

HEMORHEOLOGICAL CHANGES UNDER BLOOD LEECHING

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ABSTRACT

Blood leeching is an ancient method of bloodletting, which has been used extensively in the treatment of various disorders since centuries in the natural medicine. The purpose of the present study was to see if, apart from the bloodletting benefit, the leech treatment effects the hemorheological properties. Whole blood from 23 patients (19 women and 4 men) with different diseases (cardiovascular and cerebral diseases, peripheral vascular disorders, etc.) was investigated before and after application of ten leeches on the lumbar region and one month after the first measurement. Whole blood viscoelasticity, plasma viscosity, whole blood and plasma density and hematocrit were determined as hemorheological parameters. The results showed that the whole blood viscoelasticity is significantly decreased after leech treatment although no significant changes of hematocrit and plasma viscosity could be observed. The decrease of blood visco-elasticity values was even more marked after one month of treatment. They correlated well with changes in aggregation tendency and flexibility of the red blood cells (RBC). These results suggest, that leeching is not a hemodilution effect alone. Moreover, apart from rendering blood noncoagulable by acting as antithrombin, the salivary gland secretion of these animals can also have a lasting effect on the hemorheological properties.

INTRODUCTION

The leech has been used extensively among people for bloodletting since ancient times in the treatment of various disorders. It is still used in many countries in the folk medicine, because people believe in the healing effect of bloodletting. However, the secretion from the salivary glands of the leech,

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Hirudino medicinalis, has also proven effects in medicine and pharmacology. The physiological effect of this secretion was determined not only by hirudin which inhibits fibrinogen coagulation by thrombin binding but also by components blocking the contact stage of blood coagulation (1). Apart from hirudin other proteinase inhibitor proteins, e.g. bdellins (trypsin and plasmin inhibitors) (2), and eglins (elastase-catepsin G inhibitors) (3) and other enzymes, like destabilase (4) that can dissolve fibrin, are reported to exist in the leech salivary gland secretions. Since recombinant hirudin could be prepared by means of genetical engineering, it has been the object of pharmaco-clinical studies (s. 5 overview). In animal studies with induced experimental thrombosis, it could be demonstrated, that hirudin could prolong the occlusion time or completely prevent vessel's thrombotic occlusion and additionally it was able to delay it markedly on an extracorporeal arterio-venous shunt (6). In recent clinical studies on patients with disseminated intravascular coagulation, it could be shown that hirudin application resulted in normalisation of fibrinogen level and platelet counts and in the disappearance of fibrin monomer complexes and degradation products (7). It has also been reported, that the salivary gland secretion blocks platelet adhesion on the surface of collagens (8). Further, prostaglandins were also determined in the salivary gland and body of leeches, which, however, did not show antithrombotic and antisclerotic effects (9).

Therefore the aim of this preliminary study was to investigate if, apart from these reported effects, the leech treatment has any influence on the flow behavior of blood. For this purpose the rheological parameters of blood before and immediately after leeching and about 4 weeks after the treatment were determined.

METHODS

3 ml of venous blood were collected in K₂-EDTA-coated tubes. Viscoelasticity of whole blood, plasma viscosity and hematocrit (PCV) were determined as hemorheological parameters within 2 h after venepuncture. The measurements were done with an oscillating capillary rheometer (OCR-D, Paar, Graz) at a constant frequency of 2 Hz and at 37° C. The instrument, first developed by Thurston (10) and modified by Chmiel and co-workers (11-14) sets the samples under sinusoidal oscillating flow, and measures both amplitude and phase of shear stress $\hat{\sigma}$ and shear rate $\dot{\gamma}$. Using the complex number notation a complex viscosity is defined analogous to Newton's law of viscosity:

