LDH, lactate dehydrogenase; PUUV, Puumala virus; r, Spearmańs correlation coefficient

* Corresponding author.
E-mail addresses: christian.nusshag@med.uni-heidelberg.de (C. Nusshag), anja.osberghaus@med.uni-heidelberg.de (A. Osberghaus), alexandra.baumann@med.uni-heidelberg.de (A. Baumann), paul.schnitzler@med.uni-heidelberg.de (P. Schnitzler), martin.zeier@med.uni-heidelberg.de (M. Zeier), ellen.krautkraemer@med.uni-heidelberg.de (E. Krautkrämer).

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responsible for the stabilization of endothelial cell-to-cell contacts, whereas Ang-2 is an antagonist of Ang-1 and causes disruption of cellular junctions and destabilization of endothelial monolayers [11,12]. Changes in Ang-1 and Ang-2 levels and ratio imbalance have been described for different infectious and non-infectious pathologies that affect barrier function of organs [13–16]. Increased Ang-2 to Ang-1 ratios were observed in dengue fever and malaria and are associated with thrombocytopenia and hemorrhages [17–19]. High Ang-2 to Ang-1 ratios resulting from a decrease in Ang-1 and an increase in Ang-2 levels are associated with severe cases of malaria and may be used as predictors for worst-case prognosis [18,19]. Single angiopoietins were also examined in hantavirus infection. For hantavirus Hantaan (HTNV) reduced levels of Ang-1 were shown in plasma and serum samples of infected patients during acute phase of infection [20,21]. *In vitro* studies in hantavirus-infected HUVECs demonstrate that the infection suppresses Ang-1 expression. Treatment of these cells with Ang-1 antagonizes the negative effects on monolayer integrity, which were induced by HTNV and Andes virus (ANDV) infection [2,20,22]. Furthermore, it was shown that Ang-2 levels were elevated in PUUV infected patients and may play a role in the context of mobilization of circulating endothelial precursor cells and endothelial repair [1,23]. These results indicate that angiopoietins may influence the clinical course and play a role in the signaling induced by hantaviruses.

2. Objectives

The interaction of Ang-1 and Ang-2 during the clinical course and their role in severity of hantavirus disease are so far not known. Therefore, we analyzed the levels of Ang-1 and Ang-2 in a cohort of PUUV infected patients in Germany and a single case of infection with DOBV-Sochi in Russia.

3. Study design

3.1. Patients

Patients with PUUV (n = 31) and Dobrava-Belgrade virus genotype Sochi (DOBV-Sochi) (n = 1) infection were included. Infections were confirmed serologically. In addition, DOBV-Sochi infection was confirmed by RT-PCR and sequencing [24]. PUUV infections occurred in Germany and DOBV-Sochi infection in Russia. All patients met the case definition of acute hantavirus infection of the German Robert Koch Institute. An age-matched group of 16 healthy persons served as control. Characteristics, symptoms and laboratory parameters were analyzed through a review of medical charts of the Department of Nephrology.

3.2. Quantification of angiopoietins by ELISA

The plasma levels of Ang-1 and Ang-2 were quantified from platelet poor plasma by Quantikine enzyme-linked immunosorbent assay (ELISA; R & D Systems). Assays were performed according to the manufacturer's instructions.

3.3. Statistical analysis

Clinical parameters of two groups were compared using Student's *t*-test or Mann-Whitney *U* test. Normal distribution was tested with the Kolmogorov-Smirnov test. Correlation was assessed by calculating Spearman's correlation coefficients. *P* values of < 0.05 were considered significant. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

Table 1
Characteristics and median peak and nadir levels of laboratory parameters of 31 patients with serologically confirmed hantavirus infection during hospitalization.

	median (range)
age (years)	41 (24–70)
hospitalization (days)	7 (2–28)
serum creatinine max (mg/dl)	6.26 (1.58–12.47)
hemoglobin min (g/dl)	12.0 (8.4–15.5)
hematocrit min (l/l)	0.34 (0.25–0.45)
leukocytes max (G/l)	11.67 (8.39–16.52)
platelets min (G/l)	122 (32–434)
LDH max (U/l)	405 (217–1032)
CRP max (mg/l)	57.1 (18–154.6)
serum albumin min (g/l)	31.4 (15.4–38.3)
temperature max (°C)	39 (37–41)
hematuria max (erythrocytes/µl)	4 (0–171)

4. Results

4.1. Clinical characteristics

A total of 31 cases of HFRS caused by PUUV infection were analyzed with regard to clinical course and levels of cytokines involved in control of vascular permeability. With a median age of 41 years and a gender ratio of 21 men to 10 females, our cohort of PUUV infected patients exhibits the typical epidemiological characteristics observed for patients with PUUV hantavirus disease in Germany. The clinical course started with flu-like symptoms followed by a renal phase with acute renal failure that required renal replacement therapy in three cases. The impairment of laboratory parameters included elevation of levels of leukocytes, serum creatinine, CRP, and LDH. Levels of serum albumin, thrombocytes, hemoglobin, and hematocrit were decreased (Table 1). In some patients, microhematuria, petechiae and other bleedings were detected.

4.2. Measurement of levels of angiopoietins

Plasma levels of Ang-1 and Ang-2 were analyzed and the ratio of Ang-2 to Ang-1 calculated for the cohort of patients and a healthy control group (Fig. 1). Median plasma levels of Ang-1 differed between patients and healthy controls (3300 pg/ml [range 1167–17924 pg/ml] vs. 2443 pg/ml [range 1179–6517 pg/ml]), but without statistical significance. In contrast, median levels of Ang-2 (4502 pg/ml [range 2962–9252 pg/ml] vs. 1504 pg/ml [range 912.5–3588 pg/ml]) and median ratios of Ang-2 and Ang-1 (1.23 [range 0.28–5.34] vs. 0.61 [range 0.21–1.44]) showed a significant elevation compared to the control group. Measurement was performed between day five and 24 (median 10 days) after onset of symptoms and especially for Ang-1, a broad range of levels were observed for patients. To examine, whether angiopoietin levels changed during the acute phase, we analyzed levels over time. The analysis of levels and ratios revealed that levels of Ang-1 and Ang-2 were changed very early in the clinical course leading to an elevated Ang-2/Ang-1 ratio (Table 2). Highest ratios were observed between day 5 and 7 after onset of symptoms, when Ang-1 levels were decreased. Normalization of the angiopoietin levels after 14 days after onset of symptoms was paralleled to the recovery phase of hantavirus disease.

In addition to the patients with hantavirus disease caused by infection with PUUV, we analyzed the role of angiopoietins in a patient with DOBV-Sochi infection (Table 2). The female patient suffered from severe HFRS with fever, thrombocytopenia, acute renal failure, progressive respiratory failure and required ICU admission and dialysis during their hospital stay [24]. The plasma levels of Ang-1 and Ang-2 in DOBV-Sochi patient were decreased and elevated, respectively. The Ang-2/Ang-1 ratios in the course of DOBV-Sochi infection were much higher than the median level observed for the healthy control group.