

Biocompatible membranes must be hemocompatible: the role of heparin adsorption onto dialysis membranes

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INTRODUCTION

In the last ten years, the concept of biocompatibility applied to hemodialysis membranes has gained a general clinical acceptance with the consideration that the clinical expression of β_2^- microglobulin (β_2m) amyloidosis found in long-term hemodialyzed patients was governed by the dialysis membrane characteristics. High permeability and biocompatible synthetic mem-

branes interferes with the clinical expression of the disease and postpones occurrence of carpal tunnel syndrome and juxta-articular bone cysts^{1,2}. These beneficial properties cannot be solely ascribed to high permeability in a mechanistic perspective of improved removal of β_2m , membrane biocompatibility must be taken into account^{3,4}. Biocompatibility is a difficult concept to define in the absence of well established clinical correlates. Besides "anaphylactoid reactions"⁵ that are more frequently related to contaminated dialysate than to membrane sustained activation of the contact phase of coagulation in patients treated with angiotensin converting enzyme inhibitors^{6,7}, the concept of

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biocompatibility refers to any harmful stress induced by blood contact with dialysis membrane⁸. Insofar all the membrane are bioincompatible and we consider that a membrane is less biocompatible when the sum of humoral and cellular reactions occurring during hemodialysis is higher than for the reference membrane.

The aim of this short review is to highlight the key role played by dialysis membrane adsorption for defining biocompatibility and to extend this advantage to heparin binding.

MEMBRANE COMPONENTS AND EFFECTS OF THE EXTRACORPOREAL DEVICES

A wide spectrum of cellulosic and synthetic material has been used for manufacturing dialysis membranes. It is not necessary to point out that none of these products was nonetheless developed primarily for industrial use and later found their way to medical devices: the first dialysis membrane to be largely used in patients was derived from the wrapping material cellophan.

Historically, the first goal of hemodialysis was to treat transient acute renal failure allowing control of water and electrolyte balance. Later, iterative hemodialysis was applied in chronic renal insufficiency, and the first dialysers made for this purpose were equipped with modified cellulosic membranes. Cuprophan, a substituted cellulosic membrane, became the dialysis membrane gold standard during the infancy of chronic hemodialysis. Equipping dialysers of various shape and internal geometry, the cellulosic membranes were classified according to water permeability. Soon, it became obvious that these dialysis membranes were not permeable to peptides and low molecular weight proteins that belong to the class of middle molecular weight molecules which contains uremic

toxic molecules. The milestone studies of Baldwin Scribner's group⁹ pointed out the importance for removal of toxic molecules of middle molecular range that required more permeable membranes than the conventional cellulosic membranes available thirty years ago. The first membrane fulfilling criteria of effective removal of middle molecular range uremic toxins (measured as vitamin B12 clearance) was the polyacrylonitrile membrane¹⁰. Then appeared other synthetic membranes such as polysulfone¹¹. All these membranes were "highly permeable" and allowed secure dialysis for shorter duration because of efficient diffusive and convective transfers. Their very high water permeability rendered necessary the making of adequate monitors of ultrafiltration, able to maintain programmed ultrafiltration, limiting fluid imbalance and cardiovascular instability during the session. The use of high flux dialysers lead to the understanding of endotoxin diffusion from contaminated dialysate by backfiltration and the general acceptance of the hypothesis of "membrane bioreactor" proposed by L. Henderson¹². In short, it was suggested, that monocyte activation induced by endotoxin stimulated synthesis and secretion of the proinflammatory interleukine-1. Further, it was documented that other proinflammatory and chemoattractant cytokines are secreted in excess in hemodialysis patients, such as TNF- α , IL-6 and IL-12^{13,14} and, to come back to biocompatibility, it was demonstrated that generation of proinflammatory cytokines was also induced by the direct contact of leukocytes with cellulosic membranes in the absence of endotoxine¹⁵. The studies on the cytokine plasma levels have provided conflicting clinical results about their interest as prognostic markers in the treatment of acute renal failure. Nevertheless, the use of synthetic highly permeable membranes which bind cytokines and growth factors with high

grade purity dialysate (the lowest possible endotoxin concentration as measured by upregulation of the endotoxin receptor CD14 at the surface of circulating leukocytes is recommended by the European Best Practice Guidelines (*to be published*).