$$\zeta^* = \hat{\sigma}^* / \dot{\gamma}^* = \zeta' - i\zeta''$$

The real part, ζ' , describes irreversible energy dissipating deformations, whereas the imaginary part, ζ'' , describes elastic deformations (15). The visco-elasticity values are determined between shear rates of 0.5 and 200 s⁻¹, however, for comparative evaluation of the results, the values at $\dot{\gamma} = 10$ s⁻¹ were used. Plasma was separated from whole blood after 20 min. of centrifugation at 1000· g. Hematocrit was measured with double determinations with a microhematocrit centrifuge (Combifuge, Heraeus, Osterode) after 15 min. centrifugation at 5800 · g. Fibrinogen concentration of thawed plasma samples from 4 patients in different stages of treatment was determined with the immune diffusion method (NOR-Partigen®-Fibrinogen, Behring).

23 patients (19 women and 4 men), mean age 54.9 years (min.24 y, max.85 y.), with different diseases, (cardiovascular and cerebral disorders, peripheral vascular diseases etc.) were selected for the study. 9 of these (7 women, 2 men) had increased viscoelasticity as indication; this group will be treated seperately in the following.

Measurements were done before and after the application of ten leeches on the lumbar region of the patients. The duration of leech application varied between 1 - 2 hrs. until the leeches were fully sucked with blood. The next measurements were done about four to five weeks after the treatment. Two patients could be followed up to 5 months; these received a second leech treatment after 4 months. The patients did not use other hemorheologically active drugs during the investigation period. As control, values from healthy volunteers from the blood bank were used. The ζ' - and ζ'' - values were converted to 45% hematocrit, as described in earlier work, based on the exponential hematocrit dependence of ζ' and ζ'' (14, 16). Two indices, the aggregation index, AI and the flexibility index, FI, as a measure for the aggregation tendency and flexibility of RBC were also calculated from the ζ' - and ζ'' -values at different shear rates (16). The results were calculated statistically with paired variant of Student's t-test.

RESULTS

Fig. 1 shows the changes for the mean values of the viscous component after leech application for both groups, together with normal values. There is a significant decrease of ζ' values in both groups, both immediately after leech application and after one month. This decrease is more marked especially in the group with increased values before treatment. The same relationship applies for the elastic component of whole blood viscoelasticity as shown on Fig. 2. The values after treatment are significantly decreased, so that they lie in the normal range, especially those after one month of application.

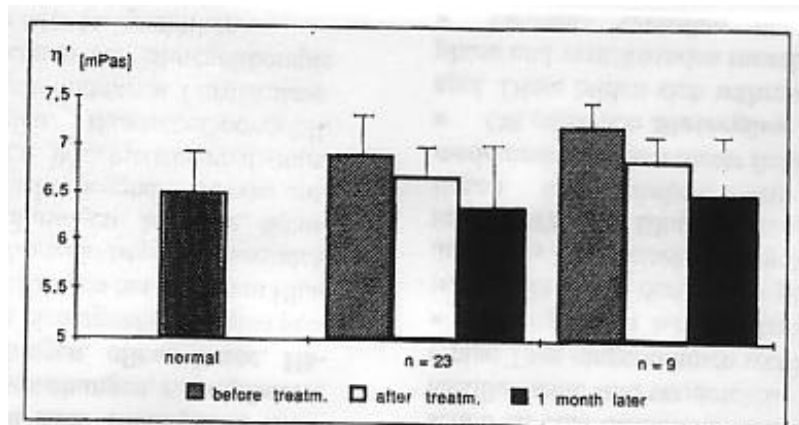


FIG.1 Mean values for the viscous component of complex viscosity for healthy donors and for both groups of patients before and after blood leeching.

