Title: IMMUNO ABSORPTION COLUMNS FOR THE SUBTRACTION OF ANTIBODIES FROM BLOOD WITH SELECTIVE PLASMAFILTRATION TECHNIQUES

Abstract: The text describes immuno absorption columns containing conjugates constituted by specifically designed modified peptides and a related support that allow the subtraction of the antibodies responsible for multiple sclerosis with selective apheresis (plasma exchange) techniques.
IMMUNO ABSORPTION COLUMNS FOR THE SUBTRACTION OF ANTIBODIES FROM BLOOD WITH SELECTIVE PLASMAFILTRATION TECHNIQUES

Field of the invention
This invention refers to immuno absorption columns containing conjugates constituted by specifically designed modified peptides and a related support that allow the subtraction of the antibodies responsible for multiple sclerosis with selective apheresis (plasma exchange) techniques.

State of the art
As it is known, in a lot of auto-immune diseases the organism produces autoantibodies that play a fundamental role in the disease’s development and worsening. A typical case is that of Multiple Sclerosis, where the myelin damage, typical of the disease, is very probably due to a synergetic action of cellular response T and of antibody response against proteins and glyco-proteins of myelin sheathes.

The procedure, in these cases, consists in the elimination of antibodies from the patient’s blood; an intervention that unfortunately has to be repeated, because the antibodies tend to form again over time.
One of the techniques used to eliminate antibodies is apheresis (plasma exchange), which consists in the complete subtraction of the patient’s blood volume and its substitution with donors’ blood, with all the problems that such an intervention brings about.
An evolution of this technique is plasmafiltration, with which all the patient’s immuno globulines are subtracted and the patient’s own blood is reinfused.
The advantages of the second procedure, compared with the first, are evident, but this procedure too implies severe disadvantages for the patient because he is uselessly deprived of the immuno globulines' defences.
It is therefore obvious how important it is to develop a selective plasmafiltration by means of equipment and methods that will eliminate only undesired antibodies, allowing a greater compliance for the patient and eliminating risks and inconveniences due to the application of the procedures described above.
In International Application No. PCT/EP02/06767 filed on 19 June 2002 in the name of the Applicant, we have described glyco-peptides formed by 11 – 24
aminoacides and their conjugates with several supports, capable of identify and capture antibodies in Multiple Sclerosis. Moreover we have described their use in the diagnostic field without, however, suggesting their possible use in selective plasmafiltration treatments.

**Brief description of the drawings**

Figure 1 schematically represents a column filled with conjugates, the enlarged details schematically illustrates the free conjugates (Figure 1 (a)) and those tied to the captured antibody (Figure 1(b)).

Figure 2 (a) and (b) schematically represent the way the column works according to the invention.

Figure 3 schematically indicates plasmafiltration apparatus.

**Detailed description of the invention**

It has recently been found, unexpectedly, that the conjugates notified and described in this application allow the construction of immuno absorption columns that enable selective plasmafiltration treatments in patients with Multiple Sclerosis, eliminating only the autoantibodies corresponsible for myelin damage with a consequent worsening evolution of nervous system diseases.

The use of such columns thus makes it possible to leave unaltered all the other parts of the serum, the elimination of which has only negative effects for the patient undergoing treatment.

As we have described at length in this application and as we summarise here, the glyco-peptides that constitute the conjugates in question have the general formula (I)

\[ X\text{-Asn(G)\text{-Y\text{-Z}}} \quad (I) \]

in which:
- \( X \) = aminoacid carrying an amino or carboxylic group on the side chain;
- \( Y \) = Pro, Gly;
- \( G \) is a sugar
- \( Z \) = Ala, Val, Ile, His

Preferred, according to the present invention, are glyco-peptides of formula (II):

\[ A\text{-B\text{-X\text{-Asn(G)\text{-Y\text{-Z\text{-C\text{-D}}}}} \quad (II) \]

in which:
Y and G are as defined above;
A = 2 – 5 aminoacids or absent
B and C = trifunctional aminoacids forming a lactam bridge between each other by
means of the respective side chains, or absent;
D = 5 – 15 aminoacids;
X = Glu, Asp, Lys, Arg, Orn, Dap;
Z = Ala, Val, Ile, His.

For trifunctional aminoacids forming a lactam bridge between each other as
defined above, is meant, for example, the pair Dap-Asp or Asp-Dap, Dab-Glu or
Glu-Dab, Orn-Asp or Asp-Orn, and the pair formed by other aminoacids, for
example non-natural aminoacids, having analogous characteristics.

For sugar is preferably meant: mono and disaccharides of type Glc, GlcNAc, β-D-
GlcP-(1→4)-D-Glc (cellobiose), etc.

