HAEMODIALYSIS IN DOGS WITH A HEPARIN COATED HOLLOW FIBRE DIALYSER

L E Lins, P Olsson, *M-B Hjelte, *R Larsson, †O Larm
Karolinska Hospital, Stockholm, *IRD Biomaterial, Stockholm, †Swedish University of Agricultural Sciences, Uppsala, Sweden

Summary

Haemodialysis was performed in non-uraemic dogs with equipment coated with a stable heparin. During a three hour dialysis a constant blood flow of 205ml/min was easily maintained. There was no increase in whole blood coagulation time and no heparin release from the surface. The platelet count was initially reduced by 15 per cent, but remained constant at this value throughout the dialysis. No increase in FPA concentration was detected. Heparin coating on inherently thrombogenic materials enables haemodialysis in the absence of systemic anticoagulation and without measurable activation of the haemostatic mechanism.

Introduction

Conventional haemodialysis requires heparin anticoagulation to prevent clotting in the extracorporeal system, but systemic heparinisation, however, is associated with enhanced bleeding tendency and long-term side-effects of repeated administration of heparin such as osteoporosis [1].

In an attempt to exclude systemic heparin we prepared for haemodialysis a hollow fibre dialyser and PVC tubings with a covalently bonded heparin coating which exhibits thromboresistant properties with regard to platelets and plasma coagulation [2]. In this paper results from dialysis in non-uraemic dogs are presented.

Materials and methods

Low density polyethylene tubings (A/S Surgimed, Oelstykke, Denmark) with an inner diameter of 3mm were used for aortic blood pressure recordings, arterial blood sampling and vascular connection to the extracorporeal circuit.

Cellulose acetate hollow fibre dialysers (CDAK 3500, Cordis Dow, Concord, USA) with a surface area of approximately 0.9 m² were used in combination with PVC blood lines (Gambro, Sweden).
Haematocrit was determined by high speed centrifugation (Wifug, Stockholm, Sweden) and platelet count by a platelet counter (Linson 431A, LIC, Stockholm, Sweden). For the calculations only haematocrit corrected values were taken into account. Coagulation time in whole blood was determined by gently tilting 2ml of blood in 10ml glass test tubes every 30 seconds. Solid clot formation was regarded as the end point.

Heparin concentration in plasma was determined by using a factor Xa inhibition test with the aid of a synthetic chromogenic substrate (Coatest, Kabi, Stockholm, Sweden). Standard curves on pooled dog plasma with heparin concentrations from 0.025 to 0.1IU or from 0.1 to 1.0IU heparin/ml were constructed.

Fibrinopeptide A (FPA) in plasma was radioimmunologically assayed essentially according to Nossel et al [3] with the aid of human rabbit antisera cross-reacting with canine FPA [4,5].

Surface heparinisation of tubings and hollow fibre dialysers

After careful rinsing with water the material was coated with a covalently bonded layer of heparin. The procedure consists of two steps. In the first step polyethyleneimine is attached to the substrate surface. The heparin to be used is partially degraded by nitrous acid whereby terminal residues of 2,5-anhydro-
D-mannose with a reactive aldehyde function are formed. In the second step of the coating procedure, these reactive residues are allowed to react with the surface bound primary amine groups to form a Schiff's base, which is reduced by sodium cyanoborohydride to form a stable secondary amine bond. The details of the binding method are described elsewhere [2].

The dialysers were replastisised using glycerine and sterilised, procedures which were kindly performed by Cordis Dow Co, Concord, USA. The dialyser performance with regard to ultrafiltration and dialysance was essentially unchanged after heparinisation procedure: ultrafiltration coefficient 5.9ml/hr/mmHg. In vitro dialysance at Q_B = 200ml/min for urea 128, sodium chloride 115 and uric acid 84ml/min respectively.

Experimental procedure

Five mongrel dogs, 16 to 22kg, were used. The animals were anaesthetised with intravenous sodium pentothal, tracheally intubated and ventilated with a mixture of oxygen and dinitrous oxide in a volume controlled respirator (Mivab, Stockholm, Sweden). A carotid artery was cannulated for aortic blood pressure monitoring and blood sampling. A jugular vein was cannulated for fluid infusion.

