SPECIFIC REMOVAL OF CIRCULATING ANTIGEN BY MEANS OF IMMUNOADSORPTION

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1. Introduction

In previous reports the feasibility of specifically removing circulating DNA antibodies in vivo with an immunoadsorbent was demonstrated [1–3]. The selective removal of antigens and antigen-antibody complexes from the circulation in immune complex disease would be another desirable therapeutic goal. Cuprophan (Enka Glanzstoff, AC, Munich, West Germany), a non-toxic cellulosic membrane commonly employed in hemodialysis, has demonstrated a capacity to non-specifically adsorb proteins [4] and would thus appear to be a potential solid support for antibody adherence. We herein demonstrate that rabbit gamma globulin (RGG) may be conjugated to Cuprophan columns and that these RGG columns are capable of withdrawing specific antigen from plasma circulated through them.

2. Methods, results and discussion

Cuprophan columns 8 cm in length and 0.9 cm in diameter (surface area 22 cm^2) as shown in fig.1. Teflon tips were prewrapped with Cuprophan strips, moistened with 0.1 M phosphate buffer pH 7.0 and dried on the tubing to facilitate attachment of the Cuprophan sheets. The latter were rolled over the prewrapped Teflon tips which were separated by a glass rod. The Cuprophan sheets overlying the Teflon tips were moistened with 0.1 M phosphate buffer pH 7.0 and dried, resulting in its firm adherence to the Teflon. The glass rod was withdrawn and the connection between the Cuprophan and Teflon tubing was then wrapped with Parafilm (American Can Co., Neenah, Wisc.). Finally, a strip of Teflon tape was applied to the exterior seam of the Cuprophan to provide stability to the column.

The retention of RGG (Pentex, Kankakee, Ill.)

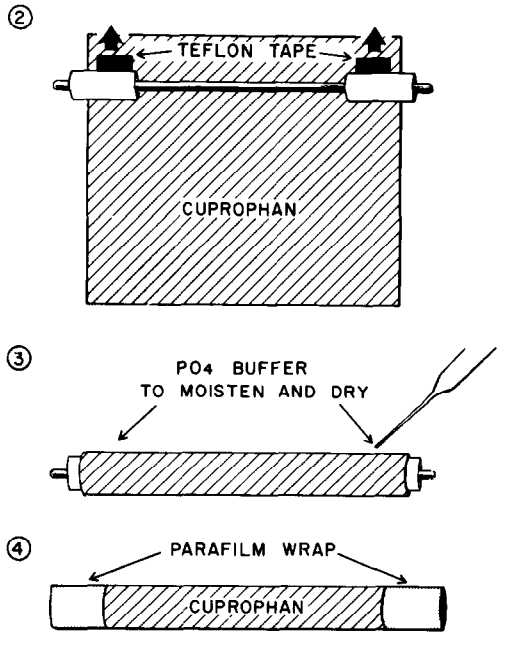

Fig. 1. Procedure for preparation of Cuprophan columns. See text for details.

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on these Cuprophan columns was studied by adding 15 mg in 3 ml of 0.1 M phosphate buffer pH 7.0 with various concentrations of glutaraldehyde to each of 3 tubes. ^{125}I RGG [5] in 0.3 ml vol was added to each tube as a marker. The Cuprophan tubes were clamped over the Teflon segments to avoid injury to the Cuprophan and gently rocked on a platform rocker (Bellco Glass Inc., Vineland, N. J.) at 4°C for 12 h. The Cuprophan tubes were then cleared of solution and exhaustively washed with 3 ml aliquots of 0.1 M phosphate buffer pH 7.0. The Cuprophan was detached from the Teflon and together with the washes and original solution counted in a Picker Nuclear gamma scintillation counter. The quantity of ^{125}I RGG retained on the Cuprophan was calculated as a percentage of the total number of counts recovered in the washes and on the Cuprophan. Results are shown in fig.2. There was a three-fold increase in RGG uptake on columns exposed to RGG alone, resulting in the retention of 3.3 mg of RGG.

As a preliminary to in vivo studies, antibodies to bovine serum albumin (BSA, Pentex, Kankakee, Ill.) were obtained from a repetitively immunized rabbit and isolated by the method of Ishizaka et al. [6]. Ten ml (9 mg) of purified antibodies to BSA exhibited an antigen binding capacity (ABC-33) [7] of 33 μg of BSA bound/ml of undiluted antibody

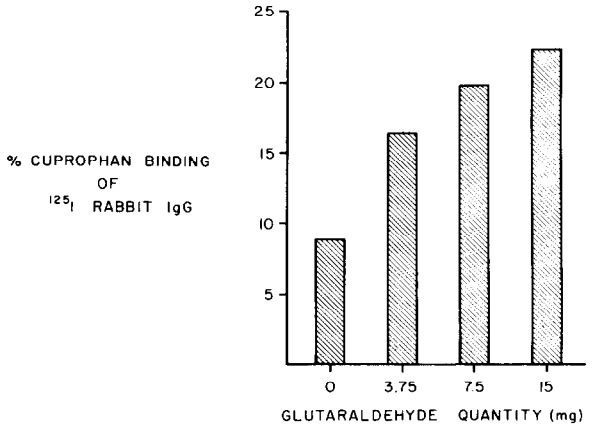

Fig. 2. Progressive rise in the percentage of 15 mg RGG retained by Cuprophan columns as it was incubated with increasing quantities of glutaraldehyde.