For aminoacids, where not differently defined, natural or non-natural aminoacids
are meant.

Obviously residues A, if present, and D may contain an appropriate alkyl spacer to
lengthen the chain, where for alkyl spacer, in the sense used herein, is meant ω-
aminoacids with linear alkyl chains (H₂N-(CH₂)n-CO₂H where n is 2 – 6.

Specific examples of glyco-peptides of formula (I) listed in this application are:

Particularly preferred, according to the invention are the peptides represented by
the following sequences:

1. H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-Val-Phe-Leu-Ala-Pro-Tyr-
Gly-Trp-Met-Val-Lys-OH
2. H-Thr-Pro-Arg-Val-cyclic[Dap-Arg-Asn(Glc)-Gly-His-Asp]-Val-Phe-Leu-Ala-
Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
3. H-Thr-Pro-Arg-Val-cyclic[Asp-Arg-Asn(Glc)-Gly-His-Orn]-Val-Phe-Leu-Ala-
Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
4. H-Ala-Lys-Thr-Ala-Lys-Asn(Glc)-Gly-His-Val-Glu-Ala-Ser-Gly-OH
5. H-Glu-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-
Pro-OH
6. H-Asp-Asn(Glc)-Pro-Val-Glu-Ala-Phe-Lys-Gly-Ile-Ser-OH
7. H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-HN-(CH₂)₆-CO-Val-Phe-
Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH

8. H-Asp-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-(βAla)₃-OH

Conjugates employed for the refilling of columns in this invention, also described in this application are, as we have said, constituted by glyco-peptides as described above, and by a suitable support for their use.

Supports preferred for this purpose include resins insoluble in water and completely compatible with organic fluids, such as: silica gel, cellulose, polyacrylate, sepharose and analogues, as well as the same resins normally used by experts in the field for the preparation of synthetic peptides, as for example Wang's resin, polystyrene-polyoxyethylene (TentaGel resin or PEG-PS) or polyethylene glycol and polyacrylamide copolymers (completely compatible with water) such as PEGA resin and more stable analogue resins such as POEPS (polyoxyethylene-polystyrene), POEPOP (polyoxyethylene-polyoxypropylene), as well as macroporous resins described for their interest for the solid phase glycosylation of peptides, such as SPOCC (PEG substituted with oxethane) [Rademann, J; Grøtli, M; Meldal, M; and Bock, K. J. Am. Chem. Soc. 1999, 121, 5459-5466] or derivatives thereof like EXPO₃₀₀₀ (copolymer with tetrakis-[4-(3-methyl-oxethane-3-ylmethyl)-phenyl]-silane) [Tornøe, C.W.; and Meldal M. In: Peptides 2000, J. Martinez and J.A. Fehrentz (Eds.) EDK, Paris, France 2001].

The immuno absorption columns in this invention are of the kind commonly used for plasmafiltration techniques; thus are normally made of plastic materials.

The section of the immuno absorption columns, as well as their size, are adapted in accordance with the space available in the equipment for the external automatic circulation through which the patient's blood must pass.

In the calculation of size one must consider the efficiency of the selective autoantibodies extraction process in relation to the blood volume, to the concentration of suspended antibodies to be extracted and to the compliance of the treated patient, which can be translated into the duration of the procedure. One normally prefers immuno absorption columns of circular section, a height of 150 mm ± 20% and a diameter of 50 - 75 mm.

Columns are filled, according to the normal techniques and procedures used for this purpose in the art, with conjugates composed of resins, insoluble in water and
completely compatible with organic fluids, and of glyco-peptides of general formula (I) as defined above and as better described in the above-mentioned Application. As one can see in figure 2 (a) the conjugates loaded in the column (see Figure 1) at the passage of the patient's blood capture the antibodies present in it until total saturation. Afterwards (figure 2 (b)), washing the column with the suitable buffers, at the pH suitable to break the bond antigen/autoantibody so as to cause the detachment and the elimination of antibodies and the column can be used for a new blood treatment.

Columns are then mounted on specific equipment having the necessary hydraulic circuits to allow the passage of the blood of the patient undergoing therapeutic treatment through the column (filtration phase) and the subsequent washing of the columns to eliminate captured antibodies (regeneration phase) as described above.

As it can be seen in Figure 3 the plasmafiltration apparatus includes:

a column (10) according to the invention,
pipes (11) connecting the patient to the peristaltic pump (12) and to the column (10) (and vice-versa) and through which run the blood that has to be treated and the blood that has been treated;
pipes (13) through which runs the liquid for column washing once the antibodies' absorption on the conjugate has been completed.

