Original Paper

Higher Serum Hepatocyte Growth Factor Concentration is Associated with Better Preservation of GFR in Hemodialysis Patients

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Key Words

Hemodialysis • Interleukin 6 • Hepatocyte growth factor • GFR

Abstract

Background/Aims: Hemodialysis induces an intravascular inflammatory reaction which may further deteriorate renal function. We studied changes of serum interleukin 6 (IL6) and hepatocyte growth factor (HGF) concentrations during dialysis sessions, and at 12 month intervals. The synthesis of these cytokines in arterial endothelial cells in the presence of serum obtained from dialyzed patients was studied. Changes of the inflammatory reaction during 12 months of treatment were correlated with GFR. **Methods:** The study was performed on a group of 30 uremic patients treated with hemodialysis. Serum samples were collected before the start of dialysis, 15 minutes, and 4 hours later, when the session was finished. Serum levels of IL6 and HGF were measured with ELISA, as was the effect of serum samples on the synthesis of these cytokines in arterial endothelial cells. **Results:** At baseline hemodialysis induced an increase of serum IL6 (+10%) and HGF(+164%) levels at the end of the session. After 12 months of treatment predialysis serum IL 6 level was increased as compared to the beginning of the study (+22%), but no change in serum HGF level was observed. At that time the dialysis-induced rise of serum IL6 level was stronger than at the start (+18%), but the observed effect for HGF was weaker (+116%). An inverse correlation was observed between the dialysis-induced increase of HGF level and decrease of GFR after 12 months of study. The same relation was seen for HGF synthesis in the endothelium, but opposite for IL6 synthesis in the endothelium. **Conclusions:** We found that a higher HGF serum level during hemodialysis treatment is associated with a slower loss of residual renal function.

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Introduction

Hemodialysis is a lifesaving procedure in patients with end stage renal failure. However, the biocompatibility of that procedure is low, which may be an important factor contributing to systemic inflammatory response [1]. We found previously that hemodialysis induces changes in serum properties which result in the inflammatory stimulation of the endothelial cells in *in vitro* culture [2]. Dysfunction of the endothelial cells in uremic patients on renal replacement therapy may accelerate progression of atherosclerosis [3]. It was shown that the mechanisms of the impaired endothelial function are different in patients treated with hemodialysis or peritoneal dialysis [4]. Both systemic inflammation induced by uremia and renal replacement therapy, as well as cardiovascular disorders secondary to dysfunction of the endothelial cells contribute to the progression of renal dysfunction [5, 6]. The decline of the residual renal function is faster during renal replacement therapy with hemodialysis as compared to peritoneal dialysis [7].

There are various clinical reports about inflammatory markers in end stage renal failure patients on replacement therapy, such as IL6, CRP or Pentraxin-3. However, their clinical significance is not well understood. Recently, Borges et al. described the relationship between residual diuresis in patients on hemodialysis and the serum level of CRP [8]. Other authors suggested a link between intraperitoneal IL6 level in patients treated with chronic peritoneal dialysis and their residual renal diuresis [9]. In another study on peritoneal dialysis patients a correlation between serum FGF21 level and residual renal function was described [10]. Malyszko et al. found a correlation between resistin level and residual renal function in uremic patients treated with chronic hemodialysis [11].

We found previously that hemodialysis-induced changes in serum levels of IL6, VEGF or HGF correlate with various aspects of quality of life in uremic patients on chronic renal replacement therapy [12]. The probable mechanisms of such interactions are probably complex, due to various systemic effects of the inflammatory mediators. The role of endothelial cells stimulated by the hemodialysis procedure in the generation of the intravascular inflammatory reaction is unclear.

We present results from our study performed on a group of hemodialysis patients, where we evaluated, at a 12 month interval, changes in serum HGF and IL6 levels caused by sessions of hemodialysis, and the role of endothelial cells in that reaction. We also analyzed the possible link between dialysis-induced intravascular inflammation and the decline of the residual renal function.

Materials and Methods

The protocol of the study was approved by the University Ethical Committee. Patients participating in the study were informed about the study, and oral consent was obtained from each participant. The study was performed on a group of 30 patients with end stage renal failure treated with hemodialysis. The mean age of the patients was 52.4 ± 10.6 years, and they had been treated with hemodialysis for 50.1 ± 34.6 months. The glomerular filtration rate was calculated in each patient with a modification of diet in renal disease (MDRD) formula. All hemodialysis treatments were performed with a polysulfone dialyzer (Fresenius, Bad Homburg, Germany), and ultrapure water for dialysis was prepared in the Gambro system WRO ROHH (Gambro, Lundia AB, Lund, Sweden). Mean values of the clinical parameters measured at the beginning of the study and after 12 months of observation are presented in Table 1.

