Galantamine treatment for alzheimer's disease

Alzheimers disease is a neurodegenerative disorder characterised by a decline in cognitive function such as memory loss, disorientation, impairment of motor skills and emotional and behavioural problems. It is the most common form of dementia observed in the elderly population and it prevalence is on the increase. This has lead to a more focused approach in the research and development of treatment for the Alzheimer’s disease.

People with Alzheimer’s have been shown to have a shortage of the chemical acetylcholine in their brains. Although there is no known cure for AD, there are treatments in place that help by maintaining existing supplies of acetylcholine systems at sites of neurotransmission in the brain. Some of the drugs used in the treatment of this disease include Rivastigmine, Galantamine and Donepezil. These treatments help slow disease progression of the disease in patients. The main focus of my research will be examining one of the treatments, galantamine.

Background

Galantamine is used for the treatment of mild to moderate AD in patients and has been shown to have benefits in cognitive and global functions. Galantamine is a tertiary natural alkoid of the amaryllidacease family and is known to act as a competitive and reversible inhibitor of the enzyme acetylcholinesterase (AChE) . In early 2001, the US Food and Drug Administration approved the compound’s hydrobromic acid salt (active ingredient) as treatment for mild to moderate AD under the name Remily. Unfortunately due to its high cost, and inability to provide a permanent cure, the UK regulatory body NICE has ruled against its provision in England and Wales.

Sources

For thousands of years, natural products have played an important role throughout the world in treating and preventing human disease. Natural products have come from various source material mainly plant, alkaloids are powerful natural substances made in plants. The plant Leucojum aestivum was the main source of galantamine for sometime. Recently, the more widespread licensing of galantamine throughout the world has caused a requirement for alternative sources. Synthetic methods have been developed and used to produce galantamine for the pharmaceutical industry, but high costs and increasing demand has made extraction from plant sources an attractive option.

The chemical name of the salt is (4aS, 6R, 8aS)-4a, 5, 9, 10, 11, 12-hexahydro- 3-methoxy- 11-methyl- 6H-benzofuro[3a, 3, 2ef] [2] benzazepin-6-ol hydrobromide (figure 2)[7]. The structure of this component (figure 2) along with the complete structure of galantamine (figure 1) is shown below:

The current synthetic approaches to galanthamine are based either on the phenolic oxidative coupling or on the intramolecular Heck reaction. However, the methodologies are particularly complicated due to the presence of three chiral centres. Common synthetic methods used in the pharmaceutical industry for the synthesis of commercial galantamine include:

Sanochemia Industrial Production Synthesis: This method was outline in 1999 by chemists at the Vienna University of Technology in Austria. The synthetic method involves a nine step procedure and forms the basis for galantamine industrial production. The most notable steps include an

oxidative phenol coupling and crystallisation-induced chiral conversion of the single enantiomer (±) narwedine to (-) narwedine. The process successfully generates multikilogram quantities of the drug that are used in phase 2 and 3 clinical studies.

Trost Galantamine Synthesis: This method involves synthesis of galantamine via an asymmetric synthesis involving a sequential palladium-catalyzed allylic alkylation and an intramolecular heck reaction.

Eli Lilly/University of Southampton Galantamine synthesis: This total synthesis method was described by Eli Lilly and the University of Southampton in 2007. This method involves an enantioselective synthesis of (-) galanthamine via a fourteen step procedure.

Many other synthetic methods exist but have not been implemented on an industrial state.

A potential natural source for large-scale extraction of the alkoid is members of the genus Narcissus that is Narcissus pseudonarcissus (Daffodils).

Structure of Daffodils bulbs:

Alkaloids are preset in daffodils at varying levels in different varieties, particularly in the leaves and bulbs where they are considered to protect the plant from herbivore and microbial infection. Levels of alkaloids in daffodils vary with environmental condition, with the part of the plant and the stage growth the plant is in. Total alkaloid levels in daffodil bulbs are generally in the range of 1-2% dry wt, but galantamine levels can vary from zero in some varieties up to 0. 2% dry wt. Other types of alkaloids found in daffodils

include but are not limited to Tozettine, Crinarine and Lycorine; these would be examined at a later stage in our research to determine whether they affect galantamine levels obtained from extraction.

Planned Experimentation

Aim

This project aims are to evaluate whether galantamine is present in Daffodil bulbs grown in Yorkshire and Humberside region.

Objectives

Through this research, I intend to achieve the following aims:

- To analyse the levels of Galantamine present in the Daffodils bulbs grown in the Yorkshire and Humberside region.
- To develop methods of quantifying the levels of Galantamine in Daffodil bulbs at various stages in the life cycle of their development.

It’s hoped by the pharmaceutical industry that producing galantamine from daffodils bulbs would bring price of remily down making it cost effective. Key to it all is not just too producing daffodil bulbs high in galantamine but also in developing efficient ways to extract it.

Research Method and Design

Chemical and Reagents

Mobile Phase: HPLC grade Methanol, Water and Acetic acid

Commercial Galantamine

Columns: Reversed phase chromatography, C18 column (this might change depending on the HPLC method to be developed)

HPLC system separation: Gradient elution separation will be used throughout the analysis

Materials

Whole plants of Narcissus pseudonarcissus at different levels in their life cycle of their development from different locations in the Yorkshire and Humberside region.