The most advantageous direction of the flow during the filtration phase is the one represented in figure 3 (up to down), whereas there is no preferred direction for the washing, even if, for simple technical construction reasons, normally the washing flow too is made to run downwards.

The equipment preferred by the invention is that which has two columns (and thus two peristaltic pumps) that work in alternative phases. While plasmafiltration goes on in one, the washing takes place in the other and vice-versa, in order to guarantee the operational continuity necessary to finish the intervention on the patient. The passage to one column to the other can be easily realised by placing in the circuit suitable three-ways cocks that isolate the column in filtration phase from that in regeneration phase.
CLAIMS
1. Immuno absorption columns containing conjugates constituted by glyco-peptides capable of identifying antibodies in Multiple Sclerosis and related supports.

2. Columns according to claim 1, in which the supports mentioned are resins insoluble in water and completely compatible with organic fluids.

3. Columns according to claims 1 and 2 in which the glyco-peptides that constitute the conjugates have general formula:
   \[ X-\text{Asn(G)}-Y-Z \quad (I) \]
   in which:
   \( X = \) aminoacid carrying an amino or carboxylic group on the side chain;
   \( Y = \) Pro, Gly;
   \( G \) is a sugar
   \( Z = \) Ala, Val, Ile, His

4. Columns according to claim 3 in which glyco-peptides with general formula (I) are chosen in the group constituted by:
   \begin{align*}
   & \text{H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH} \\
   & \text{H-Thr-Pro-Arg-Val-cyclic[Dap-Arg-Asn(Glc)-Gly-His-Asp]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH} \\
   & \text{H-Thr-Pro-Arg-Val-cyclic[Asp-Arg-Asn(Glc)-Gly-His-Orn]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH} \\
   & \text{H-Ala-Lys-Thr-Ala-Lys-Asn(Glc)-Gly-His-Val-Glu-Ala-Ser-Gly-OH} \\
   & \text{H-Glu-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro-OH} \\
   & \text{H-Asp-Asn(Glc)-Pro-Val-Glu-Ala-Phe-Lys-Gly-Ile-Ser-OH} \\
   & \text{H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-HN-(CH}_2)_5-\text{CO-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH} \\
   & \text{H-Asp-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-(\beta\text{Ala})_3-OH} \\
   \end{align*}

5. Columns according to claims 1 - 4 in which supports are chosen in the group constituted by: silica gel, cellulose, polyacrylate, sepharose, polystyrene (Wang's resin), polystyrene-polyoxyethylene (TentaGel resin or PEG-PS), polyethylene glycol and polyacrylamide copolymers (PEGA resin), POEPS (polyoxyethylene-
polystyrene), POEPOP (polyoxyethylene-polyoxypropylene), SPOCC (PEG substituted with oxethane) or derivatives thereof.

6. Equipment for selective antibody plasmapheresis in Multiple Sclerosis including:
   a column (10) according to claim 1;
   pipes (11) connecting the patient to the peristaltic pump (12) and to the column (10) (and vice-versa) and through which run the blood that has to be treated and the blood that has been treated;
   pipes (13) through which runs the liquid for column washing once the antibodies’

7. Equipment according to claim 6, including two columns in accordance with claim 1, connected so as to work in parallel (one in immuno filtration phase and the other in washing phase).

8. Method for selective antibody plasmapheresis in Multiple Sclerosis in which the patient’s blood is made to pass through a column in accordance with claim 1.

9. Method according to claim 8, in which contemporarily two columns in accordance with claim 1, working in parallel one in immuno filtration phase and the other in regeneration phase, are used.
Fig. 2(a)
Fig. 2(b)
SEQUENCE LISTING

SEQ ID. N. 1 - H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
SEQ ID. N. 2 - H-Thr-Pro-Arg-Val-cyclic[Dap-Arg-Asn(Glc)-Gly-His-Asp]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
SEQ ID. N. 3 - H-Thr-Pro-Arg-Val-cyclic[Asp-Arg-Asn(Glc)-Gly-His-Orn]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
SEQ ID. N. 4 - H-Ala-Lys-Thr-Ala-Lys-Asn(Glc)-Gly-His-Val-Glu-Ala-Ser-Gly-OH
SEQ ID. N. 5 - H-Glu-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro-OH
SEQ ID. N. 6 - H-Asp-Asn(Glc)-Pro-Val-Glu-Ala-Phe-Lys-Gly-Ile-Ser-OH
SEQ ID. N. 7 - H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-HN-(CH₂)₆-CO-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
SEQ ID. N. 8 - H-Asp-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-(β-Ala)₃-OH