Adsorption of coagulation factors on to haemodialysis membranes during standard haemodialysis

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One of the initial events upon contact of blood with artificial surfaces is the deposition of a heterogeneous protein layer on the foreign material. Proteins of the blood coagulation system are known to be involved in these reactions. In this paper the adsorption of two contact coagulation factors [high-molecular weight kininogen (HK) and factor XI (FXI)] and fibrinogen on to three different haemodialysis membranes [CA130, cellulose acetate; Prima H, cuprophane; and Filtryzer B2-1.3, poly(methyl methacrylate) (PMMA)] was addressed. Heparinized blood, drawn from healthy volunteers, was mixed with the purified radiolabelled proteins and was dialysed with an extracorporeal dialysis machine using CH_3COO^- as the dialysis medium. Haemodialysis was followed by an extensive rinsing procedure. Then the haemodialysis capillaries were tested for bound radioactivity. Fibrinogen, HK and FXI were adsorbed on to all three haemodialysis membranes. However, quantitative differences were observed for the three membranes. The adsorption data correlate well with results obtained in various *in vitro* studies using other experimental models. Pretreatment of the haemodialysis system with saline, heparin or albumin did not reduce the adsorption of the coagulation factors tested, as may have been expected from *in vitro* tests.

1. Introduction

Exposure of blood to artificial surfaces results in the deposition of a heterogeneous protein layer. The composition of the adsorbed protein film strongly influences subsequent cellular interactions involved in thrombogenesis. Numerous studies with purified proteins and platelets have been performed to elucidate and evaluate the adsorption properties of different biomaterials [1–3].

From studies with glass, Vroman concluded that plasma proteins adsorb on to glass in a sequential mode. Albumin, the first protein adsorbed, is at least partially removed by the second, immunoglobulin G, which is replaced by fibrinogen, followed by fibronectin and finally HK, providing the basis for the activation of the coagulation cascade [4].

It has been reported that adsorption of fibronectin and fibrinogen causes increased thrombogenicity [5, 6]. Albumin, on the other hand, almost completely prevents dramatic falls in platelet counts, which are frequently observed during extracorporeal circulation [7].

Few components of the protein films which are deposited from heparinized whole blood on to artificial surfaces of haemodialysis membranes have yet been identified [8, 9]. To obtain more information about the interactions occurring upon contact of heparinized blood with haemodialysis membranes, we developed an *in vitro* haemodialysis system which allows testing of the adsorption properties of different dialysis membranes under haemodialysis conditions. With this system we investigated the adsorption of the coagulation factors HK, FXI and fibrinogen on to three different haemodialysis membranes [cellulosic membranes, CA 130 (CA); cuprophane membrane, Prima H (CP); and synthetic membrane, PMMA membrane, Filtryzer B2-1.3]. We also tested whether pretreatment of the dialysis systems with saline, heparin or albumin had an effect on the adsorption of these proteins.

2. Materials and methods

2.1. Materials

The extracorporeal circuit consisted of the following components. Extracorporeal dialysis machine (SPS-Brady, Vienna, Austria), arterial and venous blood lines (VMP; Achim Schulz-Lauterbach, Iserlohn, FRG), cellulose acetate hollow-fibre dialysis membrane (CA 130; Travenol, Deerfield, USA), cuprophane hollow-fibre dialysis membrane (CP; Prima H, Bellco, Mirandola, Italy) and PMMA hollow-fibre dialysis membrane (Filtryzer B2-1.3; Toray, Tokyo, Japan). All three haemodialysis membranes had an effective surface area of 1.3 m^2.

Saline, Duoflac and dialysis buffer (STA-CH_3COO^--concentrate) were from Leopold and Co., Graz, Austria, human albumin (5% solution), Haemoderivate, was from Immuno AG, Austria, standard

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Na-heparin, unfractionated, from Serva, Heidelberg, FRG, and human albumin, crystallized and lyophilized, from Sigma, St Louis, Missouri, USA.

The radiochemicals, used for radiolabelling of proteins [10], were ^{125}INa, high affinity, low pH (NEN; Du Pont, Dreieich, FRG) and ^{35}S-Heparin (Amersham, Amersham Place, UK).