Dialysis membranes can be classified as “non permeable” and “permeable” membranes. However, this classification does not take into account their binding capacity which differ from one membrane to the other and govern, at least partly, clearance of charged molecules. Membrane biocompatibility which is mainly associated with binding characteristic of polymers are related to the distribution of hydrophobic and hydrophilic domains and electric charges at their surface and in the bulk of the membrane. As measured by Zeta potential, a membrane classification can be proposed (Table I)¹⁸.

PHYSICOCHEMICAL MECHANISMS ASSOCIATED WITH MEMBRANE BIOCOMPATIBILITY

In hemodialyzed patients the concept of biocompatibility was first coined after the discovery that the dramatic drop of circulating leukocytes during a session of hemodialysis performed with a cellulosic membranes did not occur using the synthetic membrane^{19,20}. This effect was the result of complement activation generating anaphylatoxins, and upregulation of the surface adhesion molecules and expression of leukocyte antigens, such as CD11b, CD18, complement receptor type 3 (CR3) and CD45²¹⁻²⁴. Hydrophilic biomaterial with abundant, polar hydroxyl groups such as cellulose, is recognized by the sulhydroxyl group on the C3 complement molecule and triggers its activation. When acti-

TABLE I

Measurement of dialysis membrane electronegativity, plasma kallikrein and bradykinin (BK) concentration. Membranes: polyacrylonitrile AN69 (Hospal); PANDX: polyacrylonitrile (Asahi); PMMA: polymethylmetacrylate (Toray); CT: cellulose triacetate (Baxter); CUP: cuprophane (Akzo); PS: polysulfone (Fresenius); AN69-ST: AN69-polyethyleneimine (Hospal).

Membrane	Zeta potential <i>mV</i>	Plasma kallikrein <i>U/liter</i>	BK generation <i>femol/ml</i>
AN69	- 70 5	60 ± 15	32100 (26500-41200)
PANDX	- 60 4	80 ± 20	28983 (22600-36150)
PMMA	- 25 2	10± 5	130 (50-250)
CT	- 20 2	<5	65(25-100)
CUP	- 10 1	<5	78(25-50)
PS	- 5 1	<5	62(25-120)
AN69-ST	- 3 1	<5	150 (30-450)

vation of the complement cascade pathway is initiated, highly biologically active inflammatory mediators are generated, including the anaphylatoxins C3a, C4a and C5a, the opsonin iC3b and the terminal complement complex. C5a which exert a particular strong proinflammatory activity by inducing up-regulation of granulocytes, monocytes, lymphocytes and platelets adhesion molecules and lysosomal release from polymorphonuclear granulocytes. Cellosic membranes have been substituted with hydrophobic chemical groups through acetyl groups in cellulose acetate, for example, to decrease complement activation. The residual hydroxyl groups are still sufficient to maintain this effect. Synthetic membranes are less harmful for complement, leukocytes and platelets because they upregulate adhesion to a lesser extent than cuprophan and, in addition, some of them have the unique property for binding anaphylatoxins, cytokines and enzymes re-

leased by cell activation after membrane contact^{25,26}.

Activation of polymorphonuclears by artificial membrane induce a burst of oxygen free radicals, the so-called "oxydative stress" associated with the "inflammatory stress" that induce strong biochemical transformations of lipids and proteins (peroxydation) and generate toxic adducts that activate endothelial cells and precipitate vascular injury and atherosclerosis. It is now well documented that chronic inflammation, as indicated by high plasma levels of C-reactive protein, can be generated by both mechanisms: blood contamination by bacterial endotoxin originating from contaminated dialysate and diffusing into the blood and by the single contact of incompatible membrane with IL-1-generating cells such as monocytes²². The metabolic consequences of hemodialysis performed with a non biocompatible membrane have been evaluated. They cannot be ascribed to aminoacid loss during dialysis alone²⁷. In a pioneer study, Jonas Berströmet al.²⁸, have shown that sham-hemodialysis in healthy students induced a significant increase of protein breakdown using cuprophan membrane but this harmful effect was blunted using the polyacrylonitrile AN69 membrane.