In all patients the inflammatory reaction caused by hemodialysis was evaluated during one hemodialysis session at a 12 month interval. In each case the duration of the dialysis session was 4 hours. Blood samples were drawn before the start of the hemodialysis session, 15 minutes after the start, and after 4 hours, when the dialysis was finished. Blood samples were spun down, and collected serum was stored at -86°C for further analysis. In all serum samples concentrations of IL6 (IL6 Duo Set enzyme-linked immunosorbent assay with detection limit 0.7 pg/mL; R&D, UK) and HGF (HGF ELISA with detection limit 5.0 pg/mL; R&D,

Table 1. Renal, dialysis and serum inflammatory parameters at the beginning of the study (START) and after 12 months (END). Concentrations of IL-6 and HGF were measured before the start of the hemodialysis session. (n=30)

	START	END	
GFR [mL/min]	7.9 ± 3.8	6.3 ± 2.3	p < 0.005
Urine volume [mL/24hrs]	1193 ± 932	917 ± 863	p < 0.001
Kt/V	1.4 ± 0.2	1.4 ± 0.1	n. s.
IL6[pg/mL]	17.1 ± 4.7	20.9 ± 8.0	p< 0.005
HGF [pg/mL]	743 ± 217	798 ± 247	n. s.

UK) were measured. In an additional set of experiments the effect of serum samples harvested from the patients on the synthesis of IL6 and HGF in human aortic endothelial cells (HAEC) was studied. Cells were purchased from Life Technologies Corporation (Carlsbad, USA), and cultures were established in medium M200 supplemented with 2% fetal bovine serum, hydrocortisone 1ug/mL, human epidermal growth factor 10 ng/mL, basic fibroblast growth factor 3 ng/ml, heparin 10 ug/ml (all chemicals were purchased from Life Technologies Corporation). Disposable equipment used for cell culture was purchased from Nunc (Rochester, USA). Cell cultures were propagated and grown in monolayers in 48-well plates, at 37°C, in humidified 5% CO₂ atmosphere.

Endothelial monolayers were exposed to the serum samples obtained from all patients, which were added to culture medium in an amount to obtain their final concentration of 20%. Incubation time was 24 hours. Afterwards, the medium with the studied serum samples was removed, cells monolayers were washed with standard culture medium, and afterwards exposed to the medium during the next 24 hours. At the end of the incubation supernatants were removed from the wells for measurement of IL6 and HGF concentration, as described above. Cells were harvested from the wells with trypsin 0.05% - EDTA 0.02% solution, and their number was counted in a hemocytometer. Synthesis of the studied molecules was expressed per number of the cells in the wells.

Statistical analysis

Results are presented as mean ± standard deviation. Analysis of the data was done with nonparametric tests: Wilcoxon or Friedman test. The correlation between the studied groups was calculated with the Spearman test. Linear regression was used to estimate the coefficients of the linear equation, involving four independent variables (IL6 and HGF synthesis in the endothelial cells exposed to serum from hemodialysis patients, IL6 and HGF serum levels before and after the dialysis session) that best predict the value of the dependent variable (GFR). A p value of less than 0.05 was considered statistically significant.

Results

Hemodialysis sessions induce the intravascular inflammatory reaction. In our study we found an increase of IL6 and HGF serum levels at the end of the dialysis session as compared with the predialysis values (Table 2). In the preliminary experiments we found that the intensity of the reaction is characteristic for each patient, and does not change significantly during the dialysis sessions performed during the following days. Indeed, in our study we found a correlation between the observed hemodialysis-induced changes of the measured cytokines at the beginning of the study and after 12 months of observation: IL6 (r = 0.498, p<0.01) and HGF (r = 0.676, p<0.001).

