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Immunotherapy Monitoring Through Liposomes-An Altered Form of Bio-Sensing

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Abstract

Allergic reactions are caused by allergens belongs to the category of type-I hypersensitivity. The systemic release of histamine as a result of allergic reaction can be fatal anaphylaxis, creating a necessity to improve the efficiency of the treatment that is rendered to patients suffering from this hyper-sensitivity. The generally administered drugs include anti-histamine drugs, steroids and other oral medications but these drugs neutralizes the molecules released after the pseudo-infection, but not the misconception exercised by the immune system and only a temporary relief is achieved. An alternative is allergen immunotherapy, in which the patient is treated with a large dosage of the allergens leading to hyposensitization to the allergens. However anaphylaxis may happen. Hence, monitoring this treatment is vital.

In this paper, the methodology of immunotherapy monitoring is given. The monitoring is carried out by liposomes that are made to carry address tags-monoclonal antibodies. The liposomes envelop the histamine molecules, which are emitted on reaching target site and designed in such a way that they emit fluorescence on enveloping histamine. Increased histamine secretion (higher amount of fluorescence) denotes the inefficacy of the treatment and suppressed levels denote otherwise, hence can be used as an online biosensor. This paper deals with subsequent monitoring of the same, thereby avoiding further complications.

Keywords: Allergic reactions; Anaphylaxis; Immunotherapy; Nanoparticles

Introduction

Liposomes are artificially prepared vesicles made of lipid bilayer. They can be filled with drugs for cancer and other diseases. Liposomes are composite structures made of phospholipids and may contain small amounts of other molecules. Though liposomes can vary in size from low micrometer range to tens of micrometers, unilamellar liposomes are typically in the lower size range with various targeting ligands attached to their surface allowing for their surface-attachment and accumulation in pathological areas for treatment of disease. Liposomes can be prepared by disrupting biological membranes, for example by sonication. Liposomes can be composed of naturally derived phospholipids with mixed lipid chains, like egg phosphatidylethanolamine or other surfactants.

Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region of aqueous solution inside a hydrophobic membrane as dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer. There are three types of liposomes: MLV (Multilamellar Vesicles) SUV (Small Unilamellar Vesicles) and LUV (Large Unilamellar Vesicles). These are used to deliver different types of drugs.

Liposomes, which use a form nanotechnology science, also impressively and harmoniously, use the generalized nature of the liposomes themselves to therefore increase the efficacy, bioavailability, absorption, and delivery of these certain entrapped dietary and nutritional supplements. This generalized nature and makeup of liposomes, being composed of phospholipids, adroitly complements the natural lining of nearly every cell within the human body. This therefore creates a natural bond and or affinity for the liposomes to deliver their onboard "payload" to the cells. The quality of raw lipid used in the preparation and manufacturing of the liposomes therefore precisely co-relates to this natural congruency between the liposomes and the cells of the human body.

Objective of Invention

The drugs that are usually administered to the patients include anti-histamine drugs, steroids and other oral medications. The problem usually associated with this treatment is the fact that the drugs treat the symptoms and not the causative agent i.e., these drugs suppress the action of the molecules that are released after the pseudo-infection but not the cells that are underlying it. This leads to frequent medication to the patients each time they come in contact with the allergens. Another, more permanent means of therapy for allergic reactions is the allergen immunotherapy.

Allergen immunotherapy is a form of immunotherapy for allergic disorders in which the patient is vaccinated with increasingly larger doses of an allergen with the aim of inducing immunologic tolerance. Allergen specific immunotherapy is the only treatment strategy which treats the underlying cause of the allergic disorder. It is a highly cost-effective treatment strategy which results in an improved quality of

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life and a reduction in allergy and allergen-related asthma, as well as a reduction in days off school/work. Immunotherapy has been shown to produce long-term remission of allergic symptoms, reduce severity of associated asthma, as well as reduce the chances of new sensitizations to allergens developing. This is achieved via immunotherapy modulating the immune system response to allergens.

The therapy can either reduce the need for medication, severity of symptoms or eliminate hypersensitivity altogether. Therapy can be administered under the tongue or by injections under the skin. This immunotherapy is the only treatment option that is known to modify the allergy disease process (curative), whereas other therapies merely suppress the symptoms. Subcutaneous injection immunotherapy has been shown to be a highly efficient treatment for allergic disease, but due to a serious side effect of anaphylaxis, its use is restricted to specialist centers.

Though this type of therapy provides a means of permanent relief for the individual from the allergy, there are also cases when this also leads to increase in the sensitivity towards the particular allergen sometimes also leading to anaphylactic reactions. This is a serious issue as it concerns the aggravation of a disorder while treating. Hence, monitoring of this treatment is highly necessary which is carried out with the help of a biosensor used in an online mode of operation.

