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Platelet activation and serotonin release during treatment with haemodialysis

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Activation of haemostasis in case of extracorporeal blood circulation during treatment with haemodialysis (HD) induces intravascular coagulation (1). With respect to induction of thrombin generation, important factors include reduction of blood flow, modifications in the blood vessel wall, deviations in blood composition and biocompatibility of artificial membranes. Platelets are activated due to contact with artificial membranes during treatment with HD. Platelet activation results in adhesion, platelet aggregation on the membrane surface, thrombin generation and finally less effective HD treatment. Increased concentrations of Platelet factor 4 (PF4) due to release from alpha granulae and serotonin from dense granulae are indicative markers for platelet activation (2, 3). Degree of platelet activition corresponds with biocompatibility characteristics of HD membranes. Cuprophan and polyacrylonitrile AN69 membranes induce higher intradialytic platelet activation if compared with polysulphone high-flux and cellulose-triacetate membranes (4-6).

To prevent thrombin generation in the extracorporeal circuit Low Molecular Weight Heparin (LMWH) is applicated as a bolus injection before starting HD treatment. LMWH will potentiate the function of antithrombin III and neutralize thrombin. As a consequence, fibrin clots are not readily formed. Continuous citrate anticoagulation in the rinsing solution yields a high level of efficiency when compared with anticoagulation by application of dalteparin (7). However, blood flow dependent adjustments for citrate infusions complicate the procedure, which can effect life threatening disturbances in increased concentrations of electrolytes or pH (metabolic alkalosis).

In this study release of PF4 and serotonin during HD treatment is evaluated in order to assess biocompatibility in case of extracorporeal blood treatment. Two methods for optimisation of anticoagulation are compared, more in particular Fragmin® and sodium citrate. For the purpose of appropriate comparison, investigations are also performed in an apparently healthy subjects' group.

Patients and Methods

Ten subjects (age 32-82 years) with a regular scheme for HD treatment participated in the study. The etiology of renal insufficiency included hypertensive nephrosclerosis, diabetic nephropathy, chronic pyelonephritis and membranous nephropathy. Criteria for exclusion included application of calcium antagonists, salicylates, Warfarin, dipyrinamol.

For treatment with HD a high flux polysulphone® F60 membrane (Fresenius, Bad Homburg, Germany) was used with anticoagulant Fragmin® (intravenously 2000-5000 U bolus injection) or tri-sodiumcitrate was added to the rinsing solution. In case of Fragmin®, bicarbonate dialysate (Fresenius Medical Care, Bad Homburg, Germany) was applicated with a dialysate flow of 500 ml/min. During citrate HD a Ca^{2+}-free dialysate solution (Fresenius Medical Care, Bad Homburg, Germany) was used, whereas a sterile 15% trisodium citrate solution was infused continuously into the afferent line at a flow rate of 100 mL/h per 250 mL/min blood flow. After passage through the dialyser Ca^{2+} and Mg^{2+} concentrations were corrected by infusion of $CaCl_2/MgCl_2$ solution into the efferent line. Depending on individual needs and efficacy of treatment ultrafiltration flow rates varied between 300 and 1000 mL/min. Blood flow rates were kept constant at 250-300 mL/min resulting in HD sessions of 3-4 hours. Blood samples are collected during the third session from the arterial line before starting HD (t=0) and subsequently after 5 (t=5) and 150 (t=150) minutes from the efferent line. Blood samples were drawn into vacuum evacuated tubes anticoagulated with K_2EDTA and CTAD (Vacutainer tubes, Becton Dickinson, Plymouth, UK). PF4 concentrations are established by using a commercial ELISA-kit (Asserachrom® PF4, Diagnostica Stago, Asnières, France). PRP and PPP specimens were prepared by centrifugation of EDTA-blood samples at 200g and 4000g respectively. Serotonin content in PRP and PPP is determined with application of an enzyme immunoassay (Serotonin EIA, DSL Benelux Office, Assendelft, The Netherlands).

Reference subjects' group

A reference group of 20 apparently healthy subjects (aged 20-50 years) is selected in order to establish reference ranges for PF4 and serotonin.

Statistical evaluation

Statistical evaluation of data was performed by applying multivariate analysis (ANOVA) and Student's t-test for paired results (SPSS software 11.5 for Windows).