The femoral artery and vein were dissected unilaterally and cannulated with surface heparinised tubings. The animals were dialysed for three hours using surface heparinised blood lines and a AK5 monitor (Gambro, Sweden) with an adjusted, not totally occlusive, roller pump. The dialysate compartment of the dialyser was filled with standard dialysis fluid and sealed. The arterial blood flow was measured electromagnetically (Nycotron, Drammen, Norway) with the
probe placed on the femoral artery. The arterial blood flow and the aortic blood pressure in the animals were continuously recorded (Grass Polygraph, Quincy, Mass, USA).

Blood samples for determination of haematocrit, platelet count, whole blood coagulation time, plasma heparin concentration and FPA were drawn from the carotid artery immediately after the insertion of the catheter, after 15 minutes and 30 minutes and then every 30 minutes.

Four additional dogs were merely anaesthetised for three hours. In these animals arterial blood samples for FPA determinations were taken from a carotid artery cannula at time intervals as described above.

For statistical analysis Student's 't' test or the Wilcoxon test was used. All results are given as mean ± SD, range or median values, p<0.05*, p<0.01**, p<0.001***.

Results

A three hour haemodialysis session was performed in all animals without any technical complications and with no heparin given systemically.

![Graph showing whole blood coagulation time and plasma heparin concentration during haemodialysis with a heparin coated hollow fibre dialyser (mean, SD)]

Figure 1. Whole blood coagulation time and plasma heparin concentration during haemodialysis with a heparin coated hollow fibre dialyser (mean, SD)
The roller pump was adjusted to give a blood flow of 160 to 215ml/min (mean 205±24ml/min) which was kept constant in each dog. The systemic aortic blood pressure (164±13mmHg) and the haematocrit (45±3%) were not significantly affected by dialysis.

The whole blood coagulation time did not change during the dialysis (Figure 1). There was a minor but still statistically significant elevation of the plasma heparin concentration (p<0.001) during the first 15 minutes of dialysis (Figure 1).

Figure 2. Platelet count during haemodialysis with a heparin coated hollow fibre dialyser (per cent of initial value; mean, SD)

The platelet count, ranging from 222 to 300x10⁹/L, decreased on the average by about 15 per cent (p<0.01) during the first 15 minutes of dialysis, but was then constant (Figure 2). The FPA concentration in the arterially sampled blood increased during dialysis, but not more than in merely anaesthetised animals (Figure 3).

The total extracorporeal system was after disconnection rinsed with 1000—1500ml of saline. The dialysers and tubings were almost completely cleaned by this procedure. At connections between tubings of different diameters minute clots were occasionally seen. In the air trap a small wall attached clot at the blood-air interface level was always present.

Discussion

It is evident that a stable heparin coating on inherently thrombogenic materials enables extracorporeal circulation in the absence of systemic anticoagulation treatment and without measurable activation of the haemostatic mechanism. The present covalent binding of partially degraded heparin gives a surface heparin concentration in the order of 0.5 to 1.0IU per cm² and should from the theoretical point of view result in a completely stable heparin binding [2]. At a first contact with plasma, however, minute amounts of most probably unspecifically bound heparin is released [2]. Although the coagulation time in the present investigation remained unchanged during dialysis a minor elevation of
the plasma heparin concentration was accordingly seen in the early period of the dialysis session.

Heparin surfaces are, like a number of similarly prepared glycosaminoglycan surfaces, platelet compatible [4]. The reason for this seems to be that fibrinogen is not included in the firmly attached and about 50Å thick protein adsorbate which is formed at contact with plasma [6]. The reduction in the platelet count which was found in the present study is far less than that observed during previous dialysis experiments in dogs given heparin systemically [7]. Determination
of the FPA concentration reveals that thrombin digests or recently has digested fibrinogen in blood and it must be considered as the most sensitive quantitative assay available on intravascular coagulation. This implies that FPA generated from minute coagulation in sampling catheters or on catheter induced endothelial lesions may contaminate the blood sample. The extent to which the uneven distribution and the wide range of the FPA determination were owing to such factors can not be settled. We find it rather striking that dialysis with the aid of surface heparinised equipment did not cause fibrinogen-fibrin conversion beyond that in merely anaesthetised animals.

The surface heparinisation procedure did not essentially alter the in vitro performance of the cellulose acetate membrane in the dialyser. Toxicological tests of the heparin surface have been favourable. Trials with surface heparinised equipment seems therefore to be justified in evaluating the possible clinical advantages.

References

1. Griffith GC, Nichols G, Asher JD, Flanagan B. *JAMA* 1965; 193: 91