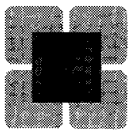

BEN H. BROUHARD, MD, EDITOR

Myasthenia gravis therapy: immunoadsorbent may eliminate need for plasma products

KOJI SAWADA, MD; PAUL S. MALCHESKY, DEng; ANNA P. KOO, MD; HIROSHI MITSUMOTO, MD

■ This in vitro study assessed the effectiveness of a new immunoadsorbent (Asahi IM-TR 350) in removing anti-acetylcholine receptor antibody from plasma with minimal loss of albumin. Plasma procured from a myasthenia gravis patient undergoing routine plasma exchange was perfused through the immunoadsorbent and recirculated in vitro to simulate a clinical treatment. To assess the temperature dependency of sorption, perfusion was performed at various temperatures. Plasma solute concentrations were taken before and after perfusion to calculate solute rejection coefficients. The immunoadsorbent has a high sorption capacity for anti-acetylcholine receptor antibody, while allowing a minimum loss of albumin. For patients with myasthenia gravis, this immunoadsorbent can provide an alternative to plasma exchange that does not require the use of plasma products.

□ INDEX TERMS: MYASTHENIA GRAVIS; IMMUNOSORBENT TECHNIQUES; PLASMA EXCHANGE □ CLEVE CLIN J MED 1993; 60:60–64

PLASMA EXCHANGE (PE) is the most effective therapy for myasthenia gravis (MG). However, many essential plasma components (such as albumin) are lost during routine PE, and in recent years the limited supply of plasma replacement products has raised serious concerns over the use of PE.[1] Moreover, the infusion of foreign plasma products carries the risk of hyperallergic reaction or viral contamination.[2,3] If on-line plasma fractionating techniques such as sorption plasma fractionation with an immunoadsorbent can be made as efficient as PE without significant loss of albumin, they will provide an alternative therapy that would avoid the limitations of PE.

MG is considered to be an autoimmune disorder[4–6] and is distinguished serologically and pathogenetically by the presence of an antibody directed against the acetylcholine receptor at the neuromuscular junction of striated muscles. This anti-acetylcholine receptor antibody (anti-AChR Ab) is found in the serum of 80% to 90% of MG patients[7–10]; the anti-AChR Ab assay is now widely used in diagnostic evaluation of MG.[11–13] The aim of PE is to quickly remove circulating autoantibodies, including anti-AChR Abs.[10,14–20] Our study evaluated in vitro the efficiency of sorption for anti-AChR Ab removal with a minimum loss of albumin. It has been reported that sorption is temperature-dependent[21–23]; therefore, we also assessed the effect of different temperatures.

From the Department of Biomedical Engineering and Applied Therapeutics (K.S., P.S.M.), the Clinical Apheresis Unit (A.P.K.), and the Department of Neurology (H.M.), The Cleveland Clinic Foundation.

Address reprint requests to P.S.M., Department of Biomedical Engineering and Applied Therapeutics, Wb3, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195.

MATERIALS AND METHODS

To simulate a clinical treatment in vitro, a recirculating circuit was used (*Figure 1*) and cell-free plasma was prepared. Plasma was procured by centrifuging blood discarded from a patient with MG undergoing routine PE. The plasma was filtered with a plasma separator (Asahi AP-05H, Asahi Medical Co, Tokyo) to remove cell components such as platelets and white blood cells.

Separate 2-L volumes of cell-free plasma were used for each of the three perfusion studies. The cell-free plasma had anti-AChR Ab concentrations 200 to 450 times greater than normal (7.81, 13.6, and 6.14 nmol/L, vs 0.03 nmol/L). The albumin contents of the three volumes of plasma were 50, 48, and 50 g (mean 49.3 g). The initial concentrations of albumin were 2.5, 2.4, and 2.5 g/dL.

The IM-TR immunoadsorbent (Asahi Medical Co, Tokyo) was used. This is a synthetic resin consisting of a polyvinyl alcohol gel (100 to 200 μm in diameter), to which the hydrophobic amino acid tryptophan is fixed as a ligand.[24–31] The adsorption of anti-AChR Ab has been shown to depend upon its affinity to tryptophan.[25–32]

The circuitry consisted of standard plastic hemodialysis tubing and a container made of polypropylene. A column of IM-TR immunoadsorbent