TABLE 1 Mean values of viscous, ζ' , and elastic, ζ'' , components of whole blood viscoelasticity plasma viscosity, ζ Plasma, aggregation index, AI, and flexibility index, FI, (\pm SD) from healthy donors and from patients before and after leech treatment. (F=2 Hz, $\dot{\gamma} = 10 \text{ s}^{-1}$, T = 37° C, PCV = 45, n = number of cases, p = probability that the null hypothesis is true)

| | ζ' [mPas] | p | ζ'' [mPas] | p | ζ Plasma [mPas] | p | AI | p | FI | p |
|------------------------|--------------------|-----|---------------------|-----|---------------------------|-------|-----------|-----|-----------|-----|
| healthy donors | 6.51±0.41 | | 1.56±0.26 | | 1.25±0.05 | | 1.01±0.19 | | 1.01±0.03 | |
| patients group 1, n=23 | | | | | | | | | | |
| before treatm. | 6.92±0.41 | | 1.83±0.36 | | 1.30±0.06 | | 1.20±0.29 | | 1.06±0.05 | |
| after treatm. | 6.71±0.31 | ** | 1.65±0.25 | *** | 1.29±0.07 | n. s. | 1.10±0.22 | ** | 1.03±0.06 | * |
| 1 month later | 6.38±0.65 | *** | 1.53±0.32 | *** | 1.30±0.07 | n. s. | 0.97±0.22 | *** | 0.98±0.09 | *** |
| patients group 2, n=9 | | | | | | | | | | |
| before treatm. | 7.24±0.27 | | 2.20±0.19 | | 1.34±0.05 | | 1.46±0.23 | | 1.07±0.06 | |
| after treatm. | 6.88±0.26 | *** | 1.88±0.09 | *** | 1.32±0.06 | n. s. | 1.25±0.13 | ** | 1.03±0.05 | * |
| 1 month later | 6.53±0.61 | *** | 1.67±0.19 | *** | 1.33±0.05 | n. s. | 1.04±0.21 | *** | 0.96±0.08 | *** |

p < n.s. : not significant, p < * : < 0.05, p < ** : < 0.01, p < *** : < 0.001

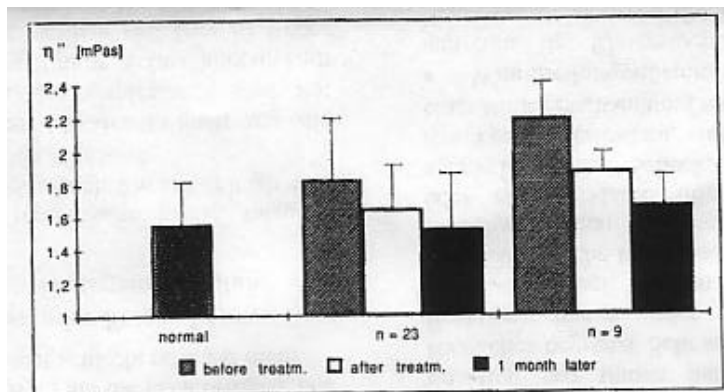


FIG. 2 Mean values for the elastic component of complex viscosity for healthy donors and for both groups of patients before and after blood leeching.

The mean values of plasma viscosity were almost constant for both groups and for both periods after treatment. The mean values of ζ , ζ'' and plasma viscosity are listed on Table 1 together with the mean values for the aggregation and flexibility indices both for healthy donors and for the two patient groups. Parallel to the decrease in ζ and ζ'' values after treatment, a significant decrease in both indices can be observed.

There were no significant changes of mean hematocrit immediately after leeching; the values after one month were slightly but not significantly increased. No changes in fibrinogen concentration could be found, whether in samples immediately after leech application or in those one month later, however, only 4 samples could have been measured.

Fig. 3 summarises the percentual changes in whole blood viscoelasticity for both groups after both periods of treatment. The mean decrease in the viscous component is up to 5% immediately after leeching and to 9.7% one month later. The changes in the elastic component are more marked: the mean values are decreased up to 15.5% immediately after leeching and to 23.9% after the lapse of one month.

The elastic component of complex viscosity is a sensitive indicator of the changes in the aggregation tendency and flexibility of red blood cells (11). Fig.4 shows the ζ'' -plots of the two patients, who could be followed up for a longer period. The arrows indicate leeching. Both patients show a decrease immediately after leeching and one month after the first leech application. The ζ'' -values are, however, raised after 4 months, but are still lower than the baseline values.