At the beginning of the study, treatment with hemodialysis induced an increase in serum HGF level mean by 696%, (p<0.001) after 15 minutes of the dialysis session, and its concentration remained elevated mean by 164% (p<0.001) at the end of the 4 hours dialysis session, as compared to the predialysis values. An increase of the serum IL6 level was seen only at the end of the dialysis session by 10% (p<0.01) (Table 2). After 12 months the hemodialysis-induced increase of the serum HGF after 15 minutes of treatment was higher by 704% as compared to the predialysis level. The serum HGF level at the end of the dialysis

Table 2. Mean values of the serum IL-6 and HGF levels measured before the start of the dialysis session (A), 15 minutes (B) and 24 minutes(C) after the start of hemodialysis. Values from the beginning of the experiment (START) and after 12 months of study (END) are presented.(n=30)

Interleukin 6 – serum [pg/mL]			
	A	B	C
START	17.1 ± 4.7	17.1 ± 5.8	18.8 ± 5.4 p < 0.01 vs A & B
END	20.9 ± 8.0	20.1 ± 8.2	24.7 ± 8.7 p<0.005 vs A & B
Hepatocyte Growth Factor-serum [pg/mL]			
START	743 ± 217	5918 ± 4498 p < 0.001 vs A	1961 ± 1020 p < 0.001 vs A & B
END	798 ± 247	6416 ± 3680 p < 0.0001 vs A	1722 ± 1062 p < 0.0001 vs A & B

Table 3. Mean values of IL-6 and HGF synthesis in the endothelial cells exposed to serum samples collected before the start of the dialysis session (A), 15 minutes (B) and 24 minutes(C) after the start of hemodialysis. Values from the beginning of the experiment (START) and after 12 months of study (END) are presented. (n=30)

Interleukin 6 [pg/10 ⁵ cells]			
	A	B	C
START	19,3 ± 4,2	20,0 ± 4,8	23,1 ± 4,4 p< 0.001 vs A p< 0.01 vs B
END	22,1 ± 3,9	21,8 ± 3,6	29,0 ± 5,6 p<0.005 vs A & B
Hepatocyte Growth Factor [pg/10 ⁵ cells]			
START	119,4 ± 32,5	125,2 ± 26,3	174,7 ± 20,4 p<0.001 vs A & B
END	110,4 ± 17,2	113,3 ± 14,7	133,2 ± 30,5 p<0.01 vs A & B

session was still higher (+116%, p<0.001) as compared to the predialysis value. However, the magnitude of that last effect was weaker than at the beginning of the study (p<0.005). At the same time serum IL6 levels at the end of the hemodialysis treatment were higher by 18% (p<0.005) as compared to the predialysis result, and that effect was stronger than at the beginning of the study (p<0.005).

Ex vivo tested serum samples obtained 4 hours after the start of the hemodialysis session stimulated the synthesis of HGF and IL6 in endothelial cells as compared to control obtained before the start of dialysis and serum samples after 15 minutes of the dialysis session (Table 3). Synthesis of IL6 was stimulated at the beginning of the study by 20%, p< 0.001 and that effect was even stronger with serum collected after 12 months of treatment (+ 31%, p<0.005). Conversely, synthesis of HGF was increased by 46% (p<0.001) when the cells were exposed to the post-dialysis serum at the beginning of the study, and only by 21% (p<0.01) in the presence of the samples obtained after 12 months of the renal replacement therapy.

After 12 months of the observation renal insufficiency progressed, as reflected by the decrease of GFR (-21%, $p < 0.005$) and daily urine volume (-23%, $p < 0.001$). The efficiency of the dialysis was not different between the two studied time periods. The blood concentration of IL6 measured before the hemodialysis session was increased by 22% ($p < 0.005$), but no change in serum HGF level was observed (Table 1).

The linear regression analysis shows that changes (end of the study vs. 12 months earlier) of HGF synthesis in endothelial cells exposed to serum obtained at the end of the hemodialysis and HGF serum concentration after the hemodialysis session predicted higher GFR values ($\beta = 0.374$, and 0.330 , respectively). Conversely, the GFR level was significantly decreased when IL6 synthesis in endothelial cells exposed to serum obtained after the hemodialysis session was higher ($\beta = -0.373$). The fourth factor of the model, IL6 serum levels, had a slight ($\beta = -0.024$) and not significant effect on the GFR level. The model including these four variables turned out to be statistically significant ($p = 0.00297$) with R square of 0.375 with a large effect size ($f^2 = 0.6$).

