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New Trends

A first screening for hemocompatibility of a universal support for selective and specific hemoperfusion

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ABSTRACT: A modified filmadsorber is presented, intended to be used for selective and specific hemoperfusion. It consists of a spirally wound cellulose nitrate film, onto which — after chemical activation with sodium periodate — albumin is bound, stabilized, sterilized and activated with glutaraldehyde. Various bioactive ligands containing amino groups can be coupled to this support. A subsequent treatment with dimethylamino borane stabilizes the bonds between cellulose nitrate, albumin, glutaraldehyde and ligand. Columns in which a second layer of albumin is bound to the support as a model for a bioactive ligand were first screened for hemocompatibility using rabbits. Leukocyte, thrombocyte and hematocrit behaviour during hemoperfusion showed that hemocompatibility of the support was good. (Int J Artif Organs, 1989; 12:63-7)

KEY WORDS: Extracorporeal detoxification, Modified filmadsorber, Hemocompatibility

INTRODUCTION

In the field of extracorporeal blood purification with plasma and hemo-perfusion, selective and specific adsorbers are gradually replacing methods like plasmapheresis and perfusion with activated charcoal. Selective procedures, such as with protein A columns (1), permit the removal of the desired pathogenic plasma constituent like circulating immune complexes and excess IgG. Other examples are columns containing dextran sulphate (2) or heparin (3) covalently coupled to a support, efficient in removing low-density lipoprotein (LDL). In the very elegant procedure developed by Stoffel et al. (4) antibodies against LDL coupled to Sepharose beads specifically remove the plasma constituent of interest.

The filmadsorber for extracorporeal blood purification, as originally developed at the Eindhoven University of Technology (5), intended for the non-selective removal of toxic substances, consists of a spirally wound cellulose fabric coated with cellulose nitrate, in which activated charcoal is embedded. This column shows good flow and adsorbing properties, combined with acceptable hemocompatibility (6). In vitro experiments with plasma containing drugs (7) like barbiturates, paracetamol, and salicylic acid, and in vivo studies with dogs with ligated bile ducts (5), and partially hepatectomized pigs (6), showed its applicability for the non-selective removal of toxic substances from whole blood.

The filmadsorber has now been modified to be used for selective and specific removal of pathogenic substances from blood. The support developed consists of a polyester mesh coated with a porous layer of cellulose nitrate onto which albumin is adsorbed, stabilized, sterilized and activated by cross-linking with glutaraldehyde. Onto this support various bioactive ligands can be coupled, such as active enzymes, poly-
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mixin B, protein A.

This report describes our first experiences in the development of the modified filmadsorber and an initial screening of its hemocompatibility, with a second layer of albumin coupled to the support.

MATERIALS AND METHODS

The support used consists of a polyester mesh, Polymon PES 150/40 (Polymon, Switzerland), 2.46 mg/cm², coated with a porous layer of cellulose nitrate, 1 mg/cm², with a nitrogen content of 11%. Coating is done with a home-made device as described elsewhere (6).

After profilation, a film of 120 x 4.5 cm² is spirally wound round a PVC mandrel with a diameter of 1 cm, and packed in a cylindrical housing of PMMA, length 5 cm, inner diameter 2 cm, capped with two PVC disks with stainless steel connections.

The support is chemically activated by treatment with 0.25M NaI₀₄ in 0.1M NaH₂PO₄ at 25°C with a pH of 4.6 during 6 hours. Next, the support is coated with rabbit serum albumin (fraction V, Sigma, USA), during 3 hours incubation with a solution of 10 mg/ml of the albumin in 1M NaCl, buffered with 0.1M phosphate, pH 7.0. This is followed by treatment with a 5% solution of glutaraldehyde in 0.05M borate buffer pH 9.0, also for 3 hours, at 25°C.

After this, the filmadsorber is considered sterile. All solutions to be used hereafter are sterilized by ultrafiltration with a 0.22µ filter (Millex—OR, Millipore, France) before use.

Next, a second layer of albumin is coupled to the support, serving as a model compound for a bioactive ligand, by aseptically filling the adsorber with the same solution as used for coating. After 24 hours at 4°C, a stabilization reaction with 0.1M dimethylamino borane (Aldrich, Belgium) in 0.1M phosphate buffer pH 7.0 is done for 24 hours to reduce the imine linkages between cellulose nitrate layer, albumin and glutaraldehyde. Finally, the adsorber is filled with a 1M NaCl solution and stored at 4°C.

To verify hemocompatibility, extracorporeal circulation (ECC) in the unanesthetized, freely moving rabbit was used (8).

