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Short communication

Selective removal of immunoglobulin E from rat blood by membrane-immobilized antibody

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Abstract

We examined the suitability of an immunoaffinity membrane [rabbit IgG specific for rat immunoglobulin E (IgE) immobilized on a cellulose membrane] for removing IgE from rat blood passed through a simple extracorporeal circulatory system. To determine the concentration of IgE in the blood, we also developed a highly sensitive chemiluminescence enzyme immunoassay for rat IgE. The IgE levels in the outlet blood from the immunoaffinity membrane module decreased to 30% of the initial concentration within 30 min.

1. Introduction

Immunoglobulin E (IgE) is an essential factor in the regulatory pathways of the immune system and is responsible for enhancing allergic reactions. Therefore, control of the IgE level in blood should lead to a significant improvement in patients suffering from allergic reactions.

Affinity chromatography using immobilized antibodies is a rapid and highly specific method for the isolation of biologically active substances from a variety of different sources [1–4]. Furthermore, this method has been used to remove antibodies [5–7], IgE [8,9] and antigens [10] for medical applications. We previously reported that β_2-microglobulin in the plasma of patients on long-term hemodialysis was removed by IgG immobilized on an Affi-Prep 10 column [11,12]. However, the disadvantages of this method are the expensive running cost for antibody and the immunoaffinity support and the lack of an efficient plasma separator for clinical use. Moreover, biocompatibility of the ligand and support materials is a very real problem in the design of systems to remove components from human blood.

Recently, the use of membranes as an affinity support has been reported; for example, monoclonal antibody was purified by the use of membrane-immobilized protein A [13]. The advantage of membranes is the good biocompatibility in many cases; furthermore this procedure does not require the separation of plasma from blood in an extracorporeal circulatory system.

In order to monitor the removal of IgE from rat plasma, we recently developed a rapid, sensitive, and reproducible new chemiluminescence enzyme immunoassay (ELISA) for rat IgE. In the present report, we demonstrate that the antibody immobilized on an immunoaffinity

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membrane (IAM) is highly effective for the removal of rat IgE passed through an extracorporeal circulatory system not requiring an efficient plasma separator.

2. Experimental

2.1. Materials

Cellulose membrane for size filtration was obtained from Sartorius (Göttingen, Germany, pore size 0.8 μm). Three female Sprague-Dawley (SD) rats (12–18 weeks of age, 350–400 g) were used with an extracorporeal circulatory system. Anti-rat IgG was produced in a rabbit and was then purified by protein-A Sepharose chromatography. Biotin- or peroxidase-conjugated anti-rat IgE monoclonal antibody was purchased from Cemicon (Temecula, CA, USA), and rat recombinant IgE from ZYMED (South San Francisco, CA, USA). Lumi-Phos 530 (chemiluminescent substrate) was obtained from Wako (Osaka, Japan). Other chemicals used were of analytical grade.

2.2. Chemiluminescence ELISA and conventional ELISA for rat IgE

The chemiluminescence ELISA was carried out in a black module microtitre plate (Nunc, Roskilde, Denmark) that had been coated with anti-rat IgE (goat IgG; Nordic, Tilburg, Netherlands). Standard solution or rat plasma was diluted in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (100 μl). After incubation for 1 h at 37°C, the wells were washed three times with PBS–Tween 20 (0.05%), and 100 μl of biotin-conjugated anti-rat IgE monoclonal antibody was added to each well and then incubated for 30 min at 37°C. After three washes with PBS–Tween 20, alkaline phosphatase-conjugated streptavidin (100 μl/well) was added and incubated at room temperature for 1 h. The plate was subsequently washed with diethanolamine buffer. As a final step, 100 μl of the chemiluminescent substrate Lumi-Phos 530 was added to each well and incubated at room temperature for 30 min. The intensity of chemiluminescence was read as relative light units (RLUs) with a LUMINOUS CT-9000 D (Dia-Iatron, Tokyo, Japan).

The conventional ELISA was used with immunoplates (Nunc, Roskilde, Denmark) that had been coated with goat IgG anti-rat IgE. Standard solution or rat plasma was diluted in PBS containing 1% bovine serum albumin (100 μl). After incubation for 1 h at 37°C, the wells were washed three times with PBS–Tween 20. Peroxidase-conjugated anti-rat IgE monoclonal antibody was added instead of biotin-conjugated antibody as in the chemiluminescence ELISA, and incubated for 30 min at 37°C. After the wells had been washed five times with PBS–Tween 20, 100 μl of the substrate (0.83 m*M* 3,3′,5,5′-tetramethylbenzidine and 0.01% of H_2O_2 in citrate buffer) was added. After a 15-min incubation, the reaction was stopped with 1 *M* phosphoric acid, and the absorbance was read at 450 nm with an automated microplate reader (Model EL 312e, Bio-Tek, Winooski, VT, USA).