Criteria of dialysis membrane biocompatibility are numerous. They are listed in the non exhaustive Table II. Many of them are documented *in vitro*. We do not know to what extent the disturbances induced at the phase contact between blood and artificial membrane trigger general defense mechanisms, since we are able to measure all the buffering systems that support our defense against the inflammatory stress. Insofar, a reduction of these pathophysiological mechanisms is mandatory to avoid or reduce reactions associated with subacute inflammation, accelerated cardiovascular disease and denutrition that characterize the patient on chronic hemodialysis.

TABLE II

Overviews of clinical and biological pathways dealing with biocompatibility in hemodialysis

<p>Clinical outcome:</p> <ul style="list-style-type: none"> - nutrition/energetics - cardiovascular risk - β2 microglobulin-related amyloidosis <p>Inflammation:</p> <ul style="list-style-type: none"> - Complement activation - leukocytes activation/oxidation stress - adhesion molecules - cytokines <p>Contact activation:</p> <ul style="list-style-type: none"> - Coagulation - Platelet activation - Fibrinolysis

HEMOCOMPATIBILITY OF DIALYSIS MEMBRANES

One aspect of dialysis biocompatibility is hemocompatibility, a perspective that has been particularly well studied for cardio-pulmonary surgery extracorporeal circulation. In the absence of anticoagulant, all membranes induce activation of contact phase and subsequent reactions of blood clotting²⁹⁻³². Blood coagulation results from the conversion of fibrinogen into insoluble fibrin.

The fibrin is deposited on the membrane surface and forms a meshwork of fibrils. In contact with fibrin, platelets are activated and aggregate, inducing a cooperative interaction that leads to blood clotting. White blood cells are attracted by the thrombus, invade it and, through enzymatic release contribute to further fibrin formation and platelet recruitment. These series of enzymatic reactions and binding of clotting molecules onto the membrane surface contribute to the biological membrane spreading over the polymer and is conventionally called "protein cake". The biologic layer contains plasma proteins such as Factor XII, fibrinogen, vitronektin, high molecular weight kininogens and others whom activation precipitate thrombogenesis further^{33,34}, inducing strong platelet aggregation and release of procoagulant contents like platelet Factor 4. We have seen that leukocyte are also involved in this process and contribute to the proinflammatory reactor of membrane incompatibility, by secreting proteases, lactoferrin and myeloperoxidase²⁵.

A particular aspect of hemocompatibility is the occurrence of contact phase activation that is implicated in the pathophysiology of so-called "anaphylactoid reactions". Conflicting results have been published in this field tested in various *in vitro* models^{24,32}. Several etiologic factors have been implicated as triggers of dialy-

sis induced-reactions: ethylene oxide, toxic leachables, acetate dialysate, bicarbonate dialysate contamination and reuse procedure. Various mechanisms have also been hypothesized, such as activation of complement system, release of histamine and recently demonstrated, release of bradykinine³⁵. The higher the membrane surface electronegativity, the highest the contact phase activation and the subsequent generation of bradykinine, as shown in Table 1¹⁸. It is well known that inhibition of bradykinine degradation by inhibition of kininase II, also called angiotensin convertase, using angiotensin-converting-enzyme inhibitors in patients hemodialyzed with high flux synthetic membranes, mainly polyacrylonitrile membranes, is a factor of anaphylactoid reactions. These reactions disappear after discontinuation of angiotensin-converting-enzyme inhibitors⁷.

