IMPROVEMENT OF POST-HEPARIN LIPOLYTIC ACTIVITY (PHLA) AFTER HAEMOFILTRATION, IN URAEMIC PATIENTS

S di Giulio, B Lacour, M Thevenin, C Basile, T Drüeke, N K Man
Necker Hospital, Paris, France

Summary

The decrease of serum PHLA in uraemic patients has been attributed to toxic effects of 'middle molecules' on lipoprotein lipase. Haemofiltration could improve PHLA by increasing 'middle molecule' clearance. Serum triglyceride concentration decreased and PHLA improved in three uraemic patients who received haemofiltration during three weeks as the initial treatment schedule. Activated PHLA of a healthy subject failed to be inhibited by uraemic sera and uraemic plasma ultrafiltrates obtained in vitro using polyacrylonitrile and cuprophane membranes.

Conclusion: haemofiltration is able to improve PHLA and to restore normal serum triglyceride concentrations. The reported toxicity of uraemic serum may affect metabolic steps preceding the activation of the circulating lipolytic enzymes.

Introduction

High plasma triglyceride levels often occur in uraemic patients both with end-stage renal failure and receiving long term haemodialysis. This trouble may account for the increased incidence of development of atherosclerosis [1]. Impaired activity of lipolytic enzymes involved in triglyceride catabolism, such as lipoprotein-lipase and hepatic triglyceride-lipase, is frequently found when measured by the test of post-heparin lipolytic activity (PHLA) [2]. Recent reports have shown an inhibitory action of uraemic serum on tissue lipoprotein-lipase [3]. This inhibition can be reduced when the serum is pre-treated by in vitro dialysis [4]. The role of possible circulatory inhibitors of 'middle molecular weight' or of high molecular weight [4] in uraemic serum has been suspected to affect the lipoprotein-lipase activity.

The aim of the present work using haemofiltration was: (a) to assess the effect of a high removal rate of 'middle molecular weight' solutes on the lipid disturbances of uraemic patients, and (b) to investigate whether the impairment of PHLA was associated with serum or ultrafiltrate toxicity.
Material and methods

Post-heparin lipolytic activity in patients with end-stage renal failure

Post-heparin lipolytic activity was measured according to the method of Fredrickson et al. [5] in fasting healthy control subjects (n = 10) and uraemic patients (n = 14) before the first session of the artificial kidney programme (creatinine clearance ≤ 5ml/min). Heparin (0.1mg/kg) was injected into the antecubital vein as a bolus. Plasma was collected 10min later and incubated immediately in the presence of 20% Intralipid (Vitrum, Sweden) as substrate. Results are expressed as nmol of free fatty acids liberated per min and ml of plasma.

Post-heparin lipolytic activity in patients with hypertriglyceridaemia treated by haemofiltration

Post-heparin lipolytic activity was measured in fasting uraemic patients (n = 3 men) with hypertriglyceridaemia (> 2mmol/L) at the first session of the artificial kidney programme. Heparin was administered as a bolus injection (5000 I.U./m² body surface area) at the beginning of the haemofiltration session, followed by a continuous intravenous infusion of 30 I.U./m² body surface area [6]. Plasma samples were tested for PHLA as previously described at 60, 90 and 240min after the beginning of the haemofiltration session.

Haemofiltration was performed by a post-dilution system using an RP 6 (Rhône Poulenc) artificial kidney with polyacrylonitrile membrane. The re-injection fluid contained sodium 145, potassium 1.0, calcium 1.75, chloride 105, acetate 40, and magnesium 1.5mmol/L. At the tenth haemofiltration session (3 times/week, 4 hours each), PHLA was measured under the same conditions as at the first one. Serum triglyceride concentration was measured before the first and the last haemofiltration session [7].

Post-heparin lipolytic activity measurement in the presence of uraemic serum and plasma ultrafiltrate

Activated PHLA of a healthy subject was measured in vitro in the presence of uraemic sera (n = 6 patients from the first group), control sera (n = 8 healthy subjects), saline (n = 9 determinations) or plasma ultrafiltrates. Ultrafiltrate at 50% was obtained in vitro using polyacrylonitrile (n = 8 uraemic plasmas) or cuprophane membrane (n = 9 uraemic plasmas). Results obtained with sera were compared to each other. Results with ultrafiltrates were compared to saline.

Statistical analysis was performed using Student’s t-test. Paired t-test was used for the patients treated by haemofiltration. Results were expressed as means ± SEM.

Results

PHLA in patients with end-stage renal failure

Post-heparin lipolytic activity was lower in uraemic patients than in healthy control
(Heparin 0.1 mg/kg I.V.)

![Bar graph showing FFA nmol/ml/min for control and uraemic subjects](image)

**Figure 1.** Post-heparin lipolytic activity in uraemic patients and in healthy control subjects

subjects: 22.9 ± 4.8 SEM nmol/ml/min versus 78.5 ± 9.8 nmol/ml/min, p < 0.001 (Figure 1).

**PHLA in patients with hypertriglyceridaemia treated by haemofiltration.**

Post-heparin lipolytic activity in the three uraemic patients with hypertriglyceridaemia was measured at 60, 90 and 240min.

