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(54) Title: METHOD AND APPARATUS FOR TREATING BIOLOGICAL FLUIDS

(57) Abstract: A method for removing an insulin binding protein (IBP) from a biological fluid which comprises contacting the biological fluid with an affinity agent capable of forming a complex with said IBP and separating complexed IBP from said biological fluid.

METHOD AND APPARATUS FOR TREATING BIOLOGICAL FLUIDS

This invention relates to methods and apparatus for treating biological fluids. The invention particularly relates to the use of solid phase affinity media in such methods and apparatus and to the use thereof in the extracorporeal treatment of blood or plasma in the treatment of patients suffering from disease states including type II diabetes mellitus (non-insulin dependent diabetes mellitus).

BACKGROUND OF THE INVENTION

One of most prevalent chronic disorder worldwide (and one which results in high financial costs for society) is Type II Diabetes also called non-insulin dependant diabetes mellitus, which occurs predominantly in adults. The disease is in general characterized by hyperglycemia resulting from a disturbed maintenance of glucose homeostasis as a consequence of the imbalance between the function of B-cells in the pancreas and the insulin sensitivity of glucose-producing and glucose-utilizing tissues (Unger et al). Non-insulin dependant diabetes mellitus is also characterized by a defect of both insulin secretion and insulin function (Defronzi RA et al). Failure to treat Type II diabetes patients result in general in an increased mortality rate by cardiovascular complications and other diabetes-related diseases such as maculopathy, retinopathy, nephropathy, and neuropathy. Type II diabetes mellitus is associated with insulin resistance at target tissues as well as reduced secretion of the hormone from pancreatic β cells. Both of these factors contribute to the high blood sugar levels commonly observed in untreated diabetics; and it is this hyperglycaemia which is a major cause of the pathology related to all forms of diabetes, not just Type II, examples of which are outlined above. As a compensation for the insulin resistance, natural insulin secretion is often enhanced in Type II diabetes. Insulin resistance is also often developed by non-diabetic patients such as individuals with polycystic ovary symptoms, a common endocrine disorder in women of reproductive age. There is also, in some Type II diabetes patients, a genetic mutation of in the gene(s) coding for insulin and/or for insulin receptors

and/or for insulin-mediated signal transduction factors, thereby resulting in an impairment of insulin action and glucose metabolism.

For many years treatment of Type II diabetes has involved a strategy aimed at the lowering blood glucose based on diet. Alternatively, treatments involving administration or injections of drugs in combination with insulin have been proposed. In view of this, several drug therapies involving different biological mechanisms, but having the objective to treat insulin-resistance patients, have been found in the prior art. The most important proposed treatments rely on use of sulphonylureas (to increase insulin secretion from pancreatic β cells), biguanides (anti-hyperglycaemic agents), α-glucosidase inhibitors (to inhibit intestinal α-glucosidase), thiazolidinediones (to increase glucose transporters on target tissues), prolactin stimulators, growth hormones, herperuetic agents, cholinergic agonists, and benzimidazole derivatives.

In US Patent No. 5 750 519, Cincotta and Andover claim a process for the long term regulation of lipid and glucose metabolism to insulin resistance, and hyperglycemia by administration of a dopamine agonist which is a prolactin stimulator.

In US Patent No. 5 597 797, a method is disclosed which involves the administering of effective amount of growth hormone in combination with an effective amount of IGF-I. In US Patent No. 5 801 117, Beyer and Karl disclosed a method of treating non-insulin-dependent diabetes mellitus by the administering of herperuetic agent. In US Patent No. 5 886 014, Takashi describes the use of benzimidazole derivatives which have valuable activity for the treatment and/or prophylaxis of a variety of disorders, including hyperlipemia, hyperglycemia, obesity, impaired glucose tolerance (IGT), insulin resistance and diabetic complications. In Patent No. US 5 561165, Lautt et al disclosed the administration of an effective amount of a cholinergic agonist for increasing insulin responsiveness and improving glucose tolerance.

Drug therapy remains the most common treatment of Type II diabetes. However it is merely a palliative treatment and does not represent a cure with many diabetics being drug dependent for the rest of their lives. It is additionally associated with a number of side effects such as weight gain, headaches and diarrhea. Although drug therapy remains still the most common in the treatment of diabetes Type II, the danger in pharmacological therapy is still the limited effectiveness in the long term and the unavoidable occurrence of side effects.

In our investigations of the mechanism involved in insulin resistance, we have discovered that the so-called "insulin binding proteins" play a crucial role in non-insulin dependent diabetes and that by removing insulin binding proteins from the blood of patients, a new treatment protocol is provided for the management of the Type II diabetic disease.

