GALANTHAMINE, A NATURAL INHIBITOR OF CHOLINESTERASES: DETERMINATION BY CAPILLARY ZONE ELECTROPHORESIS.

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Summary

The research to find better medicaments against Alzheimer's disease is going on continuos. New drugs are under different levels of study. Galanthamine, an acetylcholinesterase inhibitor, is in phase III (clinical trials) in Austria. In this communication, the results of its determination in biological fluids and pharmaceutical preparations using capillary zone electrophoresis are presented.

KEY WORDS: Galanthamine; Alzheimer's disease medicaments; Acetylcholinesterase inhibitor; Capillary zone electrophoresis.

Introduction

Alzheimer's disease (AD) is a fatal degenerative disorder of the Central Nervous System (CNS) (1). Clinically, it is characterized by psychic changes, disorder of the memory, disorientation and confusion. AD is associated with a decrease of some neurotransmitters; one of them is acetylcholine.

For the synthesis of acetylcholine, enzyme acetylcholintransferase is an important agent. Owing to a reduced activity of this enzyme, which is associated with decline of mental function, the concentration of acetylcholine is decreased. The enzyme acetylcholinesterase is responsible for the degradation of acetylcholine in cholinergic synapses (2).

For symptomatic treatment of AD, several inhibitors of this enzyme were tested, including tacrine, physostigmine and heptylphysostigmine, but therapeutic success of these compounds was limited. For example, physostigmine showed some improvements of neuropsychic functions in patients with AD, but for clinical use it is not available for its short half-time. Tacrine has also some disadvantages, for example short biological half-time, relatively low bioavailability, noncompetitive way of enzymatic inhibition, insufficient specificity for acetylcholinesterase, small therapeutic range and liver toxicity (3).

Galanthamine is the next acetylcholinesterase inhibitor, which has shown increase of clinical effectiveness. It has similar therapeutic potential as tacrine, but better pharmacokinetics and toxicological profile. That is, galanthamine shows many favourable characteristics as its own completely oral bioavailability, reversibility, biological half-time, competitive and highly specific inhibition of acetylcholinesterase. And above all galanthamine does not show any toxicity. In addition to this, galanthamine was found to be about 50 times more effective against human erythrocyte acetylcholinesterase than against human plasma butyrylcholinesterase (4) and it is evident that it can penetrate the blood-brain barrier and inhibit the brain acetylcholinesterase (5).

Galanthamine is an alkaloid of some *Galanthus* species, chemically 1,2,3,4,6,7,7a,11c-octahydro-9-2--methoxy-2-methylbenzofuro [4,3,2-efg]benzazocin-6--ol (Fig. 1). This tertiary compound was first isolated from the bulbs of first Caucasian snowdrop *Galanthus woronowii (6)* and a common snowdrop

Galanthus nivalis (7). This alkaloid has been also found in number of other sources, e.g., various species of *Narcissus* (8, 9), *Lycoris* (10) and Several South African *Amaryllidaceae* species (11). Now galanthamine is extracted mainly from *Leucojum aestivum* (12).

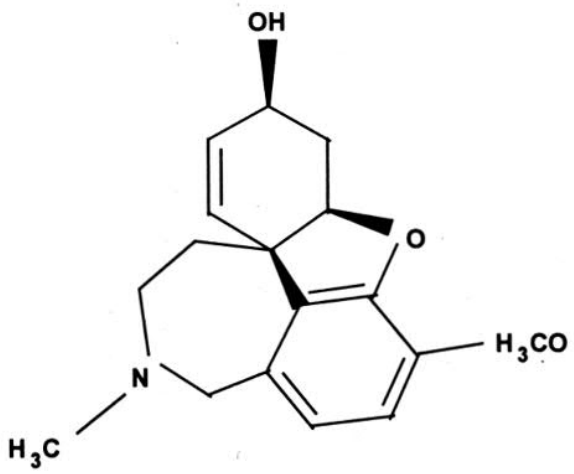

Fig. 1 Galanthamine structure

Because acetylcholinesterase inhibitors used for the treatment of Alzheimer's disease have many undesirable effects and their effectiveness are dependent on accurate drug doses, monitoring of their level concentration in biological fluids is very important (13). Up-to-date the determination of galanthamine has been performed mostly by immunoassay (11, 12), GC-MS (14) and HPLC procedure (after pre-analysis) (15).

