Heparin-induced Extracorporal LDL Precipitation (H.E.L.P) in Diabetic Foot Syndrome – Preventive and Regenerative Potential?

Authors
H. Rietzsch[1], I. Panzner[2], T. Selisko[1], U. Julius[1], N. Jabs[1], M. Reimann[1], E. Bonifacio[3], M. Bornhäuser[4], S. R. Bornstein[1]

Affiliations
[1] Technical University of Dresden, Department of Medicine III
[2] Technical University of Dresden, Department of Orthopedics
[3] Technical University of Dresden, Center for Regenerative Therapies
[4] Technical University of Dresden, Department of Medicine I, Dresden, Germany

Key words
- amputation
- diabetic foot ulcers
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- peripheral arterial disease

Abstract

Peripheral arterial disease is more aggressive in concomitant diabetes posing an increased risk for critical limb ischemia and subsequent limb loss. The majority of therapies available are not effective to prevent amputation in patients with severe disease. The current observational study reports the effect of the heparin-induced extracorporal LDL-precipitation (H.E.L.P.) as a novel therapeutic approach in patients with severe diabetic foot syndrome. Seventeen diabetic patients with septic foot lesions recruited from the diabetic outpatient clinic underwent H.E.L.P. apheresis regularly until fibrinogen levels were stabilized at 3 g/l or infection was controllable as evidenced by alleviation of necrosis. Patients were subsequently followed up for 2 to 73 months. Fibrinogen levels were reduced by 68% after H.E.L.P. treatment. No severe complications were noted. Necrosis could be confined in sixteen patients. Minor amputations were indicated in twelve patients. Three patients underwent major amputations of the lower limb and two patients received surgical reconstruction. In conclusion, H.E.L.P. apheresis may offer an alternative therapeutic option to diabetic patients with critically ischemic feet and appears to have a beneficial major/minor amputation ratio.

Introduction

The metabolic syndrome is a major challenge for the health care system due to its high cardiovascular morbidity and mortality [1–3]. Alterations in lipid metabolism are among the main determinants for the development of vascular diseases in the metabolic syndrome [4,5]. The cardiovascular risk is even more elevated with the subsequent development of diabetes mellitus [6]. Diabetic patients have a four-fold increased risk for peripheral vascular disease and are more susceptible for critical limb ischemia [7]. Diabetic foot ulcers are a major cause of hospital admittance for people with diabetes preceding 84% of all nontraumatic lower-leg amputations in this growing population [8]. Although standardized protocols for care exist, the healing process is complicated owing to the impaired peripheral sensation and perfusion, which can result in diabetic foot ulcers [9]. The patients often are immunocompromised with inadequate inflammatory responses to infection, which may subsequently lead to sepsis. Since inflammation is a strong stimulus for fibrinogen synthesis, which on becoming chronical, causes increased blood viscosity and hypercoagulopathy. This in turn aggravates already compromised peripheral perfusion of microvessels culminating eventually in a circulus vitiosus [10]. Concomitant increase in plasma cholesterol can additionally influence the release of the vasoactive endothelium-derived mediator nitric oxide to disturb blood flow [11].

Different methods aiming at acceleration of wound healing in lower extremity sites are available, such as the use of growth factor-loaded gels, hyperbaric oxygen, grafts, and artificial skin replacement [12–14]. These have in common that they do not benefit compromised peripheral perfusion – one of the main determinants of wound healing. Other methods have been developed to achieve a better hemorheologic pattern such as hemodilution, defibrinogenation, or oral medication, but these do not act in a fast, safe, and efficient way [15]. The current study therefore examined the question whether drastic lowering of plasma fibrinogen and plasma viscosity with heparin-induced

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Correspondence
Prof. Dr. med. S. R. Bornstein
University of Dresden
Department of Medicine
Fetscherstraße 74
01307 Dresden
Germany
Tel.: +49/351/458 59 55
Fax: +49/351/458 63 98
Stefan.Bornstein@uniklinikum-dresden.de

extracorporal low-density lipoprotein precipitation (H.E.L.P. apheresis) has an impact on wound healing in patients with diabetic foot lesions.