Examples of insulin binding proteins that have been reported hitherto include the insulin-like growth-factor binding proteins (IGF-receptors 1 and 3), antibodies directed against insulin (immunoglobulin) and the soluble form of the insulin receptor. A variety of factors have suggested to us that insulin-like growth factor 1 and 3 have a binding capacity for insulin and thus reducing the insulin responsiveness. For example, insulin-like growth factors (IGFs) have diverse anabolic cellular functions, and structure similar to that of proinsulin. As the possible role of the soluble form of insulin receptor, we noted that it had been found that the expression of a soluble high-affinity insulin-binding protein in the plasma of transgenic mice led to alterations in glucose homeostasis that resemble those encountered in the early stages of Type II diabetes (Schaeffer et al). We also noted that the genetic Type II diabetes-like animals presenting hyperglycemia and insulin resistance have been obtained via the expression of various transgenes under the control of insulin promoter elements, thereby targeting heterologous proteins to the β-cells of the pancreas (Epstein et al). The observed hyperglycemia may derive from the loss of β-cell function, either with or without the involvement of the immune system.

Knowledge about the structure of the insulin receptor has accumulated during the 1980s,and substantial progress was made in determining the function, structure and location of insulin receptors. Insulin receptors are present in the major target tissues for insulin such as the liver, muscle and fat. In addition, insulin receptors are also in non-insulin dependent tissues such as blood cells and cells located in the central nervous system.

The human insulin receptor (hIR) is a tetrameric glycoprotein located on the surface of target cells and consists of two identical extracellular α-subunits that contain the binding site for insulin and two identical transmembrane β-subunits with tyrosine kinase activity in their cytoplasmatic domain (Ulrich et al). The subunits are linked together by disulfide and other bonds. Insulin binding to the receptor α-subunits is an essential prerequisite for subsequent hormonal action and results in activation of tyrosine kinase activity on the β-subunit (Marshall S).

The insulin receptor is a member of the ligand-activated receptor and tyrosine kinase family of transmembrane signalling proteins that collectively are fundamentally important regulators of cell differentiation, growth, and metabolism. However, it has two main differences to the other members of this family, firstly, the majority of receptor tyrosine kinases dimerise on binding their agonist, the insulin receptor however, is permanently dimerised with this dimer being maintained by disulfide bonds. Secondly, when it is activated and tyrosine residues within it are phosphorylated, it does not directly bind to signalling proteins with SH2 domains in them, but works through an adaptor protein: the Insulin Receptor Substrate (IRS-1).

Once insulin binds to the α subunits of the receptor it causes a conformational change in the β subunits, which activates the tyrosine kinase activity of the β subunits, which in turn catalyze the transfer of phosphate groups to tyrosine residues located in the cytoplasmic domain of the receptor as well as to a variety of insulin receptor substances (IRSs) which activate a variety of "downstream" effectors.

In summary, the tyrosine kinase family of transmembrane signalling proteins, together with ligand-activated receptors (another group to which the insulin receptor belongs) are collectively fundamentally important regulators of cell differentiation growth and metabolism. The main physiological role of the insulin receptor, individually, appears to be metabolic regulation i.e. controlling blood sugar level.

A number of discoveries have led to the suggestion that the soluble form of insulin receptor is derived from the cell related receptors. Gavin et at first observed the secretion of insulin receptors from cell lines IM-9 lymphoblasts, when incubated with a buffer solution containing insulin. More recently, Papa et al found that the secretion of intact and functional insulin receptors is a common feature of several human cells in culture. Pezzino et at have described the presence of immunoreactive insulin receptors in human plasma. The observed circulating insulin receptor consisted of an intact and functional α-subunit with an altered, low molecular weight β-subunit but existing biological activities such as phosphotransferase and autophosphorylation. Therefore, the soluble form of insulin receptors found in the plasma bind insulin with high affinity.

SUMMARY OF INVENTION

In accordance with the present invention, we have found that certain non-insulin dependent diabetes can be treated by removing key endogenous toxins in the blood circulation, especially extracorporeal therapy involving removal of the soluble form of the circulating insulin receptor and/or any soluble proteins which bind to insulin. According to our knowledge, the use of adsorbent devices (or any extracorporeal therapies involving removal of soluble insulin binding proteins) has not been hitherto proposed for treating Type II diabetes.

According to one aspect of the invention, there is provided a method for removing an insulin binding protein (IBP) from a biological fluid which comprises contacting the biological fluid with an affinity agent capable of forming a complex with said IBP and separating complexed IBP from said biological fluid.

The affinity agent preferably comprises a solid support, and a substance capable of forming a complex with said IBP bound to the support, collectively known as the solid affinity medium. The manner of binding of the affinity agent to the support is not critical to the invention, but preferably the affinity agent is covalently bound to the solid support.

It is preferred that the affinity agent has specific binding characteristics for the IBP. To achieve this the affinity agent may include an amino acid sequence that forms a binding site capable of binding specifically to an insulin-binding domain of said IBP, for example the α-subunit of a soluble insulin receptor. Such an amino acid sequence may comprise at least 3, preferably at least 5 and most preferably at least 6 contiguous amino acids of a mammalian insulin, preferably human insulin.

The soluble IBP referred to herein may be selected from a soluble insulin receptor, an anti-insulin immunoglobulin and an insulin-like growth factor, wherein the anti-insulin immunoglobulin is an IgG or IgM and the insulin-like growth factor is insulin-like growth factor 1 or 3.

The biological fluid from which an insulin binding protein (IBP) is removed, may be whole blood or plasma, preferably derived from a patient suffering from non-insulin dependent diabetes.