HK and F XI were purified according to standard procedures [11, 12]. The coagulation factors HK and F XI were tested for clotting activity. The purified proteins had specific activities of the order of 25 U mg^{-1} for HK and 87 U mg^{-1} for F XI. Fibrinogen was purified according to the method of Jakobsen and Kierulf [13]. The radio-immunological determination (RID assay) showed a concentration of 6.8 mg ml^{-1} fibrinogen, which corresponded with total protein. Using the method of Clauss [14] the fibrinogen concentration was 6.5 mg ml^{-1}. Thus, our fibrinogen was highly pure and fully active in a coagulation assay. The clotting activity of each protein was tested before and after the radiolabelling procedure, and only preparations that had not lost clotting activity during the labelling process were used for the subsequent experiments. Each radiolabelled protein was analysed on reduced and non-reduced sodium dodecyl sulphate (SDS)–polyacrylamide gels and was compared with the unlabelled starting material. Since the adsorption behaviour was an important criterion, we tested each of the three coagulation factors for adsorption to glass. We also ensured that the unlabelled form of each protein could compete with the labelled material for the adsorption sites on the test surface. Only radiolabelled protein preparations that fulfilled the criteria of full functional activity, exhibited an unchanged electrophoretic pattern and showed normal adsorption properties were used for further experiments (specifications are given in Figs 1–3).

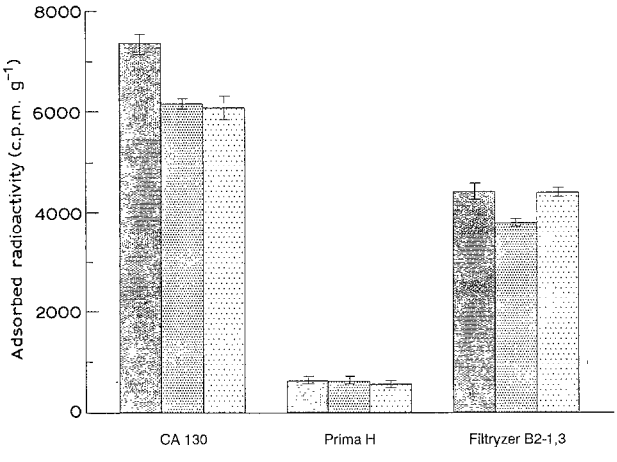

Figure 1 Adsorption of ^{125}I-HK. Each series of experiments (different capillaries and different prepriming solutions) was performed in triplicate. Figs 1–3 show the data for one representative experiment. HK [3.83×10^8 c.p.m. (100 µg)$^{-1}$ protein] was adsorbed as described in Section 2. The adsorbed radioactivity is expressed in c.p.m. g^{-1} hollow fibre. From left to right: CA 130, Prima H and Filtryzer, treated with (■) saline, (▦) heparin or (⊡) albumin. The standard deviations, represented by the vertical bars, were calculated from the 24 different portions of capillaries measured in each experiment.

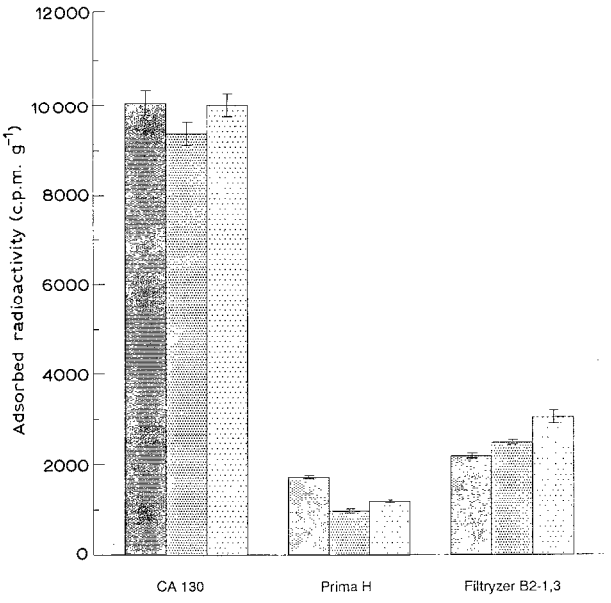

Figure 2 Adsorption of ^{125}I-F XI. F XI [8×10^9 c.p.m. (100 µg)$^{-1}$ protein] was adsorbed as described in Section 2. The adsorbed radioactivity is expressed in c.p.m. g^{-1} hollow fibre. From left to right: CA 130, Prima H and Filtryzer, treated with (■) saline, (▦) heparin or (⊡) albumin. The standard deviations, represented by the vertical bars, were calculated from the 24 different portions of capillaries measured in each experiment.

