

# High plasma endostatin level unaffected by low-molecular weight heparin in hemodialysis patients - a preliminary report

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## ABSTRACT

**Purpose:** Endostatin (ES) is a potent inhibitor of angiogenesis and neoangiogenesis, and interestingly its activity is modified by heparin. To understand if low-molecular weight heparins have different clinical profiles regarding this cytokine, we studied the effects of enoxaparin, nadroparin and dalteparin administered for hemodialysis (HD) anticoagulation on plasma ES levels.

**Material and Methods:** Seventeen chronic HD patients completed this prospective, crossover trial. They were randomized into 6 groups – each patient was administered enoxaparin (effective dose of 0.75 mg/kg), nadroparin (70.4 IU/kg) and dalteparin (78.6 IU/kg) in 3 time periods of 2 months each. At the end of each period plasma levels of ES were measured at the start and at 10 min and 180 min of the HD procedure.

**Results:** Mean predialysis plasma ES levels in HD patients were extremely high for all three heparins used. We observed no changes in ES levels during dialysis, there were also no differences in ES profiles for each of the low-molecular weight heparins used.

**Conclusions:** Plasma ES levels are unusually high in chronic HD patients and the significance of this fact needs future research. ES levels do not change after heparin administration and at least in that aspect enoxaparin, nadroparin and dalteparin are equal.

**Key words:** endostatin, hemodialysis, low-molecular weight heparin

## INTRODUCTION

Endostatin (ES) is a potent inhibitor of endothelial cell proliferation, discovered in 1997 [1]. Since then the cytokine has attracted considerable attention, mainly because it profoundly inhibits angiogenesis and neoangiogenesis; in fact it was even acknowledged in *Nature* by Folkman's group as a unique sort of „resistance-free cancer therapy“ [2]. Endostatin inhibits endothelial cell proliferation, migration, and tube formation *in vitro*, and inhibits tumor growth *in vivo*. It is a 20 kDa C-terminal fragment of collagen XVIII and its anti-angiogenic activity mainly depends on interaction with heparan sulfate, and thus heparin may increase ES blood levels by its competitive release from vascular wall [3]. Recent reports have demonstrated the potential benefits of endostatin in treating chronic inflammatory disorders such as rheumatoid arthritis,

diabetic nephropathy, proliferative diabetic retinopathy or peritoneal sclerosis [4-7].

Low-molecular weight heparins (LMWHs) are different from unfractionated heparin (UFH), mostly due to their reduced tendency to non-specifically bind to plasma proteins, platelets, endothelial and blood cells. Moreover each LMWH varies in many important aspects starting with different means of production (each depolymerization technique induces different chemical changes) and they are considered to be distinct drugs with different and unique chemical structure, pharmacodynamics, pharmacokinetics and clinical profiles. They also differ in several effects not measurable by conventional methods, such as interaction with endothelial and other cells, various blood proteins and modulation of many pleiotropic growth factors and cytokines [8].

Maintenance hemodialysis (HD) patients are a specific population with flourishing atherosclerosis, cardiovascular disease and defective vascular repair. On the other hand, they receive millions of units of heparin in their life because the drug is being administered during every thrice weekly blood purification procedure. Little is known on the biology and blood levels of ES in this particular group.

We aimed to determine the effect of three different LMWHs (enoxaparin, nadroparin and dalteparin) on the ES activation profile during HD procedures and indirectly compare the extra-anticoagulant activity of these LMWHs.

## MATERIAL AND METHODS

This study had a prospective, crossover design which helped to minimize both the study group and the interpersonal variability. The patients were randomized into 6 groups (it eliminated the carry-over effect of the preceding treatment) comparing 3 anticoagulation regimens with: enoxaparin (Clexane®, Rhone-Poulenc Rorer, France); nadroparin (Fraxiparine®, Sanofi-Winthrop, France); and dalteparin (Fragmin®, Pfizer, Switzerland) in 3 periods of 2 months each. After 2 months of observation samples were collected at the beginning of HD session (T0) and afterwards at 10 min (T10) at 180 min (T180). The study was designed to keep HD prescription, pharmacologic and dietary treatment stable.

We enrolled 21 patients (9 men, 12 women; median age 69 years) who had been undergoing maintenance HD for a median of 62 months (range 15.5-177 months). All the participants had been anticoagulated with enoxaparin for at least 3 months prior to the study. Exclusion criteria were malignancy, severe liver disease (alanine aminotransferase >50 U/l), recent acute inflammatory or infectious diseases (C-reactive protein >10 mg/l), recent surgery, immunosuppressive therapy, insulin-dependent diabetes mellitus, treatment with vitamin K antagonists, heparin (except for HD) or regularly with nonsteroidal anti-inflammatory drugs, HD vintage less than 3 months prior to the study, HD access other than native arteriovenous fistula and Kt/V less than 1.2.

Seventeen patients (7 men, 10 women; median age 71 years) finished this study. The cause of premature withdrawal was orthopedic surgery – 1 patient received heparin prophylaxis, 2 deaths due to stroke, and 1 consent withdrawal.

The local Ethics Committee approved the study, and both oral and written information was given before signed consent was obtained from all patients prior to participation. The protocol also abides by the tenets of the Helsinki protocol.