A biosensor is an analytical device used for the detection of an analyte that combines with a biological component with a physicochemical detector component.

It consists of 3 parts:

The sensitive biological element

Biological material, biologically derived material or biomimetic component interacts with the analyte under study. The biologically sensitive elements can also be created by biological engineering.

The transducer or the detector element

It transforms the signal resulting from the interaction of the analyte with the biological element into another signal that can be more easily measured and quantified.

Biosensor reader device

The associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way. This sometimes accounts for the most expensive part of the sensor. A user friendly display that includes transducer and sensitive element can be generated. The readers are usually custom-designed and manufactured to suit the different working principles of biosensors.

Scope of Invention

The monitoring is done so as to channelize the therapy in the right direction towards the cure of the allergy in the patient rather than worsening the individual's physiological condition. Through this methodology proposed which primarily aims at analyzing the condition of the patient while the treatment is being carried out in scaffolds and arrive at a decision concerning further administration of the allergen or stopping it. This methodology is of very high importance as it prevents a life saving process from taking a counter mechanism and leads to the patient's mortality. The methodology provides a means of online quantification of the compound released as a result of an allergic reaction and hence analyze the seriousness of the situation at hand.

Background of Invention

Anaphylaxis is defined as a serious allergic reaction that is rapid in onset and may cause death. It can result in a number of symptoms including throat swelling, an itchy rash, and low blood pressure. On a pathophysiologic level it is an acute multi-system type I hypersensitivity reaction. Based on the pathophysiology, anaphylaxis can be divided into "true anaphylaxis" and "pseudo-anaphylaxis" or "anaphylactoid reaction." The symptoms, treatment, and risk of death are the same; however, "true" anaphylaxis is caused by de-granulation of mast cells or basophiles mediated by immunoglobulin E (IgE), and pseudo-anaphylaxis occurs without intervention by IgE.

Anaphylaxis can present with many different symptoms due to the systemic effects of histamine release. These usually develop over minutes to hours. The most common areas affected include: skin (80% to 90%), respiratory (70%), gastrointestinal (30% to 45%), heart and vasculature (10% to 45%), and central nervous system (10% to 15%) with usually two or more being involved.

Skin involvement may include generalized hives, itchiness, flushing, and swelling of the lips, tongue, or throat. Respiratory symptoms may include shortness of breath, wheezes or stridor, and low oxygen. Gastrointestinal symptoms may include crampy abdominal pain, diarrhea, and vomiting. Due to the presence of histamine-releasing cells in the heart, coronary artery spasm may occur with subsequent myocardial infarction or dysrhythmia even in the absence of epinephrine use. A drop in blood pressure may result in a feeling of lightheadedness or loss of consciousness. There may be a loss of bladder control a feeling of anxiety or of "impending doom".

Anaphylaxis can occur in response to any allergen. Common triggers include insect bites or stings, foods, medication, and latex rubber. Foods are the most common trigger in children and young adults while medications and insect bites and stings are more common in older adults. Physical factors such as exercise or temperature may also act as trigger.

- Food: Many foods can trigger anaphylaxis. The most common are peanuts, wheat, tree nuts, shellfish, fish, milk, and eggs in Western cultures. In the Middle East sesame is common and rice and chick peas occur more frequently in Asia. Severe cases are usually the result of ingesting the allergen.

- Medication: Any medication may potentially trigger anaphylaxis. The most common medication to do so, include antibiotics (β-lactam antibiotics in particular), aspirin, ibuprofen, and other analgesics. Chemotherapy agents and herbal preparations have also been implicated. Some medications (polymyxin, morphine, x-ray contrast among others) may cause an anaphylactoid reaction (anaphylactic-like reaction) on the first exposure. This is usually due to a toxic reaction, rather than the immune system mechanism that occurs with "true" anaphylaxis. The symptoms, risk for complications without treatment, and treatment are the same, however, for both types of reactions. Some vaccinations are also known to cause anaphylactoid reactions.

- Venom: Venom from stinging or biting insects such as Hymenoptera (bees and wasps) or Hemiptera (kissing bugs) may induce anaphylaxis in susceptible people.

Allergy Statistics

A) There are up to 25 million people who are allergic to insect stings.

• Each year, up to 1000 anaphylactic deaths occur from insect stings.

• In people who have had a reaction to an insect sting, 30% to 60% will have a repeat reaction that is as severe as or more severe than the first episode.

B) Food allergies affect 15 million people.

• The incidence of food allergy in children is increasing.

