Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2067205 C 2009/10/27

(11)(21) 2 067 205

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1991/01/17
(87) Date publication PCT/PCT Publication Date: 1992/03/05
(45) Date de délivrance/Issue Date: 2009/10/27
(85) Entrée phase nationale/National Entry: 1992/04/09
(86) N° demande PCT/PCT Application No.: CH 1991/000016
(87) N° publication PCT/PCT Publication No.: 1992/003163
(30) Priorité/Priority: 1990/08/22 (CH2720/90-7)

(51) Cl.Int./Int.Cl. *A61K 39/385* (2006.01),
A61K 47/48 (2006.01)

(72) Inventeur/Inventor:
CERNY, ERICH H., CH

(73) Propriétaire/Owner:
CHILKA LTD., VG

(74) Agent: RIDOUT & MAYBEE LLP

(54) Titre : VACCIN ET IMMUNOSERUM CONTRE LES DROGUES
(54) Title: VACCINE AND IMMUNE SERUM AGAINST DRUGS OF ABUSE

(57) Abrégé/Abstract:
Addictive drugs like cocaine, heroin or amphetamines are spreading in an epidemic manner in the western world and are an important factor in the spread of the acquired immune deficiency syndrome AIDS(multiple use of infected needles among drug addicts). The present invention describes a vaccine and immune serum against drugs. The vaccine contains the drug bound to a carrier protein in order to produce antibodies against the drugs in the affected person. The use of the drug in the presence of the antibodies deactivates the drug. The desired drug effect is thus eliminated and the vicious circle between stimulation and application is broken.

Title: Vaccine and Immune Serum Against Drugs of Abuse

Abstract:

Addictive drugs like cocaine, heroin or amphetamines are spreading in an epidemic manner in the western world and are an important factor in the spread of the acquired immune deficiency syndrome AIDS(multiple use of infected needles among drug addicts). The present invention describes a vaccine and immune serum against drugs. The vaccine contains the drug bound to a carrier protein in order to produce antibodies against the drugs in the affected person. The use of the drug in the presence of the antibodies deactivates the drug. The desired drug effect is thus eliminated and the vicious circle between stimulation and application is broken.

Vaccine and Immune Serum Against Drugs of Abuse

The invention describes a procedure for the manufacturing of a vaccine against drugs that can generate a dependence, wherein one or more drugs are linked as a hapten to a carrier substance in order to get the hapten antigenic, or wherein one or more drugs are chemically cross linked as a hapten in order to get an antigenic effect without a carrier compounds. The invention describes also a procedure wherein the vaccines, manufactured according to a process wherein one or more drugs are linked as a hapten to a carrier substance in order to get the hapten antigenic, are used as antigens for the production of antibodies against drugs, which may cause a dependence.

Dependence on drugs is often understood as a physiological and psychological dependence. One or the other component can be dominant, but both components are normally present in an addict. Drugs which cause a physical dependence are e.g. opiates, barbiturates, alcohol, cigarettes, anxyolytic drugs and some sedatives.
Psychological dependence on the other hand is described as a desire for a drug, which temporarily becomes extinct, after ingestion of the drug and when the desired effect has taken over. Examples for a drug which can generate a psychological dependence are opiate and cigarettes.

A successful therapy implies a liberation of the psychological as well as the physical component of dependence. The classical therapy tries to obtain a detoxification through a successive diminution or in some cases (methadone) substitution of the drug. The psychological component of dependence is treated through a program of rehabilitation.

The vaccine of this invention allows a new approach to therapy and prevention for patients who are addicted to a drug. The continued use of the drug in the presence of the antibody after vaccination inactivates the drug and stimulates the production of new specific antibodies. The desired drug effect is therefore eliminated and the vicious circle between stimulation and application is interrupted.

The new vaccine broadens the application of vaccines into the field of drugs. There are the following fundamental differences between a classical vaccine and the present vaccine: 1) The vaccine is not directed against an infectious agent but against a drug. 2) The typical application of this vaccine is not preventive but therapeutic. 3) The antigen against which the vaccine is directed is in most cases not by itself antigenic, but must be bound to a carrier protein in order to elicit antibodies. 4) The effect of the drug which causes dependence is based on the interaction of the drug with the receptor. This interaction is eliminated through binding of the antibody with the drug.