Details of the surgical procedures and the advantage of the model are published elsewhere (8). One day after establishing blood access in the neck of two rabbits, A and B, ECC is done for 120 minutes on subsequent days. Rabbit A underwent ECC 5 times and rabbit B 3 times.

| TABLE 1 - LEUKOCYTE AND THROMBOCYTE COUNTS (GPT/I) OF RABBIT A AND RABBIT B DURING ECC |
| LEUKOCYTE COUNTS |
| experiment: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| time (min) | | | | | | | | |
| < 0 | 10.2 | 14.0 | 10.8 | 18.8 | 14.1 | 11.5 | 9.6 | 10.0 |
| 0 | 10.5 | 12.6 | 10.9 | 18.4 | 13.5 | 11.5 | 7.9 | 9.7 |
| 15 | 9.6 | 9.8 | 11.0 | 13.7 | 15.4 | 7.2 | 7.3 | 7.3 |
| 30 | 8.9 | 8.3 | 14.3 | 13.7 | 13.9 | 7.5 | 7.4 | 7.4 |
| 60 | 7.1 | 10.5 | 5.5 | 21.2 | 14.2 | 8.5 | 7.9 | 9.9 |
| 120 | 14.0 | 15.8 | 15.9 | 29.1 | 13.6 | 12.8 | 9.5 | 9.3 |

| TROMBOCYTE COUNTS |
| experiment: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| time (min) | | | | | | | | |
| < 0 | 99 | 185 | 110 | 187 | 51 | 131 | 108 | 137 |
| 0 | 143 | 255 | 134 | 148 | 42 | 130 | 106 | 135 |
| 15 | 96 | 203 | 82 | 122 | 69 | 125 | 71 | 120 |
| 30 | 66 | 61 | 67 | 169 | 55 | 122 | 69 | 120 |
| 60 | 77 | 78 | 77 | 112 | 53 | 100 | 62 | 100 |
| 120 | 94 | 109 | 111 | 172 | 57 | 103 | 43 | 87 |
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Fig. 1 - Leukocyte behaviour of rabbit A during ECC, expressed as the percentage of the value at t = 0. Experiment 5 was done with an empty column.

RESULTS AND DISCUSSION

The support of the filmadsorber has now been modified to be useful for selective and specific removal of exogenous and endogenous substances from whole blood. The newly developed support is composed of a polyester mesh with a porous coating of cellulose nitrate. The support is designed to ensure spacing between the turns when spirally wound, and is packed in a column so as to ensure unrestricted blood flow. It is chemically activated with sodium periodate to generate dialdehyde units. Next, albumin is adsorbed onto the support, which is bound both by hydrophobic interaction (9) and the formation of reversible imine linkages between the lysyl amino groups of the albumin and the aldehyde groups in the cellulose nitrate layer. This albumin layer is then stabilized (10), sterilized (11) and activated (12) by cross-linking with glutaraldehyde.

To this activated albumin layer various bioactive ligands containing primary amino groups can be coupled, such as active enzymes, polymixin B and protein A. The subsequent treatment with dimethylamino borane stabilizes the imine linkages between cellulose nitrate, albumin, glutaraldehyde and ligand by reduction. Simultaneously, the borane reagent inactivates the remaining aldehyde functions of both glutaraldehyde and the cellulose nitrate layer, which otherwise could interfere during hemoperfusion by non-selective binding of substances.

There are two reasons for attaching bioactive ligands to a previously adsorbed albumin layer: cellulose nitrate to which a ligand is coupled directly will, when in contact with blood, adsorb plasma proteins very quickly and strongly. This will lessen the accessibility of the ligand. Secondly, albumin coating of polymers, followed or not by cross-linking with glutaraldehyde, is known to improve hemocompatibility by reducing platelet adherence (13).

Table 1 gives the absolute leukocyte and thrombocyte counts just before and during ECC. Figures 1 to
4 present the leukocyte and thrombocyte behaviour expressed as the percentages of values at $t=0$. Figures 5 and 6 set out the absolute hematocrit values. During each experiment the hematocrit remained constant, indicating that erythrocytes were hardly damaged on their way through the adsorber.

In experiments 5 and 8, in which columns without a support were used, leukocyte and thrombocyte counts changed little. The curves obtained with support-filled modules indicate leukocyte and thrombocyte behaviour comparable with that shown by dialytic patients undergoing ECC: during the first 60 minutes there is a decline followed by a slow rise back towards the initial values. The thrombocyte and leukocyte counts established in our laboratory were in accordance with published data on the biology of the laboratory rabbit (14).

In all our experiments leukocyte counts never dropped more than 50%. This relatively small decrease indicates that the immune system was hardly affected. Thrombocytes were counted manually and thus showed a large spread. They never dropped more than 70%.

The leukocyte and thrombocyte behaviour was quite different from that reported by Böttcher et al. with uncoated charcoal filled modules, in which a persistent decrease was observed (8).

It is therefore concluded that the modified filmadsorber shows excellent hemocompatibility in this sensitive animal model. This is supported by the fact that the behaviour of thrombocytes and leukocytes during blank experiments hardly differed from that in experiments with support filled columns.

Further investigations, in which bioactive ligands, such as polymixin B and protein A, are coupled to the support are planned.

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