I will perform several trails to achieve objectives. Therefore various research and experiments will be done:

Introduction to HPLC

High Pressure Liquid Chromatography would be used to determine the galantamine levels in Yorkshire and Humberside Daffodils. Before commencing my research project, I will familiarise myself with the HPLC system by carrying out a repeat of a second year HPLC experiment. The aim of this experiment would be to use HPLC to separate the two components of interest in a mixture and to determine the amounts of each present in an unknown sample using a method based on the use of an internal standard. The experimental procedure will consist of analysing a number of standard solutions containing different known amounts of the two analytes anthracene, naphthalene and a constant amount of the internal standard benzene. The ratio of signal intensity of analytes to internal standard will then be determined for the unknown sample and the calibration graph used

to assess the concentration of each analyte present. The main purpose of carrying out this experiment would be to develop knowledge of the essential HPLC system components such as the solvent delivery system, method of sample introduction, a column, a detector, recording and interpreting chromatograms.

Development of the HPLC method

Commercial galantamine would be analysed to determine the best HPLC method to anaylse galantamine levels in Yorkshire and Humberside daffodils. I intend to use the HPLC method used in the “ study to quantify galanthamine levels in material from larger field trials for a number of daffodil varieties grown at several sites in Wales”, to analyse the commercial galantamine. Critical components that are going to be considered for the HPLC method include HPLC analysis conditions to achieve minimally acceptable separations, sample preparation and standardisations. The HPLC method will be optimised to determine the best conditions to detect galantamine and to obtain best peak resolution.

Analysis of commercial galantamine and identification of limit of detection of galantamine by HPLC

Preparation of the stock solution: Commercial galantamine (g) will be accurately weighed, quantitatively transferred into a volumetric flask, dissolved in HPLC grade methanol and the volume will be adjusted with the same solvent.

Standard solutions of different concentrations will be prepared from the stock solution and the volume will be adjusted with the same solvent

methanol. A UV Vis absorption spectrum will be carried out on the stock solution to determine the absorbance wavelength of the galantamine; methanol will be used as a reference. Analysis of commercial galantamine will be performed in-order to determine the retention time of galantamine but this will depend on the flow rate of the HPLC method.

Identification of an internal standard for HPLC

Tramadol is the internal standard that would be added to the different concentrations of galantamine samples. It has been used in previous galantamine studies by a student from the University of Huddersfield because of its close chemical similarity to galantamine. We will use HPLC analysis to determine if tramadol provides a signal that is similar to the analyte signal in most ways but sufficiently different so that the two signals are readily distinguishable by the instrument. The internal standard will then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. The calibration graph will be used to assess the concentration of the analyte present.

Identification of limit of quantification of galantamine and identification of linear range

The linearity of the HPLC method will be checked by injecting five mixtures of standard methanolic solutions of galantamine. The identification and quantitative determination of the galantamine will be accomplished by a comparison of retention times, peak heights and areas with standard solutions of galantamine. A calibration graph will be plotted in which the

ratios of the signal intensity from the analyte to that of the standard is plotted against the amount of analyte present in each of the standards.

Analysis of Yorkshire and Humberside Daffodil bulbs

Sample preparation: The fresh weigh of each daffodil bulb will be recorded and then a core sample will be taken with a cork borer through the centre of the bulb to provide fresh wt (g) samples for analysis. Bulbs and sample cores will be stored until extraction.

Extraction Method: Fresh Yorkshire and Humberside bulbs (g) will be extracted with methanol. I intend to use the HPLC method used in the study to quantify galanthamine levels in material from larger field trials for a number of daffodil varieties grown at several sites in Wales to extract galantamine in the daffodils. All solutions will be filtered prior to use on the HPLC system to avoid blockage in the instrument.

HPLC analysis: Developed HPLC method will be used to analyse galantamine levels in daffodils.

Identification of other compounds present in daffodil bulbs

The developed HPLC method to extract Galantamine from the daffodils will be used for quantitative determination of other alkaloids that might be present in the daffodils. Further, we could look at how the present of other alkaloids affect the levels of galantamine present in daffodils and their extraction.

Study of which part of the bulb gives high result of galantamine

Tissue samples from different parts of the daffodil plants will be examined using the HPLC method to determine galantamine levels present.

Study of how much different plant growth stages give best galantamine result

Daffodils at different development stages will be examined to identify if growth stages affect galantamine levels in daffodils.

Note: The experiments will be repeated to get best results; these would be shown in calibration graphs to be plotted.

Time Plan

The time period of the research will be at least 4months. At the beginning I will focus on familiarising myself with the Laboratory work. This will be done in the first week. In the second week, I will begin my project research. It is difficult to estimate precisely the time that different research activities will take in advance; this is the reason why we have two days to perform laboratory work.

In order to keep and be able to overview, a research diary would be kept. In this diary the observational, methodological, theoretical and analytic noted would be organised. From the start through to the end of the research, information would be recorded.