The solid support of the solid affinity medium preferably comprises a ceramic, cellulose, a glass, polymethylmethacrylate or Sepharose (e.g. in the form of beads, a hollow fibre, a hollow membrane or a flat membrane). Typical beads would have a diameter of between 50 and 800 μm, and typical hollow fibre membranes would have an internal diameter of between 80 and 200 μm.

The above described method for removing insulin binding protein and the associated solid affinity medium may, according to a further aspect of the invention, be used in treating non-insulin dependent diabetes mellitus.

Thus, according to a second aspect of the invention there is provided a procedure for treating a patient suffering from non-insulin dependent diabetes which comprises withdrawing blood from the patient, treating the withdrawn blood by a method as described above and returning the blood to the patient after removal of IBP.

According to a third aspect of the invention there is provided a device for carrying out the extracorporeal treatment of blood to remove insulin binding protein therefrom, preferably comprising an affinity medium, a means for contacting blood or separated plasma with said affinity medium and inlet and outlet conduits adapted respectively to receive blood or separated plasma from a patient and to return blood or separated plasma to the patient, after treatment to remove insulin binding protein therefrom. Such a device is preferably a dialysis machine.

The present invention more specifically relates to a novel adsorbent device for the blood detoxification in Type II diabetes patients, which provides a mayor advance in management of Type II diabetes disease. The adsorbent device may be applied to prevent insulin resistance and hyperglycemia in patients by the way of extracorporeal removal of soluble proteins which bind to insulin in the circulating blood. The adsorbent device may be applied in an extracorporeal circuit and has, suitably, a high selectivity with respect to the removal of soluble human insulin binding proteins. Thereby, the soluble insulin binding proteins (including, e.g. the soluble form of insulin receptor and/or the soluble insulin-like growth factor binding protein 1 and or the soluble insulin-like growth factor binding protein 3 and/or anti-insulin immunoglobulin which may be involved in provoking the insulin resistance in diabetes Type II) can be removed from a patient's blood.

Another aspect of the novel adsorbent device involves its application in an extracorporeal manner for a sufficient elimination of the circulating soluble human proteins having a binding affinity for insulin, in order to increase the insulin responsiveness and decrease the hyperglycemia. Thus, resulting in an improvement

in the care of Type II diabetes without affecting the patients with secondary adverse reactions.

Another aspect of the novel adsorbent device involves its application in an extracorporeal manner for a sufficient elimination of the circulating soluble complex of insulin and insulin binding proteins, in order to increase the insulin responsiveness and decrease the hyperglycemia. This can result in an improvement in the care of Type II diabetes without affecting the patients with secondary adverse reactions.

Still another aspect of the invention is the provision of an adsorbent device for the elimination of human proteins which bind to insulin in a soluble form and further block its binding to insulin receptor, in order to reduce the need for insulin therapy, control the complications associated with Type II diabetes and thus also reduce the medical costs of treating these complications.

In more detail, the present invention provides an adsorbent device with the novelty of eliminating by the way of specific adsorption a specific circulating insulin binding protein which is the soluble form of human insulin receptor. Thereby, the circulating soluble form of insulin is the protein found in diabetic Type II patients, which is characterized by its structure, which is tetrameric glycoprotein consisting of two identical α-subunits that contain the binding side for insulin and two identical β-subunits with tyrosine kinase activity in their cytoplasmatic domain . The soluble form of the insulin receptor to be eliminated may also consist of one or two α-subunits but lacking its β-subunits.

Still another preferred embodiment is providing an adsorbent device with the novelty of eliminating, by way of specific adsorption, the circulating human immunoglobulin anti-insulin which have an insulin epitope. Thereby, the circulating immunoglobulin has one or many epitopes directed against the human natural or recombinant or animal insulin , which may be an IgG or an 1gM.

Still another preferred embodiment is providing an adsorbent device with the novelty of eliminating, by way of specific adsorption, a specific circulating insulin-like growth-factor binding proteins 1 and 3 which are soluble proteins with an affinity for insulin.

Still another embodiment relates to a process for making an adsorbing device as defined by its affinity agent which has the function of binding the soluble form of insulin receptors circulating in whole blood. More specifically, the binding property of the ligand is directed again the α-subunits of the soluble form of the circulating insulin receptor. Furthermore, the binding property of the ligand is directed against the soluble form of the circulating insulin-like-growth-factor binding proteins 1 and 3. Furthermore, the binding property of the ligand is directed against the anti-insulin epitope of circulating immunoglobulin. The affinity agent of the adsorbent device may be a human recombinant insulin or insulin analogue which have a binding affinity for the α-subunits of the soluble form of the insulin receptor. The affinity agent may be immobilized on a matrix by covalent bonds at its C-terminal. A spacer may be used and may consist of a peptide which bind the ligand at the C-terminal. The matrix for the support may be synthetic material, ceramic, glass, or Sepharose. The device should be made in the way that the binding affinity between the insulin ligand and the insulin receptor is conserved even if the insulin ligand is bound to a matrix, which may have the form of beads, hollow fibres membranes or flat membranes.