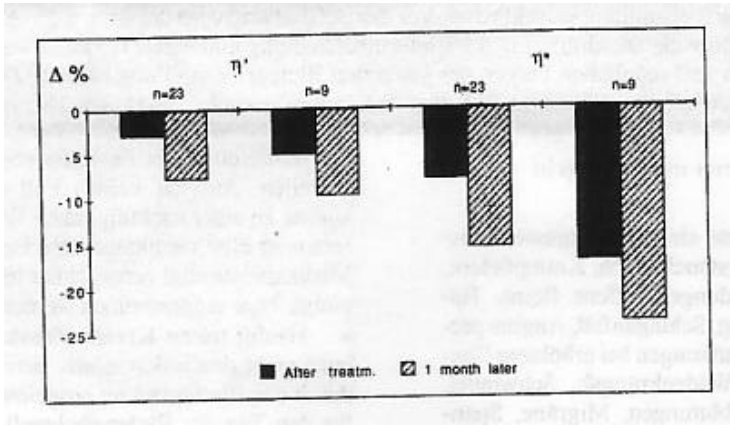


FIG. 3 Mean percentual changes for the viscous and elastic components of whole blood viscoelasticity immediately after and one month after blood leeching.

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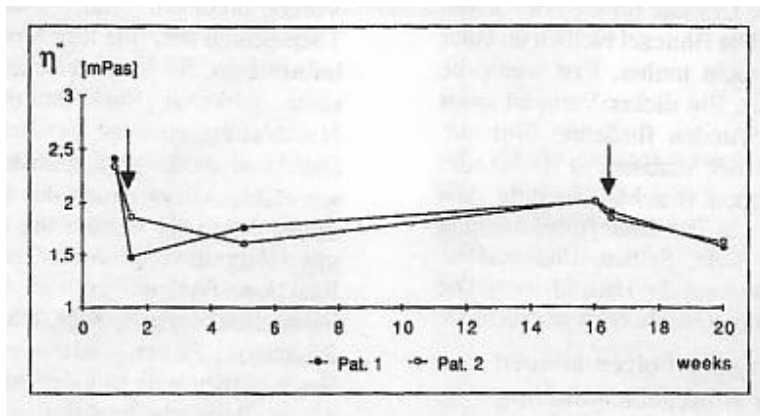


FIG. 4 Values of the elastic component for 2 patients vs. treatment period.

DISCUSSION

The results of this preliminary study show that the whole blood viscoelasticity is to a high significant extent decreased after leech application, although no significant changes of hematocrit, plasma viscosity and fibrinogen concentration could be observed. Parallel to the decrease in viscoelastic parameters, other parameters that describe the aggregation tendency and flexibility of RBC showed also a significant decrease after leech treatment. Therefore, leeching is not a hemodilution effect alone. The decrease of blood viscoelasticity values were even more marked 4 - 5 weeks after leech application. These results suggest that, apart from its antithrombotic effect, the secretion of the salivary gland of *Hirudo medicinalis* has also a remaining effect on hemorheological properties. This time-related behavior is certainly not due to the effect of hirudin alone, as its elimination half time ranges from 50 to 65 minutes (17). Leech prostaglandins as main antihemostatic agents have a protective antithrombotic ability, but their activity also diminished after 28 h (18). Salivary gland secretion of these animals have a complex structure consisting of various substances. Possibly not a single substance but a combination of several account for the benefits of this treatment. As already suggested in the earlier literature, due to its "blood cleansing and metabolism stimulating effect" (19) the treatment can induce a new formation in the bone marrow (20). Therefore, it can be suggested that, under the influence of the active substance of the leech salivary gland secretion, the erythropoiesis is stimulated and that the new formed red blood cells are possibly endowed with normalised hemorheological properties. More scientific investigation is therefore needed to determine the time-dependent effects of leech application.

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