It is therefore a goal of this invention to describe a vaccine against drugs of abuse:

1) Which is directed against one or more drugs which cause dependence and which is linked to a carrier protein in order to make the drug immunogenic.

2) Which is effective against drugs causing a physical dependance as well as against drugs causing a psychological dependence.

3) Which can be applied therapeutically (a drug addict is vaccinated in order to inactivate the drug) as well as preventively (in order to protect the fetus of a drug addict pregnant woman from the direct damage of the drug as well as a later dependence on the drug).

4) Which can be directed against all kind of drugs: for example opiates and opiate-like drugs, marihuana, cocaine, amphetamine, antipsychotic drugs, barbiturates and other sedatives, psychomimetic drugs, anticholinergic drugs as well as compounds which contaminate these drugs.

5) Which typically activates the B-cell branch of the immune system, but which may gain an additional efficiency through the activation of the T-cell arm of the immune system or through an allergenic effect.

Definitions:

Drugs which may cause a dependance: All compounds, which in the largest sense of the term may cause a physical or psychological dependence after one or repeated application. A drug elicits a physical dependency, if a withdrawal of the drug causes withdrawal symptoms. Psychological dependence on the other hand is caused by a drug to which the addict has gotten used and which causes with the addict a desire for a specific effect. Drugs which may cause a dependance are among others: cocaine and cocaine derivatives(cocaine consumed by drugs addicts contains frequently among other compounds in addition mannitol, lactose, nicotine, ephedrine, caffeine, procaine and amphetamines), barbiturates and other sedatives, benzodiazepine, methaqualone, glutethimide, chloral hydrate, methyprylon, paraldehyde and bromides, antipsychotic drugs, psychomimetic drugs like phenylcyclidine or LSD (lysergic acid diethylamide), phenylcyclidin and analog compounds, amphetamine and tryptamine derivatives, psylocybine, volatile nitrite and anticholinergic drugs.

Components of drugs: drugs are not used all the time in a chemically pure form and are often mixed with other compounds. It may under certain conditions be an advantage to direct the vaccine against more than one compound in order to obtain a better protective effect.

Vaccine: a preparation which contains an immunogen being able to activate the B-cell arm and eventually also the T-cell arm of the immune system. The immunogen of the vaccine of this invention is typically a drug, which is bound as a hapten to the carrier substance. The binding between hapten and carrier can be covalent or ionic or be based on van der Waal forces or hydrogen bridges. The linkage may also contain one or more atoms forming a bridge. A drug can also be rendered immunogenic through a simple chemical crosslinking(for example through glutaraldehyde) in

order to gain a molecular weight in excess of 5000 Dalton. It is in these cases not necessary to use a carrier compound.

The manufacturing process of the vaccine contains typically two steps: 1) Conjugation: the drug is linked to the carrier compound 2) Purification: the hapten-carrier conjugate is purified from products of the conjugation procedure and dissolved into a physiological solution. The conjugation can be performed in an organic solvent or in a aqueous environment. Substances, which are frequently used for coupling are: carbodiimides, imidoesters, N-hydroxysuccinimide ester or their water-soluble sulfo-derivatives, maleimide-derivatives and phenyl azides.

The purification is usually done by dialysis or with the help of gel- or ion chromatography. The hapten-carrier conjugate has mostly a molecular weight exceeding 100,000 Dalton and can therefore easily be purified from an excess of coupling substance, which typically has a molecular weight under 500 Dalton. Preferred methods of separation are extensive dialysis with dialysis tubing in an aqueous solution, for example through multiple exchanges of Phosphate Buffered Saline (PBS) or chromatography using a Sephadex* G 25 column (Pharmacia LKB Biotechnology, Bromma, Sweden). Sterile filtration with a filter having a pore size of 0.2 micrometer and the removal of pyrogenic material are the final steps of the purification.