2.3. Preparation of immunoaffinity membrane

Anti-rat IgE (rabbit IgG) was immobilized on the cellulose membrane (total membrane area, 26.4 cm^2) by the mixed anhydride method [14,15]. In brief, membrane was immersed in a solution of 3% succinic anhydride in pyridine, and shaken at room temperature for 24 h. The carboxylated membrane was then reacted with tri-*n*-butylamine and isobutyl chlorocarbonate in dehydrated dioxane for 30 min at 15°C. Having been washed twice with dioxane, the membrane was placed in an aqueous solution of the IgG (anti-rat IgE) at pH 9.0 and left there for 3 h at 4°C. After removal of the membrane, the amount of uncoupled IgG remaining in the solution was evaluated in terms of protein concentration by the method of Bradford with rabbit IgG as a standard [16], and the amount of the IgG coupled to the membrane was then estimated.

2.4. Extracorporeal circulatory system

Rats were anaesthetized by an intraperitoneal injection with sodium pentobarbital. For the extracorporeal treatment, the rats were catheterized with carotid artery and contralateral jugular vein catheters (0.8 mm I.D.) to gain access to the blood [17]. Since the basal level of IgE in the rat blood was very low (<7.9 ng/ml), the animals each received 10 μg of recombinant rat IgE by intravenous injection 2 h before the experiment. Heparin (500 IU/kg) was injected intravenously and the catheters were connected to a peristaltic pump (Bio-Rad). The blood was pumped at 1.5 ml/min through the module containing IAM-immobilized anti-IgE antibody.

The affinity membrane module was custom-built, and consisted of six membranes (total surface area 158.4 cm^2), each separated from the adjacent one by two nylon mesh strips (size $32 \times 5 \times 0.1$ mm). The IAMs were packaged between two heat-sealed polypropylene sheets. This system was equilibrated with heparinized saline (10 IU/ml) before use and maintained in the incubator at 37°C. The blood was passed through the membrane module and then returned to the host animal. The extracorporeal circulatory system, having a total volume of around 2.8 ml, is schematically shown in Fig. 1.

Blood samples were sequentially obtained from two sites, the outlet of IAM module and the tail as a subterminal blood, at 0.5, 1.0, 1.5, and 2.0 h after the circulation. The IgE content in each sample was determined by ELISA. Western blotting was carried out as described previously [18].

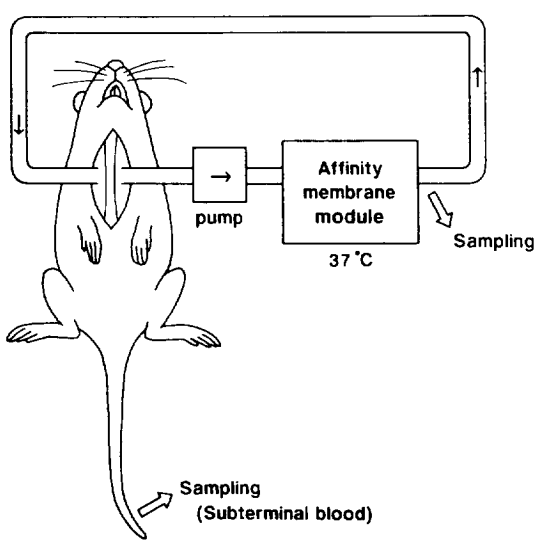

Fig. 1. Extracorporeal circulatory system.

3. Results

3.1. Evaluation of chemiluminescence ELISA for rat IgE

The standard curve (log–log scales) prepared from the data obtained by the chemiluminescence ELISA with the anti-IgE antibody demonstrated a stoichiometric relationship between IgE protein concentration and the intensity of chemiluminescence (Fig. 2). The working range of the

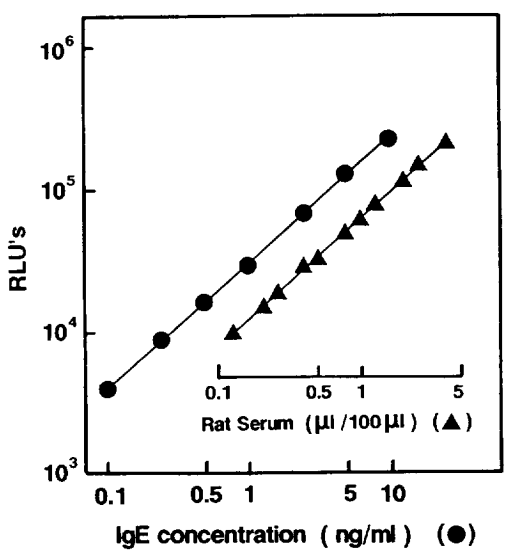

Fig. 2. Standard curve of recombinant rat IgE generated by the chemiluminescence ELISA and detection of IgE in rat plasma. Authentic rat IgE was incubated in triplicate tubes, and indicated volumes of rat serum were incubated in duplicate tubes. The intensity of chemiluminescence was determined by the luminometer in relative light units (RLUs).