Materials and Methods

For seventeen diabetic patients with septic foot lesions H.E.L.P. apheresis was considered an optimal treatment. Patients were eligible when they had diabetes mellitus with concomitant progressive foot phlegmon (Wagner stages III–V) and severe angiopathy, which did not qualify them for revascularization. Further criteria were systemic infection (leucocytosis, lymphadenitis), high risk for amputation, and plasma fibrinogen > 6 g/l. Patients with an indication for revascularization or thrombectomy or patients who had known hemorrhagic diathesis, gastrointestinal ulcers, cancer, liver disease, apoplexy, or cardiac insufficiency were not considered appropriate for this treatment. The procedure complied with the Helsinki declaration. Written informed consent was obtained from all participants.

Participants were recruited during routine visits at the diabetes outpatient clinic. Current medical history was obtained from patient files. Fibrinogen levels were routinely determined during their visits at the outpatient clinic. Inflammatory state was determined by measurement of leucocytes and C-reactive protein. All participants underwent H.E.L.P. apheresis regularly to remove low-density lipoproteins, fibrinogen, and lipoprotein from plasma until fibrinogen levels were stabilized at 3 g/l or

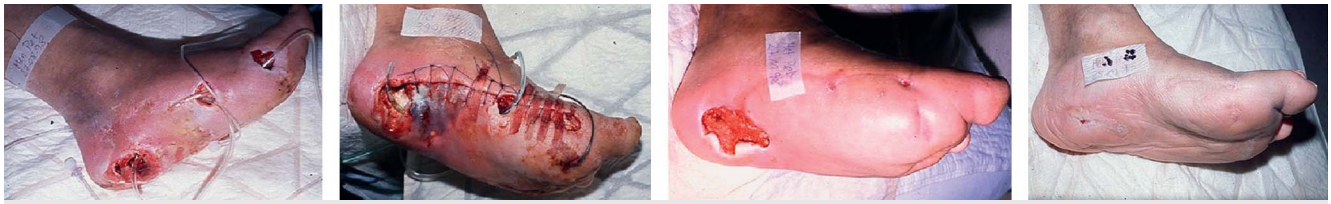

Fig. 1 Diabetic Foot at several stages of surgical interventions and after six H.E.L.P. aphereses.

Table 1 Baseline characteristics of patients before H.E.L.P. apheresis and outcome after conclusion of in patient treatment

Patient no.	Before heparin-induced extracorporal LDL precipitation				After heparin-induced extracorporal LDL precipitation			No of H.E.L.P. aphereses
	Sex/age (year)	HbA1c (%)	Fibrinogen (g/l)	Plasma viscosity (mPas)	Plasma viscosity (mPas)	Operation	Result	
1	M/68	9.6	8.0	n.m.	0.36	Amputation D5R	complete healing	2
2	M/68	9.1	8.3	1.73	2.60	1. Amputation D2D3L 2. Necrectomy planta	complete healing	3
3	M/47	8.2	10.0	1.71	2.10	1. Amputation D4R 2. Necrectomy	complete healing	3
4	M/65	10.5	8.9	1.34	1.99	Amputation D4L	complete healing	5
5	M/66	11.6	7.6	1.60	3.20	Amputation D4L	complete healing	1
6	M/75	12.3	8.4	n.m.	4.40	Amputation LL R	complete healing	1
7	M/76	8.4	7.7	n.m.	2.10	Amputation D1L (hallux)	complete healing	3
8	M/67	8.5	8.1	n.m.	2.50	1. Amputation D1R 2. Amputation LL R	complete healing	3
9	M/76	11.0	7.1	1.42	2.20	1. Amputation FF Lisfranc 2. Amputation FF Chopart	complete healing	4
10	F/76	7.6	10.4	1.56	3.20	1. Amputation FF Lisfranc 2. Amputation FF Chopart	complete healing	4
11	M/59	10.3	9.4	1.80	4.02	1. Amputation FF Lisfranc 2. Amputation FF Chopart	complete healing	7
12	M/53	anemia	13.4	1.98	5.30	Follow up resection Metatarsus	death after amputation	5
13	M/65	12.2	6.2	1.55	2.00	Amputation D1-D3R	complete healing	2
14	M/56	11.7	10.8	1.66	2.60	Necrectomy heel	remaining ulcer	6
15	M/55	10.6	6.9	1.68	2.60	Amputation FF L	complete healing	2
16	M/63	8.3	10.5	1.74	2.90	1. Necrectomy 2. Skin grafting	death (cardiac infarction)	2
17	M/54	7.1	6.4	1.60	2.80	1. Necrectomy 2. Joint resection D3R	complete healing	3