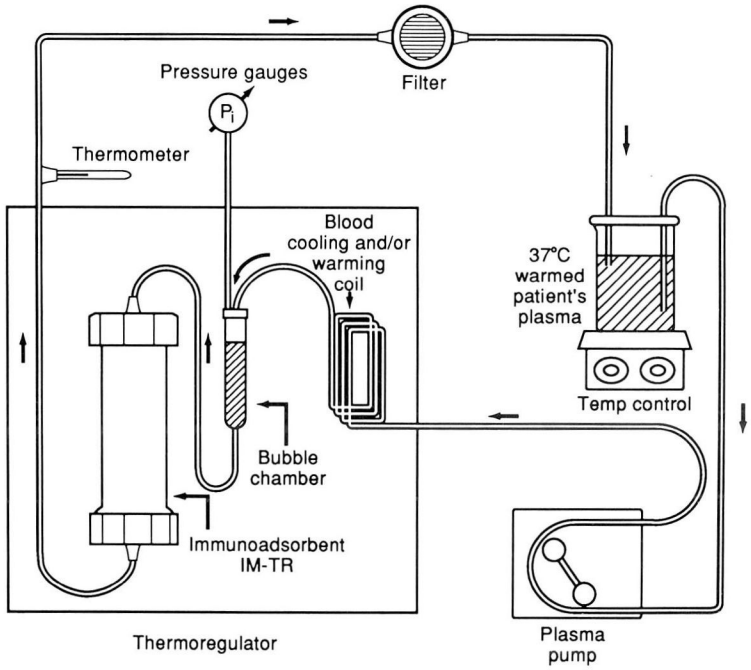

FIGURE 1. Diagram of the in-vitro circuit used in the sorption plasma fractionation study for myasthenia gravis. Cell-free plasma was circulated through the IM-TR column at 20 mL/min for 120 minutes.

$$(1)\ \text{Corrected concentration} = \text{Measured concentration} \times \frac{\text{Plasma volume + priming volume}}{\text{Plasma volume}}$$

$$(2)\ \text{Solute rejection coefficient} = 1 - \frac{\text{Concentration at outlet}}{\text{Concentration at inlet}}$$

$$(3)\ \text{Total solute removed} = \text{Plasma volume} \times \left(\text{Initial concentration} - \text{Final concentration}\right)$$

$$(4)\ \text{Percent solute reduction} = \frac{\text{Initial concentration} - \text{final concentration}}{\text{Intial concentration}} \times 100$$

FIGURE 2. Formulae for anaylsis of data. The measured concentrations of the solutes required correction for dilution (Equation 1). The corrected solute concentrations were used in subsequent calculations. The solute rejection coefficient (Equation 2) reflects the effectiveness of sorption; a rejection value of 1.00 indicates that the column completely removed the solute from the plasma; a value of 0 indicates that the column removed no solute. The amount of solute removed and the percent reduction of each solute in the plasma were also calculated (Equations 3 and 4).

TABLE 1
SOLUTE REJECTION BY ASAHI IM-TR 350 IMMUNOADSORBENT AT THREE TEMPERATURES

Solute	8°C	28°C	37°C
Anti-AChR Ab			
30 minutes	1.00	1.00	1.00
60 minutes	1.00	1.00	1.00
120 minutes	0.89	0.99	1.00
Albumin			
30 minutes	0.00	0.50	0.21
60 minutes	0.00	0.00	0.00
120 minutes	0.00	0.00	0.00
IgG			
30 minutes	0.92	0.99	0.99
60 minutes	0.78	0.89	0.91
120 minutes	0.45	0.59	0.61
IgA			
30 minutes	0.67	0.88	0.93
60 minutes	0.13	0.53	0.21
120 minutes	0.03	0.10	0.08
IgM			
30 minutes	0.56	0.93	0.90
60 minutes	0.50	0.91	0.88
120 minutes	0.20	0.63	0.67
Fibrinogen			
30 minutes	0.71	0.81	0.70
60 minutes	0.60	0.73	0.58
120 minutes	0.34	0.63	0.36

was placed in the container in the upright position. The weight of the column was 600 g, including 350 mL of gel with 0.01% aqueous solution of sodium pyrosulfite. The length of the column was 221 mm and the radius was 38 mm. The priming volume of the column was about 150 mL. The column was steam-sterilized.

The in vitro evaluations were performed according to a protocol for sorbent perfusions which is standard in our department. Before plasma perfusion, the column and the circuit were rinsed with a single pass of 2 L of 0.9% physiological saline (1 L without heparin and 1 L containing 1,000 U of heparin). The rinsing took place at ambient temperature at a flow rate of 100 mL/min.

After rinsing, 2 L of cell-free plasma was warmed to 37°C with a heater, simulating the temperature of the plasma coming from the patient. The plasma was then adjusted to the desired temperature in a thermoregulator (Hoxan Co, Sapporo, Japan). The plasma was passed through the IM-TR sorbent column and returned to the 37°C plasma pool, where it mixed with unperfused plasma.

The plasma circulated continuously in the on-line circuit at a flow rate of 20 mL/min. The perfusion time was 120 minutes. One test was performed at each of three temperatures (8°C, 28°C, and 37°C). In each test, a sample was taken from the warmed, pooled plasma at 0 minutes (before perfusion), and samples were taken from column inlet and outlet at 30 minutes, 60 minutes, and 120 minutes.