DIALYSIS MEMBRANE ADSORPTION

Exposure of blood to artificial membrane surfaces leads to adsorption of plasma proteins almost immediately. This initial adsorption profoundly influence all subsequent events, and to a large extent, determines the thrombogenicity, in other words the biocompatibility of the material. It has been proposed that they are two modes of plasma protein adsorption at the blood-membrane interface: the first occurs as a result of preferential competitive adsorption of either albumin, fibrinogen/fibrin, fibronectin or globulins; the second mode of adsorption which follows surface adsorption and activation of proteins from the blood intrinsic and extrinsic clotting sequence, consists of adhesion of platelets and leukocytes causing ultimately polymerization of fibrin onto the surface. This second membrane is a dynamic process with continuing enzymatic reactions and substrate replacement.

However, its composite turn-over rate has not been measured *in vivo*. Technique using radiolabeled proteins with counting of radioactivity entrapped into the dialyser at the end of a session in hemodialyzed patients has given some data for the .2m binding capacity of dialysers³⁷. *In vitro*, adsorption of high molecular weight kininogen (HMWK) which participates in the contact phase activation of the endogenous blood coagulation cascade has been demonstrated on the AN69 membrane^{38,39,40}. Its biological activity was maintained and correlated with the amount of adsorbed protein. The mechanism of HMWK adsorption was dependant of ionic interactions between the membrane and the protein. Electroneutralisation of surface anionic sulfonate groups scattered over the polymer by polyethyleneimine, ie, the AN69-ST membrane, significantly decreased HMWK binding and decreased kinin generation. Polyethyleneimine reduced surface electronegativity without altering the negatively charged sulfonates of the membrane core. Gain in biocompatibility of this new membrane was also documented for improving binding of C3a, C5a¹⁶, Factor D²⁶ and glycated (β 2m⁴¹).

The biological layer coating the polymer reduces membrane permeability throughout a dialysis session⁴². This event results from the concentration polymerization of plasma proteins and adherent platelets and leukocytes upon hemodialysis membranes. It depends upon a number of factors, among them are: the nature and the thickness of polymers; the type of plasma proteins; the interaction between membrane and protein electric charges and local pH; the type of anticoagulant in use; the flow properties of the system. Tailoring the physico-chemical properties of the polymer smooth surface to selectively adsorb a protein layer that include an anticoagulant such as heparin, or an enzyme such as uricase or an antioxidant such

as vitamin E has been proposed for improving hemodialysis⁴³. The degree to which any particular binding mode occurs depends on a large extent on the character of the membrane: whether it is porous or non porous, hydrophobic or hydrophylic, polar or non polar. The results of so many interactions ask the question of competitive adsorption of proteins and render difficult the design of membranes with specific sorbent properties.

HEPARIN COATING ONTO DIALYSIS MEMBRANE

Control of mural clotting can be accomplished by the use of anticoagulants. Some naturally occurring anticoagulants, such as antithrombin III contribute to the mixture of proteins adsorbed onto the membrane surface. They are quantitatively insufficient for preventing clotting. Heparin, a complex carbohydrate, is commonly used in hemodialysis⁴⁴. The presence of sulfate groups in the carbohydrate moiety gives heparin its highly negative charge. Heparin binds to antithrombin III accelerating the enzyme neutralizing effect of this serine protease inhibitor and prevents thrombin formation. In the presence of heparin the interaction of antithrombin with thrombin is virtually instantaneous. The heparin-antithrombin complex neutralizes the conversion of other coagulation proteins converted to active serine proteases (Factors XII, XI, IX, and X)

Unfractionated heparin binding to polymers has been extensively studied to render dialysis membrane more hemocompatible. Heparin binding shares chemical characteristics similar to binding of other blood components through ionic and covalent interactions. Surface-coating techniques has been developed but the informations about procedures are covered by