assay was between 0.1 and 10 ng/ml for rat IgE. The detection limit of the assay was 50 pg/ml for rat IgE, which meant a 50-fold higher sensitivity than that of the conventional ELISA. When various volumes of rat plasma (0.1–5 μl) were subjected to the chemiluminescence ELISA, the curve obtained was parallel to that of the standard IgE (Fig. 2). Therefore, this assay can be employed to determine IgE in rat plasma.

Intra- and inter-assay variation for the chemiluminescence ELISA was measured at an IgE concentration of 36–389 ng/ml ($n = 5$). Intra-assay variation based on the within-day C.V., ranged from 2.68% to 4.59% ($n = 6$). Inter-assay variation, derived from the between-day C.V., ranged from 5.08% to 9.68% ($n = 5$). Furthermore, the recoveries of rat IgE added to rat plasma were 90.6–104.6%.

We tested the validity of our method by comparing the conventional ELISA with the chemiluminescence ELISA for the determination of IgE in plasma from thirteen rats, and the correlation between the two methods was satisfactory (correlation coefficient, $r = 0.987$; Fig. 3).

3.2. Evaluation of IAM using model samples

Anti-rat IgE–rabbit IgG was immobilized on regenerated cellulose membrane by the mixed anhydride method as described in Section 2. The density of IgG coupled to the membrane was 17.0 ± 2.8 μg/cm^2. The membrane trapped the IgE efficiently, and the content was 1.30 ± 0.05 μg IgE/cm^2 when a model sample was employed (1 μg of recombinant IgE dissolved in 1 ml of PBS). As the result of calculation, the binding capacity of the support was 0.08 μg IgE/μg immobilized IgG. The time course of trapping of rat IgE by the IAM indicated that 90% of the initial concentration was complexed within 30 min (Fig. 4). Western blotting did not detect any loss of IgG from the membrane after strong washing of the preparation (data not shown).

3.3. Extracorporeal circulatory system

The aim of this study was to evaluate the effectiveness of immunoadsorption for the specific removal of rat IgE under the ex vivo condition. Aliquots of the arterial blood at the

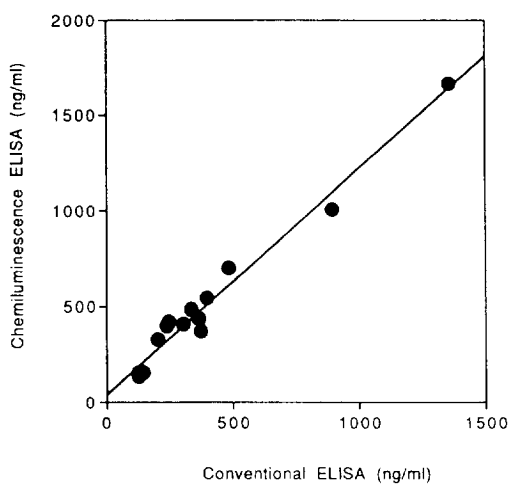

Fig. 3. Correlation between the chemiluminescence ELISA and the conventional ELISA for the determination of plasma IgE. The IgE levels in plasma of thirteen rats were measured by the chemiluminescence ELISA and the conventional ELISA. The correlation coefficient was 0.987.

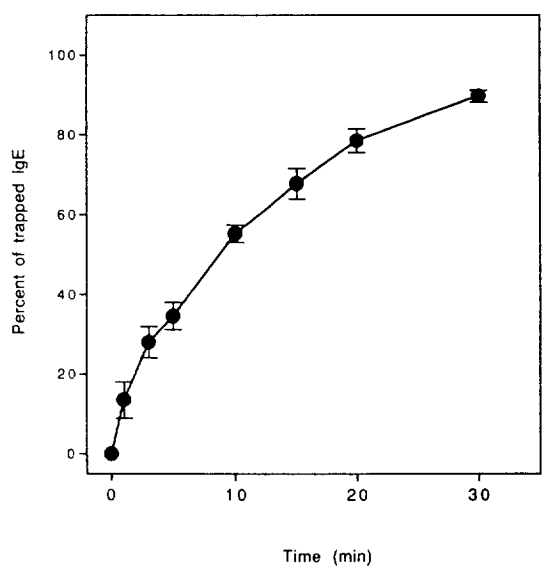

Fig. 4. Time course of trapping of IgE by the IAM. PBS buffer solution (2 ml) containing 1 μg/ml of recombinant IgE and 0.05% BSA was applied to the IAM (26.4 cm^2).