Still another embodiment relates to the use of a preferred ligand which is human recombinant insulin produced by E.coli or yeast, or a human insulin-analogue both of which have the ability to bind the α-subunits of the human soluble insulin receptor. The affinity agent is preferably bound to the matrix via its C-terminal by a chemical cross linking procedure to achieve a functional directed immobilisation of the ligand. The affinity agent when oriented in such a way that its C-terminal is immobilized, is capable of binding to the soluble form of the insulin via its α subunit

Still another preferred embodiment is the use of natural animal insulin from pigs or bovines. Such a ligand may also be immobilized at the C-terminal on the matrix. According to the invention, many commercially available forms of natural animal insulin may be used, if they satisfy the preferred criteria of binding to the soluble form of the human insulin receptors via its α-subunit. The affinity agent is preferably bound to the matrix via the C-terminal by a chemical cross linking procedure to achieve a functional directed immobilisation of the ligand.

In accordance with a preferred embodiment in which the method of the invention is applied to whole blood, important characteristics of the adsorbent device are the nature and the dimensions of the support beads material. Preferably, the diameter of the beads matrix should be between 250 and 350 µm (e.g. Type 6MB), although 100-300 µm Sepharose supports may also be used (e.g. Type "Streamline Quartz Base"). This requirement is based on the fact that the largest blood cell present in the blood has a diameter of 20 µm, so that the sieve which holds back the adsorbent desirably has a mesh of 80 pm. According to the invention, many types of commercially available matrix material may be used, if they satisfy the biocompatibility and affinity criteria. Preferably commercially available matrix beads made with synthetic material such as polymethylmethacrylate (e.g. 6µm beads), or non synthetic materials such as Sepharose, comprising spherical and entirely porous particles may be applied. According to the invention which involves a covalently binding the affinity agent to the matrix, the matrix materials should be suitable for the chemical cross linking of the affinity agent via the C-terminal amino acid.

Still another preferred embodiment is the application of the adsorbent device to plasma which has been previously fractionated by filtration of centrifugation. In this case, the diameter of the beads matrix may be between 50 and 100 µm and the sieve which holds back the adsorbent may have a mesh of 10 µm. Preferentially commercially available matrix beads made with synthetic material such as polymethymethacrylate or non synthetic materials such as Sepharose comprising spherical and entirely porous particles may be applied.

In accordance with a preferred embodiment, one specific aspect of the invention is the extracorporeal application of the adsorbent device, providing an effective and selective removal of soluble insulin receptors from the patient's blood. Conventionally, in performing such a treatment, a therapeutical device, is mounted in an extracorporeal circuit in which whole blood is circulated. The blood is taken from the patient's vein and treated by using the claimed adsorbing device to adsorb the soluble insulin receptors from the blood, before returning it to the patient. In accordance with the present invention which is restricted to the removal of the soluble form of insulin receptors in whole blood, it is suitable to use a biocompatible porous material for the matrix, in a form of beads.

Still another specific aspect of the invention is the extracorporeal application of the adsorbent device providing an effective and selective removal of soluble insulin receptors from the patient plasma. Conventionally, in performing such a treatment, a therapeutical device, is mounted in an extracorporeal circuit in which whole blood is circulated. The blood is taken from the patient's vein and treated by using the claimed adsorbing device to adsorb the soluble insulin receptors from the blood, before returning it to the patient. In accordance with the present invention which is restricted to the removal of the soluble form of insulin receptors in whole blood, it is suitable to use a biocompatible porous material for the matrix, in a form of beads.

The invention further provides a method of performing extracorporeal blood purification by removing the soluble form of insulin receptors from blood which comprises perfusing an adsorbent device with human blood as defined herein.

Description of Figures

The invention will now be described in more detail with particular reference to the accompanying drawings, of which:

Figure 1 illustrates a diagram of the extracorporeal system used in Example 3

Figure 2 illustrated results the competition-Inhibition curve of substances extracted from plasma in the presence of increasing labeled insulin (100 pM) in the presence of increasing unlabeled insulin (0-10 nM). Bound and free complexes were separated by PEG precipitation in the presence of carrier human alpha-globulin (1 mg/ml). The results are expressed through the ratio bound insulin versus unbound plus bound.

Figure 3 illustrates results of the ELISA measurements of the insulin like receptors found in diabetes type II patients versus healthy subjects. The patients were grouped as follows: group I representing 25 patients having an insulin resistance for less then 2 years, group II representing 25 patients having an insulin resistance for more then 2 years and less then 5 years; group III representing 25 patients having an insulin resistance for more then 5 years and less then 10 years; group IV representing 20 patients having an insulin resistance for more then 10 years

Figure 4 illustrates the levels of insulin like receptors before after the procedure from Example 5.

The following Examples illustrate the invention

Example 1

This Example illustrates the preparation of an affinity agent according to the invention: an affinity agent based on a sterile and pyrogen free recombinant human insulin was prepared by the following steps:

(I) Step of activating the support matrix. The activation occurs which enables the formation of functional amino groups on their surface for covalent linkage.