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Results

Application of Fragmin® resulted in an immediate increase of PF4 starting from a base level amounting to 9±4 kIU/L (mean±SD) at t=0 amounting to 101±19 kIU/L at t=5 minutes. At t=150 minutes PF4 concentrations returned to 30±27 kIU/L (Figure 1). PRP serotonin content amounted to 1.53±1.06 nMol/10^9 platelets at t=0 and 1.56±1.13 nMol/10^9 platelets at t=150 minutes. Although in the initial phase serotonin content in PRP is slightly decreased to 1.22±0.82 nMol/10^9 platelets at t=5 minutes additional release of serotonin in platelet poor plasma (PPP) could not be detected (Figure 1). During citrate dialysis a steadily ongoing increase of PF4 concentrations from t=0 till t=150 minutes was observed, whereas serotonin levels in PRP and PPP remained constant at the initial level. In HD subjects serotonin levels in PRP are obviously decreased if compared with apparently healthy subjects (3.47±1.13 nMol/10^9 platelets). Levels of serotonin in PPP are increased (142±45 nMol/L) in comparison with reference subjects (57±11 nMol/L).

Discussion and conclusions

Results of the study demonstrate that obvious deviations are due to the procedure of anticoagulation, whereas wide ranges of interindividual variations occur. When platelets are activated during HD, platelet granules will release various products, amongst others PF4 from alpha granulae and serotonin from dense granulae. Plasma concentrations of PF4 and platelet serotonin content are considered to be indicative measures for evaluation of platelet activation. Serotonin is metabolised in 5-hydroxy-indol-acetic acid (5-HIAA) by endothelial and proximal tubular cells (3). With decreasing kidney function elimination by glomerular filtration is reduced, resulting in increased levels of serotonin and 5-HIAA in plasma.

Decreased platelet serotonin content may occur in a state of hyponatraemia resulting in a disturbance of the balance in serotonin transport (8). However, in our opinion it would be more likely that decreased levels of serotonin in platelets in comparison with healthy subjects result from diminished synthesis of serotonin due to renal failure.

A dissimilar pattern for the release kinetics of PF4 and serotonin from platelets is demonstrated. Anticoagulation with citrate results in a reduced degree of activation of platelets due to limited release of myeloperoxydase from intracellular granulae of polymorphonuclear cells (9). PF4 acts as a binding protein for LMWH, neutralizing its function and thus negatively influencing inhibition of thrombin and activated coagulation factors. With respect to the immediate increase of PF4 concentrations in plasma and the only slight decrease of serotonin content in PRP during the first 5 minutes of HD treatment with LMWH anticoagulation, we hypothesize that platelet activation factors will be released more easily from alpha granulae than from dense granulae. In addition, increase of PF4 is considered to be an early sensitive marker for a mild degree of platelet damage. Therefore, PF4 is ought to be a valuable parameter for increasing knowledge concerning platelet environmental stress factors in case of HD.