Vaccine for preventive purposes: the person to be vaccinated is not yet drug dependant. A candidate suitable for a preventive vaccination may for example be a baby, which obtains the drug through the mother's milk.

Vaccine for therapeutic purposes: The person to be vaccinated is already drug addicted or has started to use the drug. A person suitable for the vaccine is for example a morphine addict, who determines to get detoxified.

---

* trade-mark

Immunization against drugs: with patients who suffer from symptoms of intoxication through drugs, there is no time to elicit with the help of a vaccine antibodies against the drug. It is possible to help in such cases through direct application of specific antibodies of human or animal origin. The specific antibody links in a short period of time after application to the drug and the immune complexes are eliminated through the reticulo- endothelial system. This leads to a removal of toxic compounds from the body. It is possible to use a mixture of antibodies against different drugs, because in lots of cases it is not clear with which drug the patient got intoxicated. The antibodies used may be monoclonal or polyclonal antibodies. Monoclonal antibodies are generated in vitro through fusion with a B-cell line containing the genetic material for the specific immunoglobulin. The hybrid cell line so generated secretes only antibodies of the desired specificity. There is also the possibility to obtain monoclonal antibodies through transformation of a B cell with the help of a Epstein-Barr virus infection.

It is possible to give the antibodies orally, because patients showing symptoms of an overdose have often not yet absorbed entirely the drug. The antibodies have to be applied in this case in a capsule protecting them from digestion in the stomach and small gut.

Antibodies: This is a class of plasma proteins, which are produced by the B-cells of the immune system after stimulation through an antigen. Human antibodies are immunoglobulins of the Ig G, M, A, E or D class.

Antigen: This is a compound with the ability to elicit a response of the immune system. Antigens are typically proteins, but they can also contain sugars or lipid moieties. Antigens typically have a molecular weight of over 10,000 Dalton.

Haptens: These are small molecules, which are not able to elicit an immune answer only by themselves. A hapten has to be linked to a carrier in order to become antigenic. The immune answer to a hapten-carrier can be directed against the hapten, the carrier or both compounds. The hapten of the present invention is normally a drug, a metabolite of a drug or a component of a drug as for example a component of cigarette smoke.

Some drugs are rapidly metabolized after absorption by the body. Morphine for example is rapidly transformed into morphine-3--glucuronate and morphine-6-glucuronate. It may therefore in certain cases be an advantage, if metabolites are used as a hapten in place of the original drug.

Carrier substance: The problem of eliciting antibodies against a small molecule (hapten) is resolved by linking the small molecule to a carrier. This linkage makes the hapten immunogenic. This means antibodies are generated after injection into the body. The binding of the hapten to the carrier protein is often covalent, but, it can be ionic or be effected through a chemical component bridging the hapten and the carrier. The carrier is typically a protein, but it can also contain sugar and fat in mono- or polymer form. Under certain conditions it is possible to crosslink the hapten and no carrier substance is needed in order to make it immunogenic.

Antigen/antibody interaction: The interaction of a specific antibody with the surface of the corresponding antigen (hapten plus carrier compound) is reversible and the complex can dissociate depending on the force of binding. There is an interaction due to the fact that the two partners have on their surface a complementary shape of their electron clouds (similar as a key with the lock). This interaction is based on hydrogen bridges, the van der Waals force, electrostatic force and hydrophobic interaction.

Adjuvant: This is a compound or mixture of compounds which are added to the vaccine in order to enhance the efficiency of the antibody production or to help generate a specific class of antibodies as for example IgM immunoglobulins or antibodies able to bind complement. Substances, which can be

used as adjuvant are for example mineral oils, derivatives of aluminium or compounds of mycobacteria. The vaccines of this invention can be used with or without adjuvant.

Different ways to apply the vaccine: The vaccine can be injected intravenously, intramuscularly, subcutaneously or intradermaly. It can also be given orally (e.g. in a capsule, protected against digestion in the stomach and small bowel). It is in certain cases also possible to give the vaccine as an aerosol or onto the skin for an absorption through the skin. The vaccine can be given one time or more than one time.