(II) Step of immobilizing the affinity protein by the carbodiimide coupling procedure under aseptic conditions on a support matrix by chemical cross linking procedures at the C-terminal of the affinity protein. Performing the covalent binding of the affinity protein at the C-terminal by forming an amide bond with the support matrix. These procedures are performed as follows:

Washing the support matrix with up to 0.1 M NaOH at room temperature, removing contaminations by washing with non-ionic detergent such as 0.1% (weight/volume) triton X-100 at 370°C for 1 mm, thus reequilibrating with buffer.

Dissolve insulin in water (adjusted to pH= 4.5) containing ethylene glycol up to a final concentration of 30%.

Dissolving a concentration of 0.1 M of carbodiimide is in water adjusted to a pH of 4.5

Mixing the insulin solution and carbodiimide with the support matrix suspension by adjusting the pH to between 4.5 and 6.0 (by addition of dilute NaOH) and stirring in a mixer at 4°C for 24 hours

Example 2

An extracorporeal system for the removal of the circulating insulin binding proteins from the blood was assembled using available dialysis equipment. The method of performing blood treating system comprises the following steps of

I) pumping the patient blood from the vein;

II) circulating the blood through the adsorbent device which may be a column;

III) and pumping the blood back to the patient in the vein.

Example 3

This illustrates diagrammatically a preferred extracorporeal system for the removal of the circulating insulin binding proteins from the blood fractionated plasma. The method of performing blood treating system comprises the following steps of

I) pumping the patient blood from the vein;

II) separating a liquid cell free fraction and a liquid cell fraction by separating the cell free plasma from the blood cell by the way of membrane filtration or centrifugation;

III) circulating the plasma through the adsorbent device which may be a column;

IV) mixing the treated plasma with the blood cell fraction;

and pumping the blood back to the patient in the vein. Features and advantages of the present invention will be apparent from the experimental results.

Example 4

The purpose of this example is to identify and characterize the insulin like receptor in diabetes type II plasma which has to be removed according to this invention. Details of the methodology is given in the Protocol following the Examples. The purification step involved use of an adsorption column as described in Example 1. We isolated from the plasma of healthy subject and patients with diabetes type II a material that reacted in a specific enzyme linked immuno assay for insulin receptors. After desorption of the material from the column, the levels in the diabetes patients were five to 10 times higher as compared with the healthy subjects. The desorbed substance has the same biochemical characteristics as a human placental insulin receptor. After

desorbtion from the insulin affinity column, the substance also reversibly bound to insulin.

Example 5

The purpose of this Example was to evaluate the ability of the method described in Example 1, using a device having a volume of 250 ml of adsorbent material based on the Streamline Quarz Base as the matrix for eliminating insulin-like receptors from he whole blood *in vitro*. A total volume of 1 000 ml of heparinized fresh blood obtained from 20 patients (same rhesus factor) having diabetes type II for more then 5 years was pooled. The blood was introduced into an extracorporeal system. The blood flow was adjusted to 50 ml/min. After 3xtime blood perfusion of the blood volume, blood samples were drawn into heparinized tubes and centrifugated at 400 x g for 30 minutes in order to separate the plasma from the blood cells. The amount of immuno reactive insulin like receptors were measured by ELISA. A control experiment was performed in which a volume of 1000 ml blood was also perfused though a passive device (adsorbent device containing the same matrix, therefore, no insulin ligand was bound on its surface).

Experimental Protocol

Extraction of insulin like receptors from plasma

200 ml blood obtained from 100 patients with diabetes type II and 20 heathy subjects were drawn into heparinized tubes and centrifuged at 400 x g for 30 minutes in order to separate the plasma from the blood cells. The plasma was perfused at 1 ml/min 3 times through a 10 ml column filled with the material described in Example 1. The column was eluted at the end of the adsorption procedure with 200 ml of 0.9% Nacl/Cl at 5.4 pH. Then, the diluted adsorbed substances was dialyzed again a phosphate buffer at 7.4 pH and thus concentrated to a volume of 20 ml by microfiltration

Labeling of desorbed material

Desorbed material were labeled with ^{125}I-Bolton-Hunter reagent (2200 Ci/mmol purchased by Amersahm, Aylesbury, Buckingshire, UK). The reaction occurred in 300 µCi in 250 µl of 50 mM borate buffer with the amount of 200 ng of material (desorbed substances).

Insulin receptor enzyme linked immuno assay

The chemicals used were: 1) coating buffer carbonate/bicarbonate buffer pH 9.6 (4.24 g Na2CO3 and 5.04 g NaHCO3 were dissolved in 1 liter of deionized H_2O and mixed) ; 2) standard buffer PBS.Tween 20 at 0.5% (weight/volume); 3) Phosphate buffer pH 7.4 containing 0.5 % Tween 20 (dissolving 1.67 Na_2PO_4, 8.5% NaCl in 1 liter of deionized H_2O and adding 0.5% Tween 20; 4 washing buffer is a phosphate buffer containing 0.05% Tween 20 and 0.02% Thimerosal.

The assays were performed as follows:

(1) Immunoplate for type Maxisorb (Nunc Danemark) were coated with 100 µl containing 10 µg/ml placental insulin receptors. The placental insulin receptor has been purified from human placenta by affinity chromatography using lectin from Triticum vulgaris as the ligand and consists of subunits (2alpha 2beta) and exhibits tyrosine kinase activity.