solution. For in vivo studies, six Cuprophan columns were prepared. To each of three, 3 mg of purified rabbit anti-BSA antibodies, 12 mg of RGG and 15 mg of glutaraldehyde in 3 ml of 0.1 M phosphate buffer pH 7.0 were added and the columns were rocked at 4°C for 12 h. Fifteen mg of RGG with 15 mg of glutaraldehyde was added to each of three control columns and similarly rocked. The Cuprophan columns were then individually washed with 200 ml

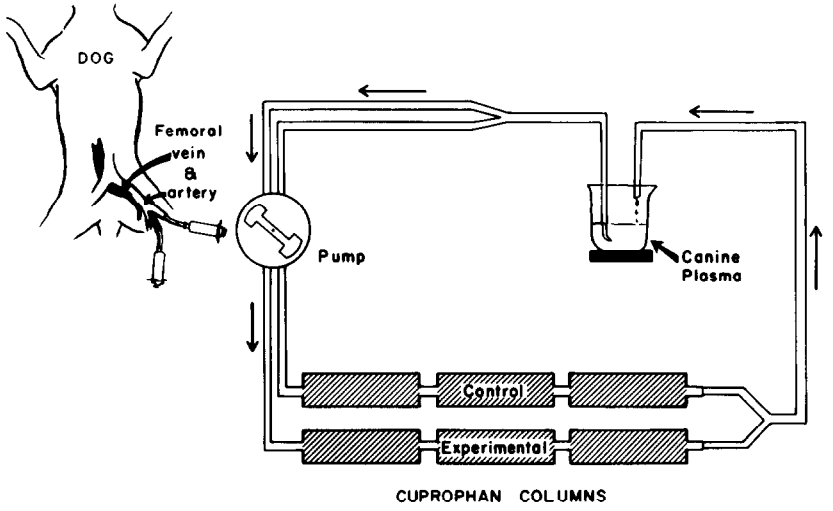

Fig. 3. Experimental design for extracorporeal system is shown. Canine plasma (100–200 ml) extracted from the femoral artery was separated from whole blood, placed in beaker and circulated by dual channel pump through experimental and control columns. See text for additional details.

Table 1
^{125}I BSA absorbed by Cuprophan columns[a]

	Background	Column I	Column II	Column III	Total ^{125}I BSA bound to Cuprophan
Anti BSA Membranes	90	408	403	437	1248
Normal RGG Membranes	90	112	107	101	319

[a]Each Cuprophon column was detached and counted for 1 min in a gamma scintillation counter. Values represent the mean of four determinations.

of 0.9% saline solution and connected with polyethylene tubing to a Travenol dual channel hemodialysis pump (fig.3). A 14 kg mongrel dog was premedicated with sodium pentabarbital and sodium heparin (1000 units/kg), and the femoral artery and vein cannulated with hemodialysis catheters (cobe Laboratories, Denver, Colo.). Ten minutes later ^{125}I BSA (0.8 μg) [5] was administered intravenously. Whole blood (100–200 ml) was withdrawn at 30 to 60 min intervals from the femoral artery. Plasma was separated from cells by centrifugation at 2000 rev/min for 7 min, placed in the beaker and circulated through the dual channel pump system for 15 to 30 min at a flow rate of 70 ml/min. The cells and plasma were then recombined, returned to the dog by the femoral vein and the process repeated until the calculated total plasma volume had been circulated through the columns. At the conclusion of the experiment, the Cuprophan columns were detached and individually counted in a Picker Nuclear gamma scintillation counter. Results shown in table 1 demonstrate that each of the experimental columns retained four-fold more ^{125}I BSA than its corresponding control column. There were no significant alterations in hematocrit, platelet, leukocyte counts, sodium, potassium, calcium, magnesium, blood urea nitrogen and creatinine values during the procedure.

The foregoing experiments demonstrate that antibodies may be conjugated to Cuprophan and that their uptake may be augmented threefold by the addition of glutaraldehyde, an intermolecular cross-linking agent [8]. When anti-BSA antibodies were coupled to Cuprophan they retained sufficient activity to withdraw four-fold more specific antigen from circulating plasma than RGG.

The surface area of the experimental columns used in this model system was 22 cm^2. We are presently investigating the use of markedly increased surface areas approximating the 18 000 to 22 000 cm^2 found in Cuprophan hemodialyzers. These will provide more antibody binding sites for withdrawal of circulating antigens.

Plasma instead of whole blood was used for passage through the Cuprophan columns since preliminary experiments had suggested that bound antibody was not functional in the presence of cellular debris that non-specifically adsorbed to Cuprophan. Indeed, mechanical systems are presently avaliable which permit separation of plasma from cells and might allow contact of plasma alone with the immunoadsorbent surface.

It might be of value to examine a similar approach as a therapeutic measure in some neoplastic diseases wherein circulating antigens appear to suppress the immune response [9,10] and in diseases mediated by immune complexes in antigen excess [11]. Perhaps, a composite system with separate columns incorporating specific antigen and antibody would be most effective in the removal of pathogenic immune substances from the blood in autoimmune and neoplastic diseases. Studies are presently underway examining the practicality of removing specific pathogenic antigens in animal models of neoplasia and systemic lupus erythemotosus.

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