manufacturer's patents. For efficient binding of heparin to cellulosic dialysers, the membrane must be first coated with an intermediate polymer. As a bridging element, polyethyleneglycol (PEG) has been extensively tested⁴⁵⁻⁵⁰. This procedure has improved hemocompatibility of artificial devices used for extracorporeal circulation in cardio-pulmonary surgery, but was not very successful in hemodialysis. More recently, a technique stretching polyethyleneimine (PEI) over the membrane surface of a polyacrylonitrile polymer has made a new membrane: the AN69-ST membrane. In comparison with the parent AN69 membrane, this new AN69-ST membrane is characterized by a lesser electronegative surface whereas the sulfonic charges present in the bulk of the membrane have not been modified by apposition of PEI over its surface¹⁸ as indicated previously. Heparin binding has been documented both *in vitro* and *in vivo*⁵¹ and once adsorbed onto the membrane heparin keeps its anticoagulant properties. This property is presently largely used at bedside, during the rinsing procedure with a solution of unfractionated heparin diluted in saline. This technique which does not require any specific membrane-manufacturing technology allows tapering heparin doses in hemodialysis, from half to two thirds of regular doses. The heparin-coated membrane has well documented anticoagulant properties for at least two hours, a delay corresponding to the biological half-life or the anticoagulant. In some patients at high risk of bleeding and under careful supervision, hemodialysis has been managed without systemic administration of heparin⁵². One wonders if lessening heparin doses in the chronically hemodialyzed patient could decrease risk of heparin-related osteoporosis. Similarly, heparin reduction should decrease hypertriglyceridemia and the increase of plasma VLDL and IDL particles, as well as hyperlipoprotein(a) found in some

80% of chronic hemodialyzed patients since these anomalies are associated with heparin-induced decrease of lipoprotein lipase activity⁵³⁻⁵⁴. These hypotheses remain to be proven. Binding of heparin should prevent further denaturation and hence activation of the adhered proteins and blood cells. This hypothesis should perfectly fulfill the concept of biocompatibility.

MEMBRANE BIOCOMPATIBILITY AND ATHEROSCLEROSIS

Presently, it is not possible to delineate the biological mediators that support the basis of the model of accelerated vascular disease presented by the hemodialyzed patient. At least, some 50% to 60% of mortality in the chronically hemodialyzed patients result from cardiovascular disease. Among many factors triggered by hemodialysis, nucleated cell activation releasing cytokines and growth factors in one hand and generating oxidative stress-associated peroxidation and glycation of proteins and lipids in the other hand may contribute to accelerated atherosclerosis^{55,56,57}. Increase of acute phase proteins and especially C-reactive protein, are presently the best biological markers of inflammation. They reflect, among the many targets of dialysis-induced proinflammatory factors, diffuse endothelium activation that may be measured more precisely by specific endothelial cell-derived markers, such as E-selectin and sICAM-1. Ridker et al⁵⁸ provided convincing evidence that the baseline plasma concentration of C-reactive protein in apparently healthy men are predictive of myocardial infarction and ischemic stroke. Consequently, if these findings may be applied to the hemodialyzed patient, we must take into account membrane biocompatibility, whatever the mechanism by which a membrane is biocompatible and hemocom-

patible. Supporting this recommendation are clinical data that suggest an antiinflammatory effect of low doses heparin during hemodialysis⁵⁹.

CONCLUSION : PROTEIN BINDING AS A SPECIFIC CHARACTERISTIC OF BIOCOMPATIBLE DIALYSIS MEMBRANE

As indicated, several biological pathways stimulated during hemodialysis should be included in any definition of membrane biocompatibility. Enzymes and substates that characterize these pathways interact together, before and after membrane coating. The relationship between the initial phases of classic complement activation and the contact phase system as well as fibrinolysis have been described. Plasmin activation that govern fibrinolysis stimulates also the complement cascade either directly or through cleavage of Factor XII. Decreasing membrane binding of complement and Factor XII as well as platelets and leukocytes with appropriate redistribution of electric charges within the surface and the bulk of the membrane appears the most efficient way for lessening the inflammatory stress of hemodialysis and rendering dialysis membranes more biocompatible. It could be possible that in the near future membrane-manufacturing technologies propose heparin-coated devices with improved hemoincompatibility. In fact, at bedside, the easy way for heparin-coating is to rinse the dialyser with heparinized saline. To conclude, biocompatibility of hemodialysis membranes is nowadays the most important challenge for improving dialysis quality which cannot be restricted to the mechanistic approach of urea removal.

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