During the first haemofiltration session, it was, respectively, 73, 63 and 60 (mean 69.7 ± 12.1 SEM) nmol/ml/min at 60min; 93, 75 and 26 (mean 73.67 ± 3.66) nmol/ml/min at 90min; and 53, 73 and 26 (mean 39.33 ± 3.62) nmol/ml/min at 240min. Post-heparin lipolytic activity was within the range of the previous uraemic group when tested at the same doses of I.V. heparin (0.1mg/kg). Serum triglycerides of the three patients before the first session were 3.30, 2.90 and 2.49mmol/L respectively. On the 10th haemofiltration session PHLA was
measured again at 60, 90 and 240 min. The values obtained were, respectively, 253, 160 and 60 (mean 193 ± 8.2) nmol/ml/min at 60 min; 226, 140 and 46 (mean 146.6 ± 6.6) nmol/ml/min at 90 min, and 100, 140 and 80 (mean 62 ± 4.54) nmol/ml/min at 240 min. Serum triglyceride values were 1.27, 1.39 and 1.9 mmol/L respectively (Figures 2 and 3).

![Graph](image)

- **Mean of the three subjects**
- **△, □, ○ Mean of PHLA at 60, 90 and 240 min for each subject**

FFA nmol/ml/min

- **p < 0.02**

Heparin i.v., 5000 I.U./m² body surface area followed by continuous infusion of 30 I.U./m².min

Figure 2. Post-heparin lipolytic activity at the first (HF1) and the 10th (HF 10) haemofiltration session in 3 uraemic patients with hypertriglyceridaemia
heparin i.v., 5000 I.U./m² body surface area
followed by continuous infusion of 30 I.U./m². min

Figure 3. Post-heparin lipolytic activity at the 1st and 4th hour of haemofiltration.
No. 1 (HF 1) and No. 10 (HF 10). Increased residual activity on the 10th haemofiltration
is shown.

Effect of uraemic serum and plasma ultrafiltrate on PHLA in vitro

Uraemic serum added in vitro failed to decrease the plasma PHLA of a healthy
subject. PHLA was $108.1 \pm 12.7$nmol/ml/min ($n = 8$) when control serum was
added, and $89.1 \pm 3.5$nmol/ml/min ($n = 6$) when uraemic serum was added.
Moreover, the addition of uraemic plasma ultrafiltrates obtained with either
polyacrylonitrile or cuprophane membranes in vitro to the post-heparin plasma
of the healthy subjects failed significantly to decrease PHLA when compared to
that observed after the addition of saline. The mean PHLA values observed were 54.6 ± 10.9 (n = 9) for polyacrylonitrile membrane, 52.9 ± 18.3 (n = 8) for cuprophane membrane (n = 8) and 54.2 ± 9.2nmol/ml/min (n = 9) for saline.

Discussion

Post-heparin lipolytic activity is decreased in uraemic patients with hypertriglycerideraemia [2]. Both hepatic triglyceride lipase and lipoprotein-lipase are impaired. The nature of the suspected toxic substances contained in uraemic plasma and their site of action are still unclear. In vitro dialysis seems to lower the inhibitory action of uraemic serum on lipoprotein lipase from adipose tissue [4]. However, no improvement of lipid anomalies is observed when haemodialysis time is increased in patients with chronic renal failure [8]. Depletion of lipolytic enzymes induced by long term heparin administration may account for lack of improvement of PHLA after prolonged dialysis treatment [6]. However, PHLA recovers within 24 hours in healthy subjects even after long term heparin administration [8]. Uraemic toxicity may involve tissue synthesis, release, activation and the circulating function of lipolytic enzymes [3]. The present work showed an improvement of PHLA with a concomitant decrease in serum triglycerides after haemofiltration. Moreover, such a treatment was associated with a lower decrease of the PHLA observed at the 4th hour when compared to the initial haemofiltration session [6].

The increase of insulin secretion which has been reported to occur during haemofiltration may account for higher lipoprotein-lipase synthesis [9].

Preliminary reports of others [10] are in accord with our data concerning the improvement of lipid anomalies of uraemic patients treated by haemofiltration [11]. The lack of inhibition of uraemic serum or plasma ultrafiltrates suggests that the metabolic step involved in the reported toxicity precedes that of the circulating activated enzymes. Lipolysis is controlled by a system of physiological activators and inhibitors of lipoprotein-lipase and hepatic triglyceride lipase such as apoproteins which are altered in chronic renal failure [12]. The influence of haemofiltration on these factors needs further investigation since increased PHLA may be related to an improved balance between such substances.

In conclusion, haemofiltration allowed an improvement of the lipid anomalies associated with chronic renal failure. However, in vitro studies failed to show experimental evidence for inhibiting activity on circulating lipolytic enzymes contained in uraemic plasma or in plasma ultrafiltrate. Further investigations are needed to identify the metabolic step involved in the decrease of uraemic plasma PHLA.

Acknowledgments

We gratefully thank the work of Miss G Lenzen for skilled technical assistance and Mrs Mavroyannis and Epaillard for secretarial help.
References

4. Murase T, Cattran DC, Rubenstein B, Steiner G. *Metabolism* 1975; 24: 1279
5. Fredrickson DS, Ono K, Davis LL. *J Lip Res* 1963; 4: 24
6. Ibels LS, Reardon MF, Nestel PJ. *J Lab Clin Med* 1976; 87: 648
7. Eggstein M, Kreutz FH. *Klin Wochenschr* 1966; 44: 262

Open Discussion

NICHOLLS (Aberdeen) As you are aware Rapoport and colleagues (New England Journal of Medicine 1978; 299: 1326) suggested the apoprotein composition of HDL was abnormal. Have you looked at apoprotein composition of HDL or VLDL before and after haemofiltration?

DI GIULIO We didn’t study the composition of apoprotein.

CHAN (London) Do the patients with different HDL cholesterol concentrations behave the same?

DI GIULIO The cholesterol concentration was low at the beginning of the study as for the group of patients on haemodialysis; generally we found a low cholesterol concentration.