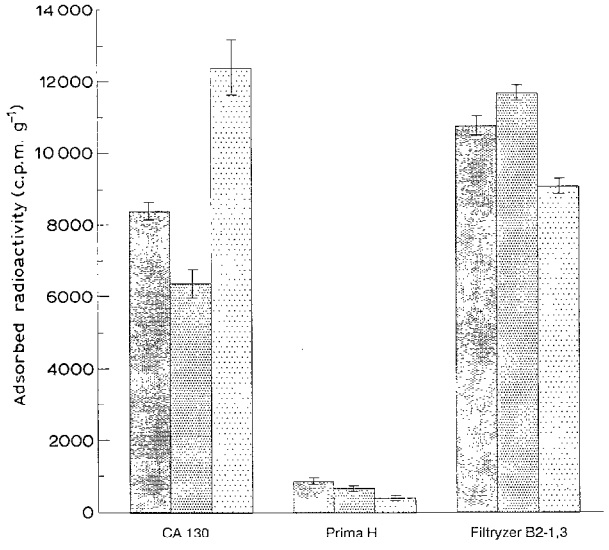

Figure 3 Adsorption of ^{125}I-fibrinogen. Fibrinogen [1.71×10^8 c.p.m. (100 µg)$^{-1}$ protein] was adsorbed as described in Section 2. The adsorbed radioactivity is expressed in c.p.m. g^{-1} hollow fibre. From left to right: CA 130, Prima H and Filtryzer, treated with (■) saline, (▦) heparin or (⊡) albumin. The standard deviations, represented by the vertical bars, were calculated from the 24 different portions of capillaries measured in each experiment.

2.2. Methods

2.2.1. Adsorption of ^{35}S-heparin and ^{125}I-albumin on to CA, CP and PMMA hollow fibres

Two separate experiments were performed for each capillary. The capillaries were mechanically opened, and several (three) defined portions (0.5 g) of the CA, CP and PMMA hollow fibres were taken and

transferred to incubation trays. The fibres were first soaked in saline for 5 min at room temperature, then saline was replaced by 20 ml heparin–saline solution (2.5 U heparin ml^{-1} and 70 nCi ^{35}S-heparin ml^{-1}) or human albumin solution (1 or 5% albumin containing 225×10^4 c.p.m. ^{125}I-albumin) and the fibres were incubated for 2 h at room temperature. The radioactive incubation solution was carefully removed and the hollow fibres were washed in 4×10 ml saline ($3 \times$ for 1 h, $1 \times$ overnight), dried and the adsorbed radioactivity was measured in a β-counter (^{35}S-heparin) or a γ-counter (^{125}I-albumin).

2.2.2. *Preparation of heparinized blood samples*

Blood from young healthy volunteers (aged 24 ± 7 years) was drawn from the anticubital vein with a 15-gauge needle into bottles containing unfractionated standard heparin (final heparin concentration 7 U ml^{-1}). Then the radioactive proteins ^{125}I-HK 50 000 c.p.m. ml^{-1} (2611 ng), ^{125}I-F XI 30 000 c.p.m. ml^{-1} (75 ng) and ^{125}I-fibrinogen 30 000 c.p.m. ml^{-1} (3509 ng) were added and the blood was used for *in vitro* dialysis experiments immediately.

2.2.3. In vitro *dialysis experiments*

The dialysis unit, consisting of an extracorporeal dialysis machine (SPS-Brady), a dialysis membrane (CA, CP or PMMA) and arterial and venous blood lines, was filled with saline, heparin in saline (3 U ml^{-1}) or 5% human albumin solution. After an incubation at 37 °C for 1 h, the prepriming solution was replaced by heparinized blood containing the radiolabelled protein (generally 200 ml). Arterial and venous blood lines were connected (short circuit) and dialysis was performed for 1 h at 37 °C with 200 ml min^{-1} blood flow using an STA-CH_3COO^--concentrate as the dialysis medium.

The dialysis procedure was followed by extensive rinsing of the system with saline (at least 1 l). Each series of experiments (different capillaries and different prepriming solution) was performed in triplicate.

2.2.4. *Measurement of adsorbed radioactivity*

Following the rinsing step the dialysis membranes were opened mechanically. Several portions of hollow fibres were removed and dried at 60 °C for about 2 days. For each experiment 24 portions of dried fibres were weighed and the radioactivity was measured in a γ-counter. The bound radioactivity (c.p.m.) per gram hollow fibres was calculated.