(2) The plates were washed x 4 times with the described washing buffer, 300 µl per wells, and them dried by tapping in the inverted plate on paper toweling.

(3) each well was blocked with 200 µl of coating buffer containing 0.5% of Roti-Block (a blocking agent purchased by Roth Germany) and incubated for 1 hour at room temperature.

(4) After washing and drying, we add a volume of 50 µl of unknowns samples or knows standard samples based on insulin receptor at the following concentration: 1 ng/ml; 5 ng/ml; 10 ng/ml; 25 ng/ml; 50 ng/ml; 100 ng/ml. Thus, we added a volume of 50 µl of HRP conjugated monoclonal anti-insulin receptor alpha subunit (AB-1 clone 83-14) purchased by Neomarkers, USA).

(5) After 1 hour incubation at room temperature, the plates were washed and dried followed by the addition of 100 µl/well of peroxidase enzyme substrate (Sigma). Thus the photometric reading was performed at 450 nm.

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CLAIMS:

1. A method for removing an insulin binding protein (IBP) from a biological fluid which comprises contacting the biological fluid with an affinity agent capable of forming a complex with said IBP and separating complexed IBP from said biological fluid.

2. The method according to Claim 1 wherein the affinity agent comprises a solid support, and on said support, a substance capable of forming a complex with said IBP.

3. The method according to Claim 1 or 2 wherein the affinity agent is covalently bound to the solid support.

4. The method according to any preceding claim wherein the affinity agent includes an amino acid sequence that forms a binding site capable of binding specifically to an insulin-binding domain of said IBP.

5. The method according to Claim 4 wherein said amino acid sequence comprises at least 3, preferably at least 5 and most preferably at least 6 contiguous amino acids of a mammalian insulin.

6. The method according to Claim 5 wherein said amino acid sequence comprises at least 3, preferably at least 5 and most preferably at least 6 contiguous amino acids of human insulin.

7. The method according to any preceding claim wherein the soluble IBP is selected from a soluble insulin receptor, an anti-insulin immunoglobulin and an insulin-like growth factor.

8. The method according to claim 7 wherein the anti-insulin immunoglobulin is an IgG or IgM.

9. The method according to claim 7 wherein the insulin-like growth factor is insulin-like growth factor 1 or 3.

10. The method according to any preceding claim wherein the biological fluid is whole blood.

11. The method according to any of claims 1 to 9 wherein the biological fluid is blood plasma.

12. A method according to any preceding claim, wherein the biological fluid is derived from a patient suffering from non-insulin dependent diabetes.

13. A method according to Claim 12, wherein the biological fluid is returned to the patient after removal of IBP.

14. A procedure for treating a patient suffering from non-insulin dependent diabetes which comprises withdrawing blood from the patient, treating the withdrawn blood by a method according to any of Claims 1 to 11 and returning the blood to the patient after removal of IBP.

15. A solid affinity medium for use in the method of claim 1 comprising a solid support, and bound to the support an affinity agent capable of forming a complex with insulin binding proteins.

16. A solid affinity agent according to Claim 15, which comprises a solid support, and on said support, a substance capable of forming a complex with said IBP.

17. A solid affinity agent according to Claim 15 or Claim 16, wherein the substance capable of forming a complex with said IBP is as defined in any of Claims 4 to 6.

18. A solid affinity medium according to any of Claims 15 to 17 wherein the solid support comprises a ceramic, cellulose, a glass, polymethylmethacrylate or Sepharose.

19. A solid affinity medium according to any of Claims 15 to 18 in the form of a bead, a hollow fibre, a hollow membrane or a flat membrane.

20. A solid affinity medium according to any of Claim 19 in the form of a bead with a diameter between 50 and 800 μm.

21. A solid affinity medium according to any of Claim 19 in the form of a hollow fibre membrane with an internal diameter of between 80 and 200 μm.

22. A device for the extracorporeal treatment of blood to remove insulin binding protein therefrom, comprising an affinity medium as claimed in any of Claims 15 to 21, and means for contacting blood or separated plasma with said affinity medium.

23. A device according to Claim 22, including inlet and outlet conduits adapted respectively to receive blood or separated plasma from a patient and to return blood or separated plasma to the patient, after treatment to remove insulin binding protein therefrom.

24. A device according to Claim 23, incorporated into apparatus for removing blood from a patient and reinfusing treated blood or separated plasma into the patient.

25. A device according to Claim 24, wherein the apparatus is a dialysis machine.

26. A solid affinity medium as claimed in any of Claims 15 to 21 for use in treating Non-Insulin Dependent Diabetes mellitus (NIDDB).