The samples were assessed for anti-AChR Ab, albumin, total protein, immunoglobulins (IgG, IgM, IgA), and fibrinogen. Anti-AChR Ab was quantified by radioimmunoassay at the Mayo Clinic Medical Laboratories (Rochester, Minnesota). Albumin and total protein were measured by the Technicon (Biuret) quantification methods at 630 and 550 nm, respectively (Technicon SMA II system, Technicon Instruments Corp, Tarrytown, New York). Immunoglobulins were quantitated by the single radial immunodiffusion method (Kallestad Labs Inc, Austin, Texas). Fibrinogen was measured by the BBL Fibrinometer (Becton Dickinson and Co, Rutherford, New Jersey).

Data reduction and analysis

The formulas used to analyze the data are shown in *Figure 2*. The measured concentrations of the solutes required correction for dilution caused by priming saline remaining in the circuit. The priming volume of the circuit was measured in each test; these volumes ranged from 135 to 153 mL. The priming volume was used in calculating the corrected concentration of each solute. Dilution correction effects were less than 8% of the total volume. The corrected values for solute concentrations were used in subsequent calculations. The solute rejection coefficient was calculated[33,34]; a rejection value of 1.00 indicates that the column completely removed the solute from the plasma, while a value of 0 indicates that the column removed no solute. The amount of solute removed and the percent reduction of each solute from the pool were calculated.

RESULTS

Solute rejection coefficients for anti-AChR Ab were nearly 1.00 at all times and temperatures, while those for albumin were zero, except at 30 minutes at higher temperatures (*Table 1*). Solute rejection coefficients of immunoglobulins and fibrinogen decreased during 120 minutes of perfusion. The amounts of solutes removed from the plasma pool and percent reduction are shown in *Table 2*. The percent reductions of anti-AChR Ab were about the same in all three studies.

TABLE 2
AMOUNT OF SOLUTES REMOVED FROM PLASMA BY ASAHI IM-TR 350 IMMUNOADSORBENT AND PERCENT REDUCTION OF SOLUTES IN PLASMA AT THREE TEMPERATURES

Solute	8°C	28°C	37°C	Mean for three temperatures
Anti-AChR Ab	9.77 nmol (62.5%)	15.78 nmol (58.0%)	6.78 nmol (55.0%)	10.77 nmol (58.5%)
Albumin	7.30 g (14.6%)	5.00 g (10.4%)	6.94 g (13.9%)	6.4 g (13.0%)
IgG	2,182 mg (58.3%)	1,696 mg (49.1%)	1,664 mg (50.4%)	1,847 mg (52.6%)
IgA	692 mg (33.3%)	693 mg (27.5%)	527 mg (28.6%)	637 mg (29.8%)
IgM	173 mg (61.9%)	168 mg (49.4%)	151 mg (53.9%)	164 mg (55.1%)
Fibrinogen	1,529 mg (65.3%)	1,320 mg (57.8%)	1,280 mg (60.4%)	1,376 mg (61.2%)

DISCUSSION

Neurologic diseases, including MG, now account for a large proportion of all apheresis procedures in the United States and Canada. In Canada, apheresis for neurologic disease increased from 28.6% in 1982 to 54.9% in 1988,[35] and the United States has seen similar tendencies. The number of apheresis procedures performed for neurologic diseases at the Cleveland Clinic has increased from 27 in 1984 to 318 in 1990. An average of 98 PE procedures were performed annually for MG between 1986 and 1990.

PE can remove anti-AChR Ab efficiently; however, the separated plasma which is discarded contains many essential proteins, including albumin. In PE, the large discarded volume requires infusion of plasma substitution fluids such as 5% albumin solution to maintain the patient's fluid and protein balance. Sorption plasma fractionation therapy was developed to obviate the need for plasma replacement products by selectively removing anti-AChR Ab. This technique addresses the problems of limited supply of plasma replacement products[1] and the disadvantages and risks associated with PE.[2,3]

In this study, nearly complete removal of anti-AChR Ab was accomplished with minimal reduction of albumin. The average loss of albumin of 13%, despite its low overall rejection, suggests that the albumin adsorption by the column occurred in the early minutes of the perfusion (typically less than 30 minutes).

Another objective of this study was to determine whether the efficiency of sorption for anti-AChR Ab removal could be improved by selecting the temperature of its operation. The IM-TR immunoadsorbent column can remove anti-AChR Ab selectively and nearly completely at all temperatures assessed; this indicates its high capacity.

The IM-TR immunoadsorbent has been used outside the United states to treat patients with MG, rheumatoid arthritis,[24] Guillain-Barré syndrome, multiple sclerosis,[25] and chronic inflammatory demyelinating polyneuropathy.[26] The removal of anti-AChR Ab was relatively higher than that of other autoantibodies because of the affinity of anti-AChR Abs to the amino acid ligand tryptophan, a component of the IM-TR immunoadsorbent.[25]

The IM-TR sorbent column will be evaluated in a multicenter trial in the United States for the treatment of MG. Our results show that it is as efficient as PE (100% rejection) in removing anti-AChR Ab and that it does so with minimal loss of albumin. If the clinical problems of MG are caused by anti-AChR Ab, then sorption plasma fractionation[31] with this immunoadsorbent at ambient temperature may be as effective as PE in treating MG patients, while obviating the need for replacement plasma products.[25–32]

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