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Original Article

Hepatocyte Growth Factor, Vascular Endothelial Cell Growth Factor and Tumor Necrosis Factor - a Release During High Flux Haemodialysis

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Abstract

Background. The aim of this study was to compare the effect of low molecular weight heparin (LMWH) on circulating levels of hepatocyte growth factor (HGF), vascular endothelial cell growth factor (VEGF) and tumor necrosis factor-a (TNF-a) during high flux haemodialysis session.

Methods. We studied 12 haemodialysis patients (62, 6 ± 13, 2 years), who were on HD for 19-218 months with high flux filters. Following washout of the filter with normal saline, HD was started with zero hyperfiltration and without heparin. LMWH enoxaparin, was administered 10 min from the beginning of HD. HD was continued without hyperfiltration for 10 more minutes. Four blood samples were evaluated: at the beginning of the HD session (t 0), 10 and 20 mins (t 10, t 20) from the beginning and 5 min after HD was completed (t - post).

Results. Compared to t0 HGF levels, t10 HGF serum levels were not altered. Following heparin administration a significant increase in HGF serum levels was noted. Compared to t10 HGF levels, the t20 levels were also significantly increased ($p<0.003$). Tpost HGF serum levels didn't differ from t0 levels. VEGF serum levels showed no significant alterations, whereas TNF-a serum levels decreased significantly.

Conclusions. HGF increased selectively during HD session, possibly due to heparin administration. TNF-a serum levels decreased and VEGF remained unchanged, independently from heparin administration.

Keywords: enoxaparin, haemodialysis, heparin, hepatocyte growth factor, tumor necrosis factor-a, vascular endothelial cell growth factor.

Introduction

HGF was first identified as a potent stimulator of hepatocyte growth and later as a pleiotropic cytokine with mitogenic, motogenic and morphogenic effects on several cell types [1]. Serum concentration of this protein is markedly increased in patients with chronic renal diseases and has been related to the extent of arteriosclerosis and viral hepatitis. More over it has been reported that haemodialysis stimulates HGF release [2]. Previous studies have established that HGF directly stimulates proliferation and migration of cultured endothelial cells and promotes development of capillary like structures. Besides HGF, several other heparin binding growth factors (HBGFs) such as VEGF have been shown to induce angiogenesis [3].

The proinflammatory cytokine system is activated in ESRD patients, and various lines of evidence suggest that decreased renal clearance might play an important role in cytokine retention. On the basis of the given interactions of heparins with HBGFs, and possibly with TNF-a, we studied the influence of LMWH on circulating levels of HGF, VEGF and TNF-a during high flux haemodialysis session.

Patient and methods

We studied 12 haemodialysis patients (7 men, 5 women, mean age 62,6 ± 13,2 years), who were on chronic HD treatment for 19-218 months (mean 73,5±51,5), using high flux dialyzers (FLX-180, membrane polyester polymer alloy, surface 1.8 m²). LMWH enoxaparin sodium was used for anticoagulation, in a mean dose of 85, 96±16, 64 IU/Kg BW. Patients with active or chronic hepatitis and patients with recent infection and symptomatic heart failure were excluded from the study.

The dialyzers were prerinsed with 2000 ml of normal saline without heparin. HD was started at zero hyperfiltration, without anticoagulation. LMWH was administered as intravenous bolus infusion, 10 min after the beginning of HD. HD was continued without hyperfiltration for 10 more minutes and then as prescribed. Blood samples for the measurement of HGF, VEGF and TNF-a serum levels, were drawn before the beginning of the HD session (t0), and then 10 and 20 minutes after the beginning (t10 and t20 respectively) and 5 minutes after HD was completed (t-post). Elisa kits for determination of HGF, VEGF and TNF-serum concentrations were purchased from R&D Systems Inc. Minneapolis, USA.