27. A device as claimed in any of Claims 22 to 25 for use in treating Non-Insulin Dependent Diabetes mellitus (NIDDB).

Fig. 1

Extracorporeal system for the removal of the circulating insulin binding proteins from a fractionated plasma

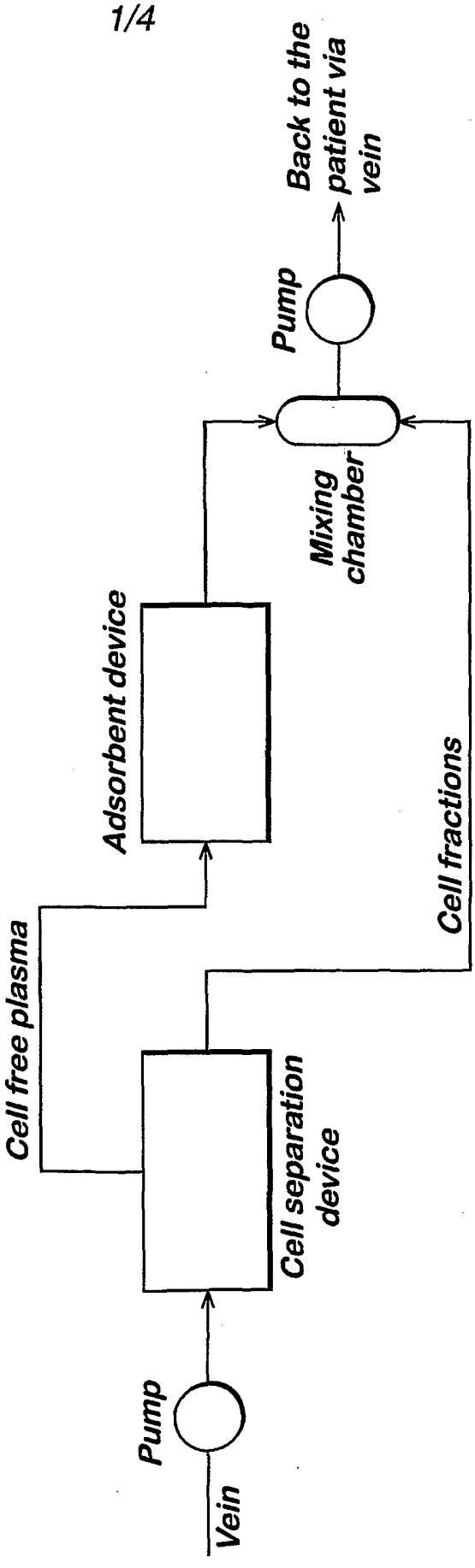

Fig. 2

Competition insulin curve of insulin binding to extracted material: placental material (n= 10) versus plasma from diabetes type II patients (n= 100)