Abbreviations: D: distal; FF: forefoot; L: left; LL: lower-leg; n.m.: not measured; R: right

infection was controllable as evidenced by alleviation of necrosis. The number of HELP treatments varied between the patients ranging from 1 to 7. At the stage of fibrinogen stabilization, necrotic tissue was removed by operation and the patients were subsequently followed up for 2 to 73 months (**Fig. 1**). During follow up, patients were treated with antibiotics and heparin to prevent sepsis and thrombosis. Ulcers were treated according to the stage of wound healing and surgical intervention was applied when indicated. Blood glucose and blood pressure were tightly monitored to keep target values within or close to normal ranges. Additionally all patients received physiotherapy and appropriate foot wear.

H.E.L.P. apheresis was performed at the LDL-Apheresis Center of the Department of Internal Medicine III, University Hospital Carl Gustav Carus using the Plamat Futura (B. Braun, Melsungen, Germany). The entire procedure has been described in detail elsewhere [16], but a short description is given below. After separation of plasma by heparin coagulation, apolipoprotein B-containing lipoproteins and fibrinogen are precipitated at a pH-value of 5.12 by the addition of a mixed acetate-heparin buffer. Before returning the plasma to the patient, the excess heparin is adsorbed and the pH normalized. A total of 3 l of plasma per patient was treated. Blood samples were obtained directly before and immediately after each H.E.L.P. apheresis for laboratory measurements of fibrinogen and plasma viscosity. Clinical chemistry and coagulation tests were measured with standard procedures. Plasma viscosity was determined in a capillary tube plasma viscometer. Data are presented as mean and range.

Results and Discussion

All investigated patients, sixteen men and one women aged 47–76 years, were affected by peripheral artery disease with septic foot lesions and polyneuropathy (determined by tuning fork test) and had a poorly controlled diabetes mellitus with mean HbA1c values of 9.8 (7.1–12.3%) (**Table 1**). Systemic inflammation was reflected by a mean leukocyte concentration of 16.0 (7.7–35.3) GPT/l and C-reactive protein values of 129.6 (17.7–374.0) mg/l. At baseline (prior to H.E.L.P. apheresis), the fibrinogen concentration was 8.7 (6.2–13.4) g/l and the plasma viscosity was 1.64 (1.34–1.98) mPas. Plasma total cholesterol and triglycerides were fairly normal even before H.E.L.P. apheresis with concentrations of 4.14 (2.69–6.66) mmol/l and 1.63 (0.8–3.78) mmol/l, respectively. However, physiological lipid values are not indicative of pre-existing or current risks regarding H.E.L.P. apheresis particularly during acute infection. Mean fibrinogen levels dropped to 2.76 (0.36–5.30) after H.E.L.P. apheresis treatment corresponding to an overall reduction of 68%.

There were no severe complications during the H.E.L.P. treatment. Necrosis could be confined in sixteen patients. Minor toe amputations were required in eight patients, and forefoot removal was indicated in four patients. Three patients underwent major amputations of the lower limb. In one of them amputation of the upper limb could be prevented. Two patients received surgical reconstruction and another two patients passed away before complete wound healing (post operative infarction) was achieved. The intensive inpatient health care took an average of 111 days. During follow up, relapses could be timely treated thereby preventing additional amputations. Five patients died due to severe concomitant diseases (cancer, CVI).