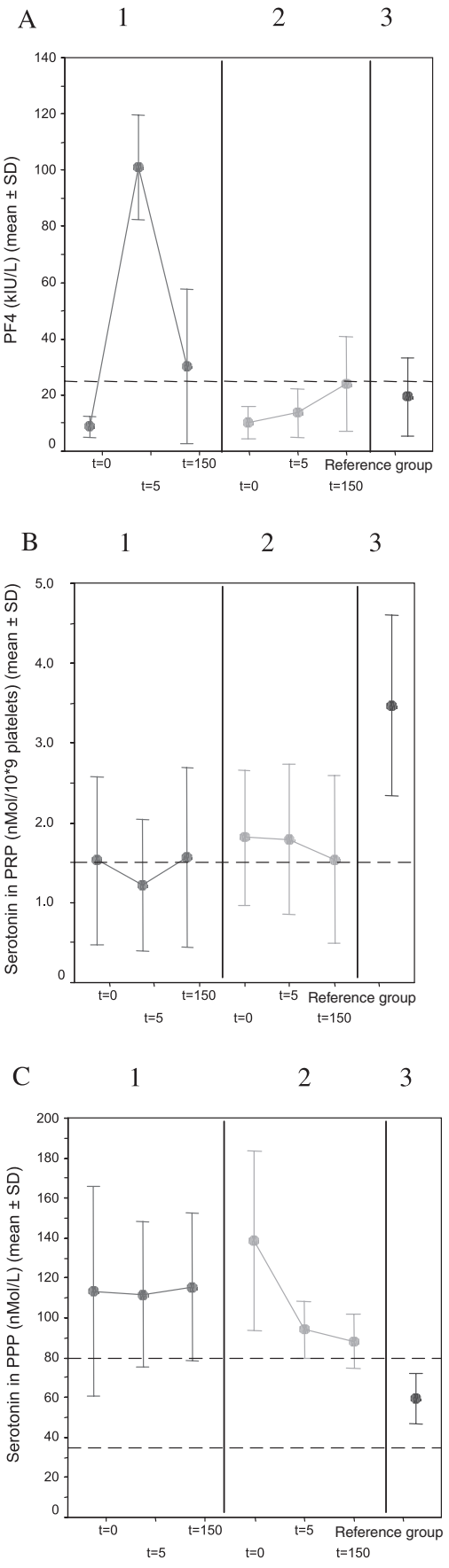

Figure 1. Release of PF4 and serotonin (mean value ± SD) at t=0, t=5 and t=150 minutes of HD treatment. A = PF4 (kIU/L); B = serotonin content in PRP (nmol/10^9 platelets); C = serotonin concentration in PPP (nmol/L). Anticoagulant: 1 = Fragmin®; 2 = citrate; 3 = Reference group. Dashed lines (-----) indicate lower and upper level of reference range.

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Lamotrigine in dried blood spots by HPLC

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Lamotrigine is an anti epileptic drug which blocks voltage-dependent sodium channels, thereby stabilizing neuron membranes. Moreover, it acts as a glutamate antagonist thereby preventing excitatory neurotransmitter release. Lamotrigine is as effective as carbamazepine and phenytoin against partial and secondarily generalised tonic-clonic seizures as well as idiopathic (primary) generalised epilepsy (1, 2). Lamotrigine can be used as monotherapy, but is particularly effective and generally well tolerated as a broad spectrum agent for adjunctive treatment of both partial epilepsy and idiopathic generalised epilepsy in adults and children (3).

Lamotrigine, is standard monitored in plasma by HPLC. Therapeutic drug monitoring of anti epileptic drugs from blood spots is far from general practice. Diagnosis of inborn errors of metabolism from blood spots in stead of plasma or whole blood in containers on the other hand is well known (4,5). Dried blood spots have the advantage of a smaller volume and are easy to obtain by the patient self. The sample can be transported by mail without any precaution.

We designed an assay in which lamotrigine is measured in dried blood spots and compared this assay with the standard plasma analysis.

Methods

Lamotrigine is measured in plasma or dried blood spots obtained from patients blood by HPLC. Discs of ¼ inch were purched of paper (Schleicher and Schuell, RC 55, 0.45 µm). Samples (10 µL) of blood, lamotrigine and/or internal standard (A725C 78, Wellcome) were added to the disc. The sample size of the plasma analysis is 200 µL. Lamotrigine and the internal standard were extracted out of the plasma sample or the dried blood spot by 200 µL dichloromethane. The extraction is performed at a pH of 10.4 and 3% propanol-2 is added to the dichloromethane to optimize the extraction-recovery. After evaporation and reconstitution of the residue in 200 µL mobile phase solution 20 µL is injected to the HPLC system. The reversed phase HPLC system (Agilent 1100) contains a stationary phase of an ODS Hypersil, 5µm, 100 x 4,6 mm column and a mobile phase of a phosphate buffer pH = 7,0 containing 32% methanol. The lamotrigine concentration is measured by a 4-point standard curve of lamotrigine and is internal standardized with A725C 78.

Results

The concentration of lamotrigine in 17 patients is measured in the following different blood components: whole blood, lysed whole blood, red blood cells, lysed red blood cells and plasma (reference). No significant difference in the lamotrigine concentration is found in these blood components when compared to the plasma concentration. Comparison of whole blood versus plasma according to Passing and Bablock shows the following equation: whole blood value = 1.09 x plasma value -0.07. This indicates that whole blood can be used in the assay in

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