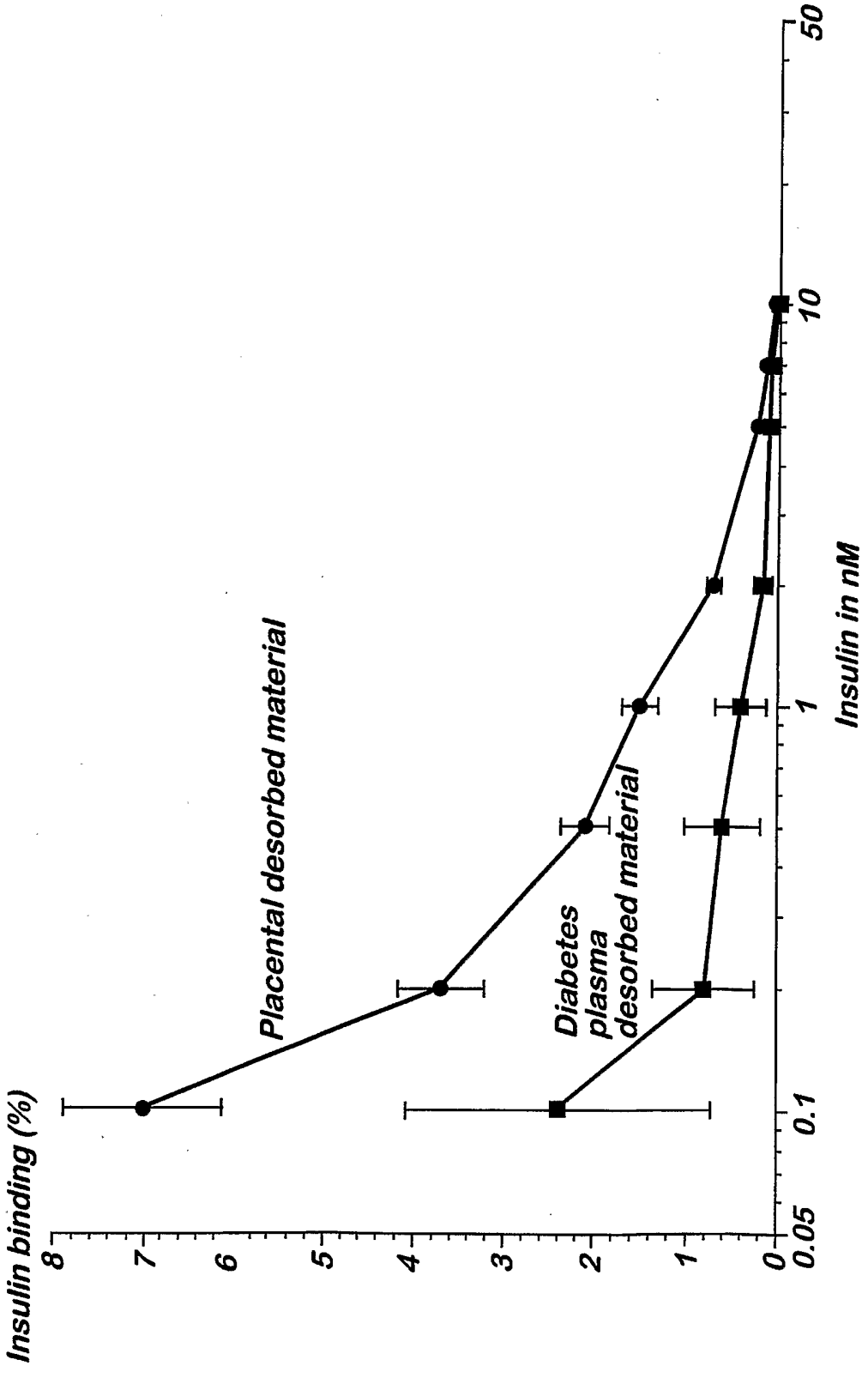

Fig. 3

Levels of the desorbed insulin like receptors in different groups of diabetes patients versus a population of healthy subjects

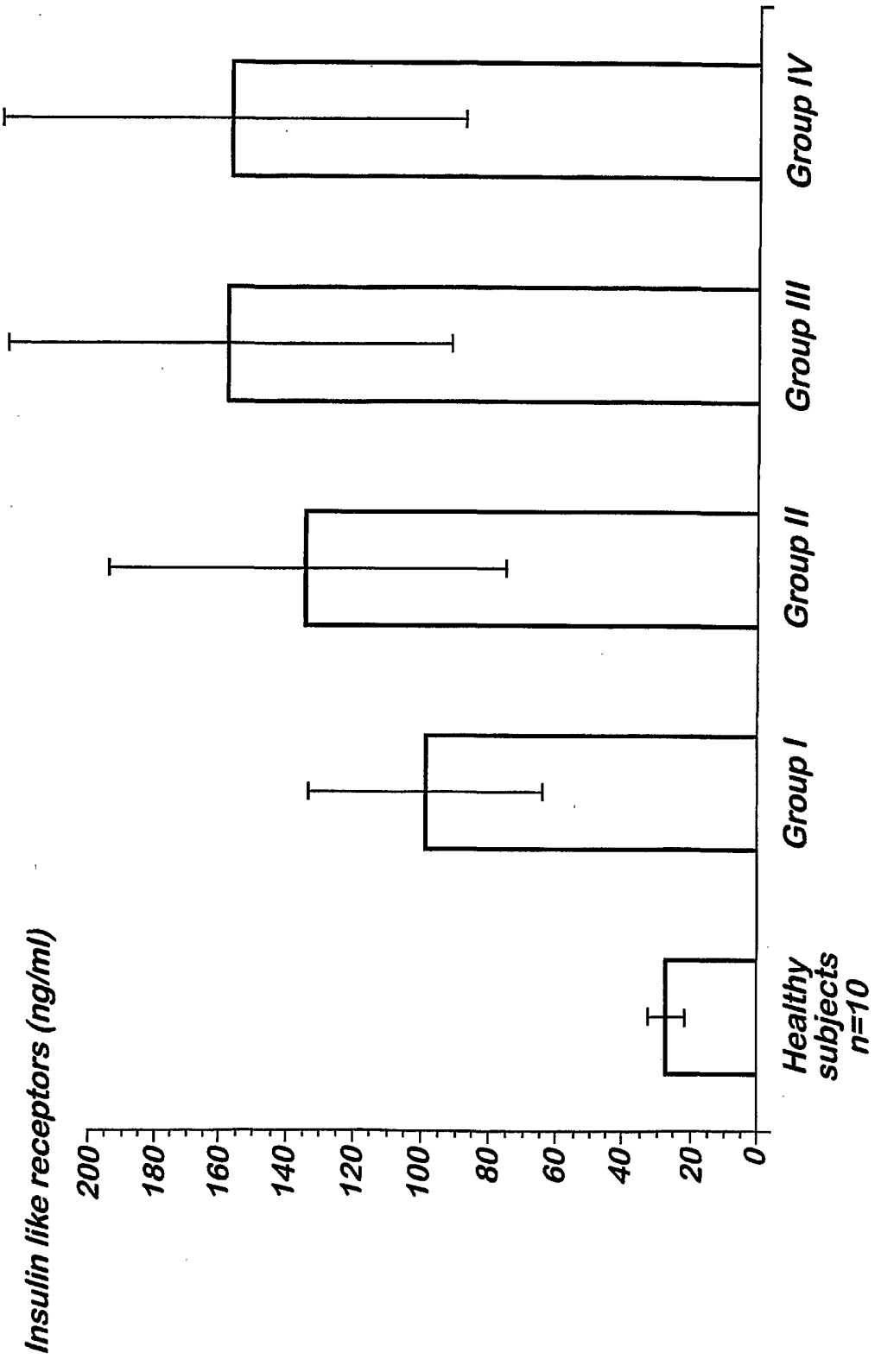

Fig. 4

Plasma levels of insulin like receptors during the procedure of adsorption