• More than 4 million, or 8% of children under three years old have food allergies.

• There are 10 million, or up to 2%, who are allergic to peanuts or tree nuts.

• Each year, 250 deaths are attributed to food-related anaphylaxis.

C) There are up to 40 million people who are allergic to latex.

• In health care, up to 17% workers is latex sensitive.

D) Up to 60 million people are allergic to penicillin.

• Penicillin is responsible for about 10,000 cases of fatal anaphylaxis per year, which accounts for an estimated 75% of anaphylaxis deaths.

• Most deaths occur among individuals with no history of drug allergies.

E) Asthmatics are at particular risk for experiencing anaphylaxis.

Anaphylaxis Incidence

Anaphylaxis occurs at a rate of 21 per 100,000 people each year.

• Nearly 200,000 episodes of anaphylaxis may occur each year in the United States and Europe.

• More than 120,000 may experience anaphylaxis each year.

Speed of potentially fatal anaphylaxis:

▪ It takes only 1 to 2 minutes for a mild allergic reaction to escalate to anaphylaxis.

▪ The faster the onset of an anaphylactic reaction, the greater the likelihood that it will be severe [1-6].

Late Phase/Biphasic Reaction

As many as 25% of people who have an anaphylactic reaction will experience a recurrence in the hours following the beginning of the reaction and require further medical treatment, including additional adrenaline injections. This delayed reaction is called late phase or biphasic, meaning two phases.

Description of the Invention

The normal reaction that occurs when a person is subjected to hyper-sensitization (allergic reaction) is there is an increase in the histamine levels in the blood stream. Histamine is an organic nitrogen compound involved in local immune responses. Histamine triggers the inflammatory response. As part of an immune response, histamine is produced by basophils and by mast cells found in nearby connective tissues. The histamine brings about bronchoconstriction leading to asthma, worsening the patient's condition. This histamine's quantity determines the seriousness of the allergic reaction in the patient and its consequent control. On subjecting the patient to allergen immunotherapy, there is an increase in the exposure of the patient to the allergen. This leads to the proceeding of the normal immune response triggering by the immune system and release of histamine into the blood stream. An increase in the histamine levels imply that the allergen immunotherapy has taken an adverse effect on the patient and is deviating from its destined path. Instead of curing the patient, the allergy is being aggravated. This might even lead to a life threatening circumstance of an anaphylactic reaction. Therefore, its monitoring is essential.

The level of efficiency of the allergen immunotherapy treatment can be monitored by the usage of liposomes. The liposomes are made to encapsulate biological molecules which act as address tags to target them to specific sites in the body. The address tags that can be used for this specific treatment are monoclonal antibodies. The monoclonal antibodies are utilized for the purpose of only targeting the vesicle and the allergen molecules to the specific site were the allergic reaction occurs. As soon as the allergen arrives at the target site, it triggers the manifestation of the allergic reaction consequently leading to the release of the histamine molecules in that region. These vesicles are designed in such a way that, on reaching the site and on release of histamine, they ooze out the monoclonal antibodies that are enclosed by them and allow the histamine molecules to adhere to their surface. The design is carried out in such a way that liposomes are more stable while histamine is adhered to its surface than they were while monoclonal antibodies performed the same. The affinity of the liposomes towards the histamine molecules must be higher than the affinity of the liposomes towards the monoclonal antibodies. Similarly, the affinity of the liposomes towards the histamine molecules must be higher than the affinity of the histamine receptors towards the histamine molecules. This can be achieved easily as liposomes contain hydrophilic regions that form stable hydrogen bonds with the nitrogen atom in the histamine molecule.

The liposomes are engineered to contain a fluorescing compound. The fluorescent molecules that may be employed include fluorescein, erythrosine, eosin, bilirubin, coumarin derivatives, acridine derivatives, etc. The structure of the vesicle is designed in such a way as to contain a fluorophore and a quencher located adjacent to each other while holding the monoclonal antibodies. As the flourophore's presence adjacent to the quencher brings about a sharing of energy of the two molecules, the fluorophore is unable to exhibit its property of emitting fluorescence. The same situation follows on release of monoclonal antibodies as it is designed to take place by opening the liposome in the diagonally opposite region from where the quencher and fluorophore is present. But, on releasing the antibodies and allowing the adherence of histamines to their surface, they undergo a conformational change in their structure which pulls apart the fluorophore from the quencher leading to the fluorescing of the liposome. This property of the liposomes is analogous to the property of molecular beacons that are used to determine the sequence of nucleotides. While the latter serves the purpose of sequencing of structures, the former serves the purpose of quarantining the organic nitrogen molecule.

