Original Article

An Immunoabsorption Column Configuration for Hepatitis B Virus

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Objective To configure an immunoabsorption column for hepatitis B virus.

Methods Being activated by epichlorohydrin, the human antibody HBsAb-IgG was bound to the carrier of agarose gel. The configuration process was as follows: the synthesis of epoxide matrix, the synthesis and activation of amino matrix, the synthesis of aldehydic matrix, the synthesis of immunoabsorption matrix, the end capping and reduction of unbound aldehydic, the blocking of unbound mass and the filling of the column.

Results The bound rate of activated agarose gel and antibody HBsAb-IgG is 85.07%. By plasma adsorption experiment, it is revealed that the immunoabsorption column can absorb and eliminate 58.97% of HBsAg and 53.1% of hepatitis B virus particles in extracorporeal plasma.

Conclusions The immunoabsorption column for hepatitis B virus can absorb and eliminate HBsAg and hepatitis B virus particles in extracorporeal plasma.

Key words: Hepatitis B virus; Sepharose; HBsAb-IgG; Linkage; Immunoabsorption

The incidence of hepatitis B Virus (HBV) infection in our country is high, about 350 million people in the world were infected with HBV. According to the serological epidemic investigation of viral hepatitis in the whole country, the average positive rate of HBsAg was 9.75 %, about 120 million people had viral hepatitis, 30 million out of which were chronic hepatitis B patients, and 10-30% of chronic hepatitis B patients will possibly progress for cirrhosis, or even liver cancer. However, most of the current treatments of chronic hepatitis B were limited to the application of medicine with unsatisfactory curative effects. Based on the principle of immunology, together with the theory of blood purification, the author has configured human antibody HBsAb-IgG immunoabsorption column by binding human antibody with active agarose gel, and then observed its absorption function to HBsAg plasm containing HBV. This technology has been applied for the national invention patent (application No. 03110948.9).

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MATERIALS AND METHODS

Reagent and materials

Sepharose CL-4B gel (Amersham Pharmacia Biotech AB, Sweden), human antibody HBsAb-IgG (Chengdu Rongsheng Pharmaceuticals Co., Ltd.), epichlorohydrin (Analytical reagent, Shenyang Lianbang Reagent Plant), glutaric dialdehyde (Beijing Chemical Reagents Company), sodium borohydride (Analytical reagent, China National Medicine Group Shanghai Chemical Reagent Company), glycine ethyl ester (Analytical reagent, China National Medicine Group Shanghai Chemical Reagent Company), 50 ml absorption column shell (Hangzhou Kerui Enterprise Weikang Medical Instrument Co., Ltd.), HBV DNA positive patients plasma 500 ml (provided by blood purification center of our hospital), peristaltic pump (NIPRO FP-960 of Japan), special DHP060 Constant-Temperature Incubator (Shanghai Laboratory Instrument Work Co., Ltd.).

Method

Synthesis of epoxide matrix

One hundred and fifty ml sepharose CL-4B gel was measured using the graduated flask, then was poured into the sand funnel for filtering, then the funnel was washed with physiological saline and all the material were poured into a 500 ml beaker after drying, 180 ml

physiological saline, 70 ml of 2 mol/L NaOH and 50 ml epichlorohydrin were added in and mixed together. This mixed material was put into a beaker flask and sealed up. It was reacted for 2 hours inside the incubation chamber under 40°C with 200 rpm, then it was filtered in the sand funnel and washed by anhydrous ethanol and water successively, after that, it was dried.

Synthesis of amino matrix

The well prepared epoxide gel was removed into a 500 ml beaker, then 50 ml physiological saline and 450 ml 13 g/L hexamethylene diamine - NaHCO₃ buffer solution were added in and mixed together. This mixed material was put into a beaker flask and sealed up. It was reacted for 2 hours inside the incubation chamber at 50°C-55°C with 200 rpm, then it was filtered in the sand funnel and washed with anhydrous ethanol and water successively, after that, it was dried.

Synthesis of aldehydic matrix

The above amino gel was removed into a 500 ml beaker, 370 ml physiological saline and 30 ml 25% glutaric dialdehyde were added in and mixed together. This mixed material was put into a beaker flask and sealed up. It was reacted for 2 hours at room temperature with 200 rpm in the incubation chamber, then it was filtered in the sand funnel and washed in turn using water, 2 mol/L acetic acid and water, finally 0.1 mol/L pH 8.2 borate buffer was used for equilibrium and then it was dried.

Synthesis of immunoabsorption matrix

The above aldehydic gel was removed into a 500 ml beaker, and 50 ml physiological saline was added in, then 600 mg HBV antibody IgG was dissolved into 250 ml 0.1 mol/L borate buffer, poured into the beaker and mixed up. This mixed material was put into a beaker flask and sealed up. It was reacted for 20 hours at room temperature with 200 rpm in the incubation chamber, then it was filtered in the sand funnel after reaction completed.

Endcapping of unbound aldehydic

The above immunoabsorption gel was removed into a 500 ml beaker, added in 50 ml physiological saline and 250 ml 40 g/L pH 8.2 glycine ethyl ester buffer solution and mixed up. This mixed material was put into a beaker flask and sealed up. It was reacted for 2 hours at room temperature in the incubation chamber with 200 rpm. Finally 5 g sodium borohydride was put into the material at 4 °C for 4 times and reacted for 6-8 hours under room temperature. After that it

was filtered in the sand funnel, washed by water, 0.2 mol/L pH 2.3 glycine-hydrochloric acid buffer, 0.1 mol/L pH 7.4 phosphate containing 1 mol/L sodium chloride and water successively, and HBsAb immunoabsorption gel was got after drying.