outlet of the IAM and of the subterminal tail blood were taken for the determination of IgE concentration. Three experiments were performed, and similar results were obtained in each case. As shown in Fig. 5, the plasma level of IgE in the blood passed through the system equipped with a blank membrane (without immobilized anti-rat IgE) did not change for up to 2 h. On the other hand, when the membrane bore the anti-IgE, the IgE level in the blood showed a sharp decrease. The subterminal plasma IgE level decreased to 55% within 30 min and reached 20% of the initial concentration within 2 h of circulation, while the IgE level in the outlet sample from the IAM decreased to 30% within 30 min. Low IgE concentrations in the system's effluent remained constant, and the immunoadsorption of IgE did not saturate the capacity of the immunoadsorbent during the duration of the experiment. The flow-rate of pumping did not affect the trapping rate for rat IgE over the range of 0.5–1.5 ml/min.

Neither clotting nor hemolysis was observed under our experimental conditions. The IAM could be regenerated by desorption of IgE with 0.1 *M* glycine-HCl buffer (pH 2.5) and immediate equilibration with PBS, and could be stored at 4°C in PBS containing 0.01% NaN_3. Under these conditions, no loss of IgE binding capacity of the membrane was observed over a period of 6 months as assessed by the same type of ex vivo experiment. Moreover, when we subjected samples eluted by 0.1 *M* glycine-HCl buffer to SDS-polyacrylamide gel electrophoresis and Western blotting using anti-rat IgE monoclonal antibody, each sample showed two major spots (molecular mass, 28 and 90 kDa), and their mobility was identical to that of authentic rat IgE (data not shown). These findings indicate that the IAM procedure is very effective and useful for specific removal of IgE from rat blood.

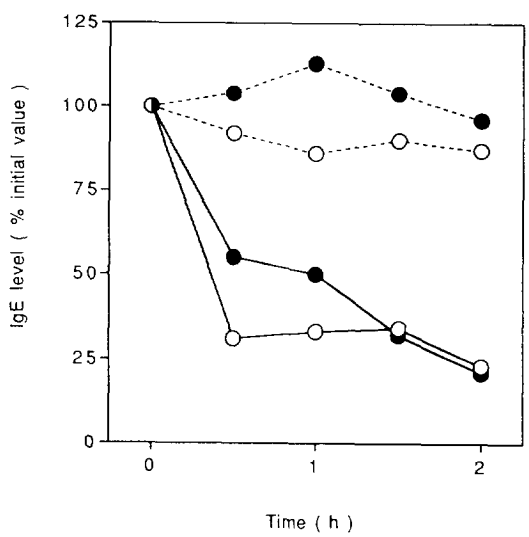

Fig. 5. Blood levels of rat IgE during extracorporeal circulation past IAM (test) and blank membranes. Blood IgE level is given as a percentage of the initial concentration: (○) for the outlet of membrane module, (●) for the subterminal blood, (dashed line) for the blank membrane, and (solid line) for the membrane with immobilized anti-IgE.

4. Discussion

The use of IAM provides an excellent method for removing rat IgE directly from the blood in a short time. This technique can be used in any facility, because it is a simple system. Compared with β_2-microglobulin removal by high-performance immunoaffinity chromatography [11,12], the present system is simpler and easier to maintain. Furthermore, it is a low-cost and practical procedure. The estimation of other plasma components was tested in the control experiment with the blank membrane. There were no significant differences in IgG, complement C3, or albumin levels in the IAM compared with blank membrane. These proteins remained constant at >96% throughout each circulation.

Another important aspect of the present study is the chemiluminescence ELISA system for rat IgE that we established. This ELISA offers two advantages. First of all, it is more highly sensitive than the previously reported RIA [19] or our conventional ELISA for rat IgE. Our chemiluminescence ELISA has made it possible to determine 50 pg/ml of IgE in rat plasma within 3 h. Secondly, this chemiluminescence ELISA can

be used as a monitoring system for any type of chromatography.

In conclusion, the present techniques should prove useful for medical application and may have broad applicability for the elimination of any unwanted plasma component with antigenic or any other storage disease.

Acknowledgements

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