In order to improve the understanding of the invention the following examples are given.

Example 1

This example shows with the help of a mouse model how antibodies are generated and how the drug is eliminated from the circulation after vaccination against morphine derivatives or barbiturates.

Conjugation of the hapten to the carrier:

The method of Wainer is used in order to synthesize morphine 6-hemisuccinate (Wainer B.B. et al., 1972, Science 176, 1143-45, Wainer B.B. et al., 1972, Science 178, 647-8) and to bind it to Bovine Serum Albumin (BSA). Another preparation is made by transforming morphine into 3-0-carboxy-methylmorphine by reaction of the free base of sodium-beta-chlorazetate in absolute alcohol. Carboxymethyl-morphine (8 mg) is dissolved in 2 ml of distiled water containing 10 mg BSA and 8 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide are added after adjusting the pH of the solution to 6. After incubation at room temperature, the mixture is extensively dialysed against Phosphate Buffered Saline (PBS).

The following method is used in order to obtain a barbiturate-Keyhole Lympet Hemocyanin (KLH, Sigma, St. Louis, USA) conjugate for the vaccine:

5-allyl-5-(1-carboxyisopropyl)barbituric acid is transformed into 5-allyl-5(1--p-nitrophenyloxycarbonylisopropyl)barbituric acid through reaction of 10 mg of the free base with p-nitrophenol (12mg) in a N,-N-Dimethylformamide solution during 24 hours at 4°C. The coupling to KLH (19) (mg) is thereafter done in a solution containing equal volumes of glycerin and water and 10 mg dicyclohexylcarbodiimide. After 24 hours incubation at 4° C., the mixture is dialysed with extensive exchange of Buffer against PBS pH 7.6. The degree of substitution of the drug at the carrier substance is calculated as an increase in optical density of the conjugate at 202 nm as compared to a KLH control solution (molar extinction coefficient of barbiturate is 19,500).

Immunization: 5 female Balb/C mice are injected subcutaneously 8 times in intervals of 2 weeks with 40, microgram per mouse and dose of the morphine-BSA conjugate. Another group of the mice receives the barbiturate-KLH conjugate under the same conditions and a control group of 10 female Balb/C mice of the same age is treated with the buffer of the vaccine preparation without the vaccine.

Bleeding: The blood is taken from the tail vein.

Assay of the drug concentration: The High pressure Liquid Chromatography (HPLC) method of Joel et al. (1988, Chromatography, 430: 394-9 is used as a reference method in order to measure Morphine, morphine -6-glucuronide and morphine 3-glucuronide. The morphine as well as the barbiturate are likewise measured with a commercial enzyme immunoassay (EMIT, Shyva Corp., Palo Alto, USA) in accordance with the manufacturers instructions (EMIT operator's manual, Shyva Corporation, Palo Alto, USA). It is important for the evaluation of the vaccine to differentiate free drug from antibody-bound drug. The EMIT assay is

therefore modified as follows: the already diluted serum is purified from antibodies before the EMIT assay is executed. The sample is incubated at room temperature for two hours in a PBS Buffer, pH 7.6 which contains CnBr-activated Sepharose* 4 B, to which polyclonal rabbit antibodies against mice light chain immunoglobulins are linked (Pharmacia LKB Biotechnology, Bromma, Sweden).

Five mice, which have been vaccinated and 5 mice of the control group are 2 weeks after the last immunization injected with 0.5 mg per mouse. The serum samples are taken immediately after injection and 2 hours after injection and the quantity of the drug in the serum is determined with the help of the modified EMIT assay. It is still possible to detect the drug in the samples which were taken immediately after the injection of the drug, but 2 hours after injection the drug is no longer detectable. The discrimination in the test system between positive and negative sample is determined to be 5 ng/ml as well for the barbiturates as for morphine. The interpretation of the test results is, that the antibodies have bound to the drug after a successful vaccination and the free drug is therefore no longer detectable.