The liposomes on arriving at the target site, release the monoclonal antibodies and histamines get adhered to the liposomes. This leads to a conformational change in the structure of the liposomes and emits fluorescence. The fluorescence emitted is quantified. This purpose is solved by using a biochip, similar to the chip that serves as a GPS in locating the position of the animals in sanctuaries, etc. these biochips are specially designed to sense and transmit the fluorescence emitted in a quantitative manner. This biochip, sensitive biological material of the biosensor, transfers the signal to an external signal receiver. This

external signal receiver then transfers the signal to a transducer which converts the biological signal to electrical signal and this electrical signal is received by the reader component of the biosensor device which quantifies the signal. The quantified electrical signal is directly proportional to the fluorescence emitted. The major difference between a normal biosensor and this invention is the fact that, the traditional biosensors require a sample to be taken and analyzed like in glucose biosensor while in this invention, the sensor works in an online fashion. The readings are taken while the therapy was being carried out in a simultaneous fashion and the readings received every second give a better outlook of the situation at hand of the patient.

The intensity of the fluorescence, that is measured indirectly by measuring the electrical signal, gives the amount of histamine that were present in the target site, released as a result of the presence of allergen molecules in the target site. If the intensity of the fluorescence is less or nil, it implies that the immune response got hyposensitized to the allergen, i.e., the immune system of the patient did not consider the allergen to be a foreign invader considered to harm the normal functioning of the body. If the intensity of the fluorescence is high and increasing with time and increase in the quantity of allergen administered, it means that the immune system persists in considering the allergen to be an infectious agent that has to be eliminated. This could lead to the development of the inflammatory responses eventually leading to anaphylactic reaction. This indicates that the time has arrived to pull the plug. The dosage of allergen given to the patient must be reduced drastically or completely stopped to save the individual's life. Thus the individual's life can be saved.

The liposomes also serve another important purpose. It serves to quarantining the histamines and avoiding them from making any contact with the histamine receptor-H1. These histamine receptors are responsible for bringing about immune response in the body. When the histamine fails to make any contact with the H1 receptor and so the H1 receptor is under the perception that there isn't any invader present. Therefore, the receptor remains in its native state without responding to the release of the histamine in the region. This averts the triggering of immune response and the consequent worsening of the patient's condition.

Benefits of the Invention

This methodology serves two purposes:

1. The analysis of the immune response: the allergen's release can lead to only one of the following two consequential actions-the immune system either remains non-responding to the entry of the invader or the immune system responds to the allergen's entry. This can be analyzed by fluorescence of liposomes and its subsequent quantification, the quantity of which directly denotes the graveness of the situation at hand.

2. The quarantining of the histamine molecules released as a result of the allergen's arrival and hence, preventing it from signaling the H1 receptor and provoke the immune response. Thus the benefits yielded are very important and is a life saving mechanism. The monitoring of the therapy is vital as it deters the life saving treatment from getting converted to a life threatening mechanism as it takes only about 1-2 minutes for an allergic reaction to transform into anaphylactic reaction.

Conclusion

This proposal is not allergy-specific, i.e., this methodology can be used while treating any allergy, be it food allergy, medication allergy, insect allergy or environmental causative agents. Hence, the monitoring of the allergen immunotherapy of any allergy can be performed via this means.

It serves both the hypo-sensitization towards the allergen by the immune response and quarantining of the signaling molecule, histamine and prevents it from contacting the H1 receptor.

Future Scope

It is estimated that the number of cases of anaphylaxis from foods in the US increased from 21,000 per year in 1999 to 51,000 per year in 2008, based on long term population studies of anaphylaxis from the Mayo Clinic in Minnesota. From 2003 to 2006, food allergies resulted in approximately 317,000 visits to hospital emergency departments, outpatient clinics and physicians' offices, according to Branum and Lukacs [6] using data from multiple US national surveys collected by the National Center for Health Statistics. Food allergy related hospital admissions increased from 2,600 per year (1998-2000) to 9,500 per year (2004-2006), according to a study from Branum and Lukacs [6]. It is estimated that food allergies cause approximately 150 to 200 fatalities per year, based on data from a five year study of anaphylaxis in Minnesota from the Mayo Clinic. Fatal food anaphylaxis is most often caused by peanuts (50-62%) and tree nuts (15-30%). This has been the case in the year 2007. With the passage of years, the number of people enduring the agony also keeps increasing drastically. Hence, with the ever increasing maladies associated with allergies, its treatment and cure is indispensible. The therapy leading to permanent cure is mandatory and so does the monitoring of the efficacy of its treatment. Therefore, this proposal is sure to be of use for the present as well as the future generations.

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