The patients were treated with bicarbonate buffered HD thrice weekly, they were dialyzed for 4 to 5 hours using the double-needle technique, native arteriovenous fistulas and low-flux dialyzers. The dialyzers were primed with normal saline, then LMWH was administered as a single bolus via the first access needle. The effective dose of enoxaparin

( $0.75 \pm 0.35$  mg/kg), nadroparin ( $70.4 \pm 8.6$  IU/kg) and dalteparin ( $65.8 \pm 10.1$  IU/kg) was established on the basis of common clinical guidelines: no visible fibrin clots in the arterial and venous bubble traps during HD, no clotted filters after HD, no bleeding from the fistula puncture sites after compression.

Five ml of fasting blood was drawn into ethylenediaminetetraacetate (EDTA)-coated vacutainers during a midweek morning HD. At T0 it was drawn from the access (before heparinization) and at T10 and T180 from the pre-dialyzer port after slowing the blood flow to 100 ml/min for 1 min. Blood samples were chilled in ice water and plasma was obtained by centrifugation at 3800 g for 10 min within 30 min of collection. Afterwards the samples were aliquoted and stored at  $-70^{\circ}\text{C}$  until further needed.

Plasma ES levels were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits purchased from R&D Systems Inc., Minneapolis, USA (cat. DNST0) according to manufacturer's instructions. All samples were measured in duplicate. The within- and between-assay coefficients were <8%.

Shapiro-Wilk W test of normality was used for data distribution analysis. Analysis of variance (ANOVA) for crossover study design was used. Paired analyses were performed for significant differences using the Wilcoxon signed rank test. The Spearman rank correlation test was used to evaluate relationships between variables. Two-tailed p-values of <0.05 were considered statistically significant. Analyses were performed with Stata 9.0 for Macintosh software.

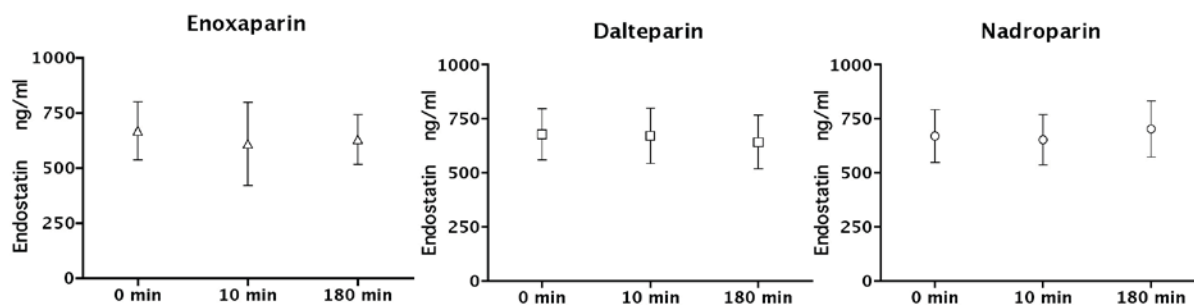
## RESULTS

Mean predialysis plasma ES levels in HD patients were  $669 \pm 124$  ng/ml for enoxaparin,  $670 \pm 121$  ng/ml for nadroparin and  $678 \pm 119$  ng/ml for dalteparin. We observed no changes in ES levels during dialysis (measured at 10 minutes and 180 minutes after the beginning of the procedure). There were also no differences in ES profiles for each of the LMWH used (*Fig. 1*).

## DISCUSSION

This is, to our knowledge, the first report showing remarkably high endostatin levels in hemodialyzed patients. There are of course limitations to our study caused by its crossover design – the main one being lack of control group – that's why this is only a preliminary report. Nevertheless it is interesting that ES levels in end stage renal disease patients are at least ten times higher than in healthy people. According to literature they are often not detectable in control groups and amount to  $49.2 \pm 11.7$  ng/ml in patients suffering from acute myocardial infarction [9]. In acute myeloid leukemia median ES levels were 14.8 ng/ml pre-treatment and 35 ng/ml post-treatment

Figure 1. Overdialytic plasma endostatin levels during anticoagulation with three low-molecular weight heparins.



[10]. PubMed and Scopus database searches with the MeSH headings „chronic kidney disease“, „renal failure“, „dialysis” and “endostatin” as key words revealed only one relevant study. In this paper, concerning early-stage chronic kidney disease patients only, serum ES levels were only  $0.36 \pm 0.13$  ng/ml and still higher than in healthy males ( $0.12 \pm 0.02$  ng/ml) [11].

The phenomenon of very high endostatin levels in chronic HD patients is very intriguing. It seems that it is not connected to heparin administration as it was partly suggested by Seko *et al.* who observed decreased ES levels not after coronary arteries reperfusion and heparin administration but also in response to hypoxia itself [9,12]. Another work indicates that ES can be targeted as a cause of worse collateral vessel formation and can partly reflect the higher rate of genitourinary malignancies in chronic HD patients. Feldman *et al.* found that ES levels were  $29.1 \pm 1.9$  ng/ml in renal cell carcinoma patients and significantly higher than in healthy controls [13].

The consequences of high endostatin levels in hemodialyzed patients are unknown and deserve further studies. Partly they may reflect „defective” angiogenesis in chronic kidney disease and be a potential therapeutic target, probably in every stage of CKD.

## CONCLUSIONS

In conclusion: plasma ES levels are unusually high in chronic HD patients and the significance of this fact needs future research (both a healthy control group and patients hemodialyzed with addition of unfractionated heparin or without heparin – dialyzers with heparin-grafted membranes - are needed). Furthermore, we did not observe the expected change in ES levels after heparin administration and at least in that aspect enoxaparin, nadroparin and dalteparin are equal.

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