Example 2:

This experiment demonstrates that the "immunity" against the drug is still present even after months. Two of the immunized mice of example I and three mice of the control group are injected 2 and 4 months after the first experiment with 0.5 mg morphine. One blood sample is taken as in the first experiment immediately after injection and 2 hours after injection and the serum is tested for morphine in the EMIT assay. The serum of the mice, which were prior immunized with the morphine -BSA conjugate is again negative and only the samples, which were taken immediately after injection show a positive result. The group of controls show in all samples detectable levels of morphine. The conclusion

---

* trade-mark

is made that the vaccine is efficient over an extended time span.

Example 3:

This example serves to demonstrate the protective effect of specific antibodies, respectively specific immune serum containing the antibodies.

Two mice each of the control group receive 0.5 mg morphine mice (A and B) and 0.5 mg phenobarbital (mice C and D). Mice A and C receive each 0.5 ml of the serum of the mice which were previously vaccinated with the anti-morphine vaccine. Two hours after injection of the immune serum the blood samples are taken and examined for the presence of drug (modified EMIT assay). The mouse which has received morphine and anti-morphine serum shows no free morphine in the assay. The mouse which got morphine and anti-barbiturate serum, shows free morphine in the serum. An analogous phenomenon is seen with mice which have received phenobarbital: no free drug can be detected with the mouse which received the specific antibodies (mouse D). The mouse with the anti-morphine serum (mouse C) shows still traces of the drug in her serum.

This experiment can likewise be done with an anti-serum consisting of monoclonal antibodies or a mixture ofmonoclonl antibodies: as an analogous result is expected.

Claims :

1. A process for the manufacture of an immunogenic preparation for use as a vaccine, comprising the steps of:

providing at least one substance linked as a hapten to a carrier compound in order to obtain a conjugate, wherein the hapten is a drug of abuse;

processing the conjugate by purification and sterile filtration; and

preparing the purified and filtered conjugate in a number of doses in a physiologically acceptable solution, so that the cumulative dose of the conjugate per vaccination treatment is in excess of 0 micrograms and does not exceed a total amount of 320 micrograms absorbed by a body and induces the lasting protective effect of a vaccine no later than 4 months and over a time span of at least 8 months after the first application of said vaccine, despite repeated drug challenges.

2. A process according to claim 1, wherein said drug of abuse is selected from the group consisting of opiates, marijuana, amphetamines, cocaine, barbiturates, sedatives, mataqualone, benzodiazepine, LSD, psychomimetic drugs, nicotine, anticholinergic drugs and antipsychotic drugs.

3. A process according to claim 1, wherein said carrier compound is selected from the group consisting of proteins, glycoproteins, polysaccharides, liposacharides, lipoproteins, metal-containing proteins, and mixtures thereof.

4. A process according to claim 1, wherein said hapten is linked to said carrier substance by a bridging compound.

5. A process according to claim 1, wherein an adjuvant is added to the vaccines in order to enhance the immunogenic effect.

6. A process according to claim 1, wherein the linking of said carrier substance to said one or more haptens is carried out and the vaccine is rendered in a physiologically compatible form and sterilized by filtration.

7. A process according to claim 1, wherein an aqueous solution is used with a pH value between 5.5 and 8 which is maintained through a buffer system.

8. A process according to claim 1, wherein the vaccine is dialyzed following linking of the hapten to the carrier compound, in order to remove by-products of the manufacturing process.¨

9. A process for producing antibodies against drugs which may cause a dependence for passive immunization treatment characterized in that the vaccine manufactured according to the process of claim 1 is used as an antigen for the production of said antibodies.

10. A process for manufacturing a vaccine capable of activating at least the B-cell branch of the immune system of a mammal against a drug which may cause a dependence in a vaccinated animal having specific antibodies against said drug as a result of a vaccination, so that an administration of said drug to said mammal elicits antibodies in said mammal's body, said method comprising the steps of:

linking a compound selected from said drug or a component of said drug, as a hapten, to a carrier substance having immunogenic properties, thereby synthesizing a hapten carrier conjugate;

purifying said hapten carrier conjugate; and

providing the purified conjugate in a physiologically acceptable solution in doses containing an effective amount for vaccination by said purified conjugate.