Discussion

The results of the present study confirm our previous findings: the hemodialysis session increases intravascular concentration of IL6 and HGF [12]. The observed early increase of serum HGF level, as early as 15 minutes after the start of the dialysis session, could be caused by heparin [13] and the stimulation of blood leukocytes [14]. Libetta et al. reported that the release of HGF was different when various types of dialysis membrane were used, which suggests the significance of the mechanical effect caused by the contact of blood with the membrane [15]. We cannot exclude that observed increased serum levels of IL6 and HGF at the end of the dialysis session could also be caused by hemoconcentration due to ultrafiltration during treatment. However, the different magnitude of the observed effects for IL6 and HGF suggests that intravascular inflammation played a major role in these effects. Hemodialysis induces inflammatory changes in the serum that may affect the function of the endothelial cells [2, 16]. We found that serum samples obtained after 4 hours of dialysis stimulate (as compared to the effect of samples harvested before the start of treatment) synthesis of IL6 and HGF in the endothelial cells. These results suggest that endothelial cells can contribute to the intravascular inflammatory reaction induced by the process of hemodialysis. An interesting observation was the fact that in serum samples obtained after 12 months of treatment with renal replacement therapy the concentration of IL6 was increased, and also the stimulatory effect of the serum samples on the synthesis of that cytokine in the endothelial cells was stronger. Previously, it was shown that inflammation increases in patients with chronic renal failure proportionally to a decrease of GFR [17]. At the same time a more intense systemic inflammatory response accelerates loss of the residual renal function in that group of patients [18]. Our results suggest that arterial endothelial cells can contribute to the intravascular inflammatory reaction in patients with end stage renal failure. Furthermore, the intensity of IL6 synthesis in endothelial cells correlated with the intensity of GFR decrease during 12 months of observation. However, a surprising finding was the lack of correlation between increase of serum IL6 during the whole study and decrease of GFR. Also, Jelacic et al. found in peritoneal dialysis patients that the effluent IL6 level correlated with residual diuresis but not with GFR [9]. It seems that the stimulated synthesis of IL6 in endothelial cells observed in our experiments after their exposure to serum samples harvested from the patients could reflect their activation by progressing uremia and treatment with hemodialysis [19]. Such changes in the endothelium may accelerate damage to the kidney [20]. Lichtenberg et al. showed that intradialytic increase of IL6 in hemodialysis patients is associated with higher risk of mortality [21].

An important finding in the present study was the discovery of the protective effect of HGF against a decrease of GFR in patients with renal failure. GFR was measured in our

study with the MDRD formula, which is not the most accurate parameter. Despite that fact, the observed relation between HGF serum level was strong, as shown in the results of the regression analysis. HGF is a pleiotropic cytokine whose concentration rises secondary to various tissue injuries, promoting the process of healing and regeneration [22]. It has a potential protective effect in various organs [23]. Hemodialysis induces an increase in the serum level of HGF, which results in amelioration of the liver function in patients with HCV infection [24]. There are reports from experimental studies showing the protective effect of HGF against acute and chronic kidney injury [25-27]. Esposito and co-workers demonstrated that HGF reduced, in *in vitro* conditions, the expression and synthesis of TGF- β and collagen type IV in normal human glomeruli [28]. There are also clinical observations suggesting an HGF stimulating effect in recovery of renal function in patients with acute renal failure [15].

The results of our study show that GFR decrease in patients with chronic renal failure is inversely proportional to the magnitude of the increase of HGF serum level at the end of the dialysis session. Predialysis mean serum HGF concentrations were similar at the beginning and at the end of the study (Table 1). However, each hemodialysis session, performed three times per week, triggers the release of a large dose of HGF, which may contribute to slower progression of renal failure, as suggested by Libetta et al [15]. A significant rise of the serum HGF concentrations was also observed at the end of the 4 hour session. However, we found that the magnitude of the hemodialysis-induced increase of serum HGF level measured at the end of the session decreases with time in renal replacement therapy: an increase by 164% at the beginning of the study, and by 116% after twelve months ($p < 0.005$).

Results from the *in vitro* experiments suggest that endothelial cells can contribute to the rise of the intravascular HGF level during hemodialysis. Synthesis of that cytokine in endothelial cells is stimulated by serum obtained at the end of the hemodialysis session. However, its magnitude decreases with time: stimulation by 46% at the beginning of the study, and by 21% after twelve months ($p < 0.01$). After 12 months of the disease, despite the increased systemic inflammatory reaction as reflected by the rise of the predialysis IL6 level, HGF level in serum at that moment was not increased. Additionally, the hemodialysis-induced rise of HGF serum level was smaller, and the effect of that serum on the synthesis of HGF in the endothelial cells was weaker. Our results suggest that during chronic renal replacement therapy the potential beneficial effect of HGF for preservation of the residual renal function becomes weaker. One can speculate that exogenous supplementation of HGF may be a desirable procedure, stabilizing renal function in patients with end stage renal failure, as shown in other studies [29, 30].

Conclusion

We conclude that GFR decrease in patients with end stage renal failure is inversely proportional to the serum level of HGF. Serum concentration of HGF decreases with time, which may result in accelerated decrease of the residual renal function.

Disclosure Statement

The authors declare they have no conflicts of interest.

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