Statistical analysis

Values are expressed as mean ± SD. Friedman's ANOVA and Kentall's concordance for within group analysis and the Wilcoxon matched pairs test were used for statistical analysis. Differences were accepted as significant if $p<0.05$.

Results

Analysis of variance by Friedman's ANOVA and Kentall's concordance, showed that the values in t0, t10, t20 and t-

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post samples were differed statistically for HGF and TNF-a values, but not for VEGF values ($p<0.01$).

Mean t0 HGF values were above normal limits (671-1992 pg/ml). Mean t10 HGF serum levels were not significantly altered, but 10 min after heparin infusion (t10), a significant increase of HGF values was noted in comparison to t0 HGF levels (81.8±10.5% increase with $p< 0.003$). The t20 HGF levels were also significantly higher than t10 HGF levels (88.1±5.7%, $p<0.003$) (Table 1, 2).

Table 1. Mean values during HD, for HGF, VEGF and TNF-a

M.v. ± SD pg/ml	t 0	t 10	t 20	t - post
HGF	2966,6±1385.8	2008,3±1000,6	17144,8±2177	5291,9±3615,3
VEGF	127.6±49.5	126.3±35.6	98.3±60.8	124.8±59.7
TNF-a	5480.6±4434,4	4393,9±3852,7	4460,3±3360,2	2568,1±1075,3

Table 2. Alterations during HD session

HGF LMWH	p	VEGF	p	TNF-a	p
t 0 VS t 10	NS	t 0 VS t 10	NS	t 0 VS t 10	0.003
t 0 VS t 20	0.002	t 0 VS t 20	NS	t 0 VS t 20	0.04
t 0 VS t-post	NS	t 0 VS t-post	NS	t 0 VS t - post	0.006
t 10 VS t 20	0.002	t 10 VS t 20	NS	t 10 VS t 20	NS
t 10 VS t - post Corrected	0.01	t 10 VS t - post Corrected	NS	t 10 VS t-post Corrected	0.04
t 20 VS t - post Corrected	0.002	t 20 VS t - post Corrected	NS	t 20 VS t post Corrected	0.006

Mean VEGF values were within normal limits before the beginning of HD (n.v: 0-120 pg/ml), and didn't alter significantly there after. Results were corrected for body weight loss.

Mean TNF-a values were within normal limits before the beginning of HD (ND-4.71: M0 2.07 pg/ml), and significantly decreased during HD. The t10 and t20 TNF-a serum levels didn't differ significantly.

Discussion

Soluble heparin molecules can bind and replace more than 85% of the HGF from heparin sulphate proteoglycans on the cell surface and in the extracellular matrix. By this way heparin administration induces an immediate rise of circulating serum HGF levels [4].

From the results of our study it is clear that high-flux HD does not induce increase in serum HGF levels during HD and that the observed increase in HGF levels is exclusively due to heparin administration. Borawski J *et al*. [5] have also reported an abrupt increase in serum HGF 10 min after HD initiation using the LMWH enoxaparine for anticoagulation.

In contrast to HGF, VEGF serum levels were unaffected by HD treatment, as well as by heparin administration. This was also noted in a paper of P. B. Salbach *et al.* [6].

The literature on cytokines in haemodialysis is full of controversy. Extracorporeal circulation in HD is associated with leukocyte activation and the release of cytokines in blood. High levels of circulating soluble receptors during dialysis make the understanding of the role of cytokines in the haemodialysis inflammation more complex. In fact, specific inhibitors of cytokines are concomitantly produced after immune system challenge [7]. According to our study, a significant decrease of TNF-a serum levels was noted during HD, independent of heparin administration.

In conclusion, it seems that high-flux haemodialysis procedure per se does not stimulate HGF and VEGF production, but decreases TNF-a serum levels. However the possibility that a certain amount of these substances could be absorbed on the synthetic high-flux polymer alloy membrane, altering mildly their levels, cannot be excluded entirely.

Conflict of interest statement. None declared.

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