3. Results

3.1. Adsorption of ^{35}S-heparin and ^{125}I-human albumin on to CA, CP and PMMA hollow fibres

In the experiment in which we tested the adsorption of purified heparin on to the three different types of capillaries we were able to show that heparin was adsorbed to all three dialysis membranes (Table I): CA hollow fibres bound 13.76×10^3 c.p.m. g^{-1}, Prima H bound 4.36×10^3 c.p.m. g^{-1} and PMMA hollow fibres bound 2.72×10^3 c.p.m. g^{-1} of the total ^{35}S-heparin present in 20 ml incubation solution.

We also demonstrated adsorption of ^{125}I-human albumin (Tables II and III) on to all three haemodialysis hollow fibres. In the presence of 1% unlabelled albumin CA bound 20.86×10^3 c.p.m. g^{-1}, CP bound 21.60×10^3 c.p.m. g^{-1} and PMMA bound 19.01×10^3 c.p.m. g^{-1} capillary. In the presence of 5% unlabelled human albumin the adsorption of the

TABLE I Adsorption (means ± standard deviation) of ^{35}S-heparin on to CA (CA 130), CP (Prima H) and PMMA (Filtryzer) hollow fibres

	CA 130	Prima H	Filtryzer
Total radioactivity in incubation solution [$\times 10^3$ c.p.m. (20 ml)$^{-1}$]	2.34 ± 0.074	2.40 ± 0.040	2.32 ± 0.074
Adsorbed radioactivity ($\times 10^3$ c.p.m. g^{-1})	13.76 ± 1.18	4.36 ± 0.91	2.72 ± 0.35

TABLE II Adsorption (means ± standard deviation) of ^{125}I-human albumin from 1% human albumin solution on to CA (CA 130), CP (Prima H) and PMMA (Filtryzer) hollow fibres

	CA 130	Prima H	Filtryzer
Total radioactivity in incubation solution [$\times 10^3$ c.p.m. (20 ml)$^{-1}$]	2.06 ± 0.056	2.05 ± 0.106	2.20 ± 0.063
Adsorbed radioactivity ($\times 10^3$ c.p.m. g^{-1})	20.86 ± 1.3	21.60 ± 4.74	19.01 ± 4.9

TABLE III Adsorption (means ± standard deviation) of ^{125}I-human albumin from 5% human albumin solution on to CA (CA 130), CP (Prima H) and PMMA (Filtryzer) hollow fibres

	CA 130	Prima H	Filtryzer
Total radioactivity in incubation solution [$\times 10^3$ c.p.m. (20 ml)$^{-1}$]	1.88 ± 0.016	1.90 ± 0.035	1.84 ± 0.012
Adsorbed radioactivity ($\times 10^3$ c.p.m. g^{-1})	13.15 ± 1.36	11.65 ± 1.75	13.46 ± 2.02

TABLE IV Adsorption of various proteins to three different dialysis membranes

	CA			CP			PMMA		
	HK	FXI	Fibrinogen	HK	FXI	Fibrinogen	HK	FXI	Fibrinogen
Saline	2.1	4.8	4.0	0.1	0.5	0.3	1.4	1.2	5.9
Heparin	1.8	3.4	3.1	0.1	0.2	0.2	1.2	1.0	6.4
Albumin	1.8	4.8	6.0	0.1	0.4	0.1	1.4	1.7	5.0

Results are expressed as % initial protein per total surface area.

radioactive tracer decreased in all cases (CA 13.15 $\times 10^3$ c.p.m. g^{-1}, CP 11.65×10^3 c.p.m. g^{-1} and PMMA 13.46×10^3 c.p.m. g^{-1}). Thus, binding of radioactivity to all three haemodialysis membranes is competed by unlabelled albumin and can be considered specific.

3.2. Dialysis experiments

3.2.1. Blood donors

All blood donors had normal haematocrit and normal levels of fibrinogen (NOR Partigen plates) and HK- and FXI-clotting activity.

3.2.2. Adsorption of ^{125}I-HK on to haemodialysis membranes

Although the adsorption of HK to the three dialysis membranes was different for each membrane type (CA > PMMA > CP), it was not influenced by pretreatment of the capillaries with saline, heparin or human albumin (Fig. 1 and Table IV).

3.2.3. Adsorption of ^{125}I-FXI on to haemodialysis membranes

The adsorption of ^{125}I-FXI to CP haemodialysis membranes was low. Binding to PMMA and CA membranes was clearly higher (Fig. 2 and Table IV). Up to ten times as much ^{125}I-FXI was bound on to CA membranes than on to CP membranes. Treatment of the system with heparin or albumin had no effect.