The complex process of wound healing is disrupted in diabetic patients and inflammatory reaction appears prolonged resulting in excess fibrinogen disposition and increased blood viscosity [17]. Impaired blood viscosity plays a major role in residual microvascular perfusion in diabetic peripheral arterial disease and seems to be an important determinant of wound healing and adverse outcome. There is strong evidence that therapies aiming at reduction of plasma fibrinogen significantly improve the perfusion of compromised microcirculation [18, 19]. Compared to conventional fibrinolytic methods, H.E.L.P. apheresis represents a safe modality to rapidly remove fibrinogen and lipid fractions from the circulation providing acute improvements of whole-blood and plasma viscosity and thus microcirculation [20]. Walzl and colleagues reported that H.E.L.P. treatment in patients suffering from cerebral multi-infarct disease significantly improved regional cerebral blood flow by 9.7–19.9% compared to untreated control patients [19]. Our observational data show convincingly that fibrinogen was dramatically reduced after H.E.L.P. treatment, which may have resulted in decreased blood viscosity and improved perfusion. Unfortunately, viscosity was measured only in one out of all the treated patients after each H.E.L.P. apheresis (**Fig. 2**). However, strong reductions of viscosity could be observed in this patient. This may give some indication for an effective reduction of plasma viscosity by H.E.L.P. Thus, H.E.L.P. apheresis may offer an alternative therapeutic option to diabetic patients with critically ischemic feet and appears to have a beneficial major/minor amputation ratio. The beneficial effect of H.E.L.P. on hemorheological factors and thus perfusion could be one explanation for improvement of wound healing in diabetic patients. In addition, it is suggested that the removal of plasma cholesterol by H.E.L.P. may beneficially impact on endothelial function via reduction of oxidative stress and stimulation of eNOS thereby restoring autoregulation of microvessels [11]. However, in the current observational study total cholesterol and triglycerides were

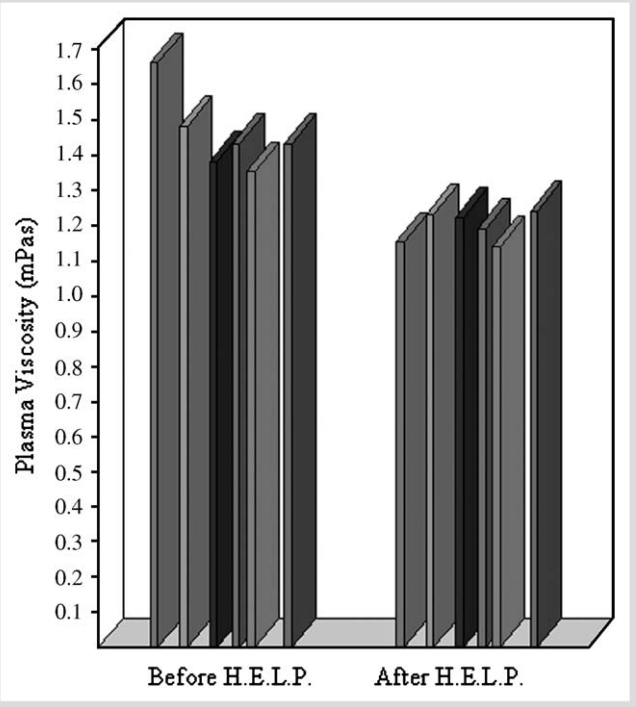

Fig. 2 Plasma viscosity of a patient with septic foot lesions before and after H.E.L.P. apheresis treatment.

more or less in normal ranges before the treatment, therefore, reductions were limited and did consequently happen within physiological ranges. Conclusively, lipid lowering may not have significantly contributed to the improvement in wound healing in our patients. From a theoretical point of view, H.E.L.P apheresis may modulate the number of stem cells in the blood, which could represent a novel mechanism of progressive wound healing. Although our own preliminary results did not reveal significant induction of progenitor cells before and after H.E.L.P (unpublished data), a potential effect cannot be ruled out particularly since this issue has been very poorly investigated so far. This, however, seems an exiting novel and relevant hypothesis to be investigated in future with a specific focus on endothelial progenitor cells.

A major limitation of this study is the absence of a control group receiving standard therapy and the lack of quantitative measures such as lesion size and ankle brachial index. However, the severity of the disease often complicates the assessment of quantitative data on wound healing and thus outcome evaluation is often reduced to descriptive or quantitative level (comparison of pictures and assessment of pain). Nevertheless, this observational study suggests that drastic reductions of fibrinogen and cholesterol accompanied by an elaborate care regimen may improve wound healing and reduce the risk of lower-leg amputations in critically ischemic feet. In a future approach different treatment regimens need to be compared to H.E.L.P. in order to directly measure the difference in outcome.

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