Column filling

Fifty ml immunoabsorption gel was put into a 50 ml polypropylene column in an asepsis chamber and sealed. The above reaction procedure was shown in Figure 1.

Extracorporeal immunoabsorption experiment

The absorption column and the blood transfusion set was connected in P2 laboratory, then the absorption column was put into the special DHP060 constant temperature incubation chamber which was preset at 37 °C, set the peristaltic pump between the absorption column and the plasma absorbed, its flow rate was adjusted to 180 ml/h. Fifty ml equilibrium solution and eluent was put in flow through the absorption column at a rate of 180 ml/h and finally let 500 ml plasma absorbed flow through the absorption column at the same rate. To detect the change of HBsAg and HBV in plasma with different amounts of HBsAb-IgG bound after absorption. The results are as following.

RESULTS

Binding rate of sepharose CL-4B gel and HBsAb-IgG

Using 150 ml sepharose CL-4B gel, the bound HBsAb-IgG can be determined by examining the content of HBsAb-IgG in the reaction solution before and after the binding, the result was shown in Table 1, following the continuous increasing of the amount

Figure 1. Anti-HBs immunoabsorption gel binding composition.

Table 1. The binding rates of HBsAb-IgG and sepharose CL-4B gel

Sepharose CL-4B gel (ml)	before binding (mg/ml)	after binding(mg/ml) HBsAb-IgG	binding rate (%)
150	150	47.25	68.50
150	200	52.60	73.70
150	600	89.58	85.07
150	800	157.76	80.28

Table 2. The absorption rates of HBsAg and HBV by different binding amount of HBsAb-IgG

HBsAb-IgG (mg/ml)	HBsAg absorption rate (%)	HBV absorption rate (%)
150	23.1	12.6
200	31.6	27.5
600	58.97	53.1
800	43.8	35.1

of HBsAb-IgG, its binding rate was also increasing, however, limited by the amount of the sepharose CL-4B gel used and the volume of the shell of the absorption column, 600 mg/ml HBsAb-IgG had the highest binding rate, which was 85.07%, which could satisfy the needs for the experiment.

Binding amount and absorption rate of HBsAb-IgG

In order to observe the absorption effects on HBsAg and HBV after the binding of different amounts of HBsAb-IgG, 500 ml plasma of HBV DNA positive patient was absorbed using the absorption column under the different dosages of HBsAb-IgG, and quantitative detection of HBsAg and HBV was made. The results were shown in Table 2, following the continuous increasing of the amount of HBsAb-IgG, its binding rate was also increasing, when HBsAb-IgG was 600 mg/ml, the absorption rate of HBsAg and HBV reached the highest point which were 58.97% and 53.1%, respectively.

DISCUSSION

Immunoabsorption is a method to purify the blood using the immunoabsorption reagent, various pathogenic factors in the body were eliminated through the special antigen-antibody reaction so as to cure the disease.^{1,2} It is a new technology for highly selective immunity treatment, its procedure is to fill the immunoabsorption reagent specific antibody (or antigen) into a column-shaped device which is also referred to as a immunoabsorption column.³ Then it was connected with a extracorporeal circulation device to enable plasma passing through the device within a regulated time and get in sufficient contact with the antibody (or antigen) in the absorption column, and finally the harmful antigen (or antibody)

inside the plasma was eliminated thoroughly.⁴ Recently, the treatment effects of the absorption column on lupus nephritis, organ transplantation, nephrotic syndrome, multi-arthritis, glomerulonephritis, haemophilia and Guillain-Barre syndrome etc., have been proved effective, and predicted clinic treatment effects have also been proved.⁵

In the research field of biomedical materials, epichlorohydrin activation is a normal method, the activated material being fixed has good stability and high fixing rate.⁶ In the serum of the chronic hepatitis B patients or HBV carrier, the number of Dane particles can be as much as 10^{13} /ml (about 100 μ g/ml), where the complete (mature) Dane particles are about 10^9 copies/ml. HBV envelope was mainly composed of lipoprotein-HBsAg, it was composed of polypeptide, which is not infectious. The envelope of each Dane particle contained 300-400 major protein molecules which is also called envelope protein. Its main functions were: 1. Immunogenicity of protective immunity was provided. 2. Virus was absorbed and then invaded into the cells. 3. Cover virus, promote virus maturation and secretion. 4. Block the host immune response to make a persistent infection. The best concentration of HBsAb-IgG bound in the experiment is 600 mg/ml, while the binding rate reach the highest point of 85.07%. Each HBsAg can be combined with multi HBsAb-IgG,^{8,9} as shown by the extracorporeal experimental results, the HBV immunoabsorption column can absorb and eliminate HBsAg and HBV particles in HBV DNA positive plasma, the absorption rates are 58.97% and 53.1%, respectively, indicating the application value of the absorption column in eliminating HBV and HBsAg inside blood. HBV and HBsAg in blood can be reduced or even eliminated through immune absorption and the repeat infection of HBV in blood and liver can be eased or even blocked, then immune

damage inside body caused by relevant immunity compound and the pressure of HBV can be decreased. Immune absorption is of extremely significance to early HBV acute infection, liver transplantation and chronic HBV. The research of this thesis provides an experimental basis for the immunoabsorption treatment on HBV infectious patients.

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