3.2.4. Adsorption of ^{125}I-fibrinogen on to haemodialysis membranes

Fig. 3 (Table IV) shows that little fibrinogen was adsorbed on to CP membranes from heparinized blood. Ten times more fibrinogen was bound to CA and PMMA capillaries under the same experimental conditions. Interestingly, albumin pretreatment of CA membranes led to a 50% increase in fibrinogen adsorption. Prepriming of the system with heparin did not change the fibrinogen adsorption.

4. Discussion

In this study we measured the adsorption of the contact coagulation factors HK and FXI and the coagulation protein fibrinogen on to haemodialysis membranes under haemodialysis conditions. The aspect we pursued was the fact that clinical haemodialysis is still associated with undesired processes such as activation of cells, the coagulation and the complement system [15–17]. These events are preceded by the deposition of a heterogeneous protein layer on the artificial surfaces. The components of these protein films strongly determine the platelet adhesion, activation of the clotting and complement system, and ultimately thrombus formation. Therefore, identification of the proteins composing the protein films deposited on haemodialysis membranes is of major interest.

To learn about the adsorption of these proteins, several studies have compared the levels of coagulation factors in plasma from patients before and after dialysis treatment. However, the results are controversial [18–20].

The differences that have been observed between predialysis and post-dialysis values may be due to adsorption of the proteins on to the dialysis membranes, or may be caused by structural changes followed by loss of functional activities. An additional complication of measurements of coagulation factor activities before and after dialysis is the presence of heparin and changes in haematocrit during the dialysis procedure. Therefore, it was realized that the adsorption of proteins on to the dialysis membranes needs to be studied directly.

A number of adsorption studies of coagulation proteins to glass and to synthetic polymers such as CA and cellulose nitrate are available. However, most of these were done with purified protein solutions or plasma [21–24]. Some recent papers have reported the importance of the adsorption of fibrinogen and HK on to dialyser membranes for the activation of the coagulation, kallikrein–kinin and the complement system. Still, very little is known about protein adsorption on to dialysis fibres from whole blood. Therefore, we measured the adsorption of the three coagulation factors on to different haemodialysis membranes during standard haemodialysis.

Our finding of quantitative differences in the binding of FXI to the three dialysers was not completely unexpected, as Mannhalter and Schiffmann have shown that different mechanisms are involved in the binding of this protein to glass and various plastics [25]. Interestingly, in a recent paper Mulzer and Brash [9] were not able to elute FXI with SDS to any significant extent from four different haemodialysers. However, as those authors pointed out, this could be due to the relatively low concentration of FXI in

plasma, resulting in a very small amount of adsorbed material and very little eluted F XI, which does not allow detection in the eluate. It might also be due to unsuccessful elution of F XI with SDS. Our studies clearly showed that F XI is adsorbed to all three dialysers, although the adsorption of F XI to CP membranes is very low. Apparently, low adsorption is a general property of CP for all three proteins tested. We also found adsorption of HK to all three membranes, again significantly lower to CP than to CA. Vroman *et al.* [26] and later Schmaier *et al.* [27] demonstrated that HK plays a key role in the adsorption of plasma proteins on to negative surfaces. HK also influences the binding of fibrinogen (Vroman effect).

In our system fibrinogen was detected on all three dialyser membranes. The highest concentration was again found on CA. Mulzer and Brash [9] observed in their elution experiments that the amount of fibrinogen that was eluted from all dialysers was surprisingly low. Even though the study was not designed for quantitative determinations, we also got the impression that the adsorption of fibrinogen was rather low. We analysed the fibrinogen adsorption after 1 h dialysis procedure. Vroman *et al.* [26] showed that fibrinogen adsorption to various materials is transient (the Vroman effect), and adsorbed fibrinogen is replaced by other proteins such as HK. This may explain the relatively small amount of adsorbed fibrinogen that we detected after 1 h dialysis. When we tested the influence of saline, heparin and albumin, we found that pretreatment of the haemodialysis system with these agents did not significantly change the adsorption of the coagulation factors studied, with one exception. Albumin pretreatment of the CA 130 membrane was followed by increased binding of fibrinogen. One possible explanation for this unexpected observation could be that the 5% (w/v) albumin solution that we used contained traces of immunoglobulins, e.g. γ-globulins. These could bind to the membrane and could induce fibrinogen binding.

In summary, we were able to monitor the adsorption of proteins on to haemodialysis membranes under haemodialysis conditions, even when they were bound only in trace amounts. As some of the adsorbed proteins (e.g. F XI) may express sufficient clotting activity even if present only in trace amounts, a system that allows studies of the adsorption of trace proteins in the presence of all other blood components can supply valuable information.

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