In this communication, the results of capillary zone electrophoresis (CZE) for the determination of galanthamine in pharmaceutical preparations and biological samples are presented. Full procedures and method of optimization was already reported (16).

Chemicals

Galanthamine ampoule - Nivalin (10 $mg \cdot mL^{-1}$) - (Pharmachim, Bulgaria). Sodium phosphate, phosphoric acid, sodium tetraborate (borate), boric acid, sodium hydroxide, ethanol and methanol, analytical-grade purity (Lachema, a. s., Brno, Czech Republic). Mesityl oxide (Fluka, Buchs, Switzerland). Lyophilized urine (Heintel Diagnostika, Wien, Austria). Standard lyophilized serum (Boehringer, Mannheim, Germany). Double distilled water, quartz still (Heraeus, Hanau, Germany).

Equipment and Conditions

SpectraPHORESIS 2000 (Thermo Bioanalysis Corporation, CA, U.S). Ultrasonic cleaner (Branson, U.S). Uncoated fused-silica capillary; 36.0 cm to detector, inner diameter 75 µm, (Composite Metal Services Ltd., The Chase, Hallow, UK). pH-meter PHM 64 (Radiometer, Copenhagen, Denmark). Glass crucible S4 filters (Kavalier, Czech Republic). Solid phase extraction C18 columns were from J.T. Baker (Phillipsburg, NJ, U.S).

Capillary conditioning: 5 min with 1 M NaOH at 60 °C, 10 min with water at 30 °C and 10 min with the background electrolyte (BGE) at 25 °C. Before each measurement, the capillary was washed with the working electrolyte. Voltage between 10-25 kV and temperature controlled at 25 °C were usually applied. Detection at 210 nm. Electrokinetic injection of the samples.

Results

The absorption spectra of galanthamine (water solutions) at different pH values showed that maximum absorbance was always 210 nm. This wavelenght was used for the detection in CZE analysis.

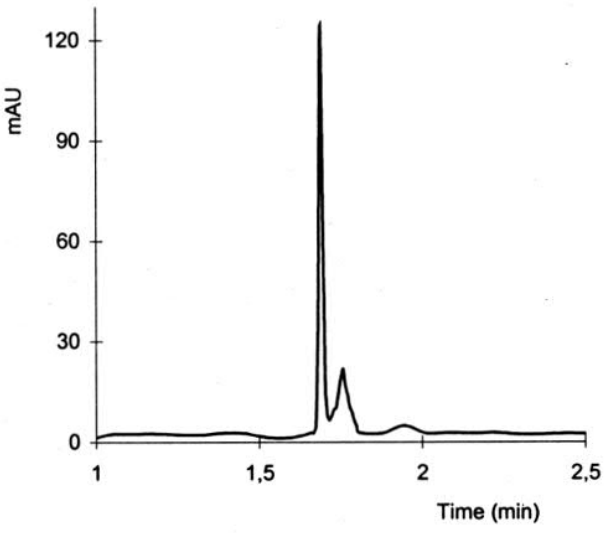

Fig. 2 Electropherogram for a galanthamine ampoule sample. CZE conditions: 22 kV as separation voltage, 20 mM tetraborate buffer (pH 7) as BGE. Electrokinetic injection (5 s, 10 kV).

1) Pharmaceutical Preparations

Figure 2 illustrated that two peaks have been obtained for the ampoule sample under using the following conditions: electrokinetic injection (5 s, 10 kV), 22 kV separation voltage, 20 mM borate

pH 7. The highest peak belongs to galanthamine while the other one has not been identified yet. A detection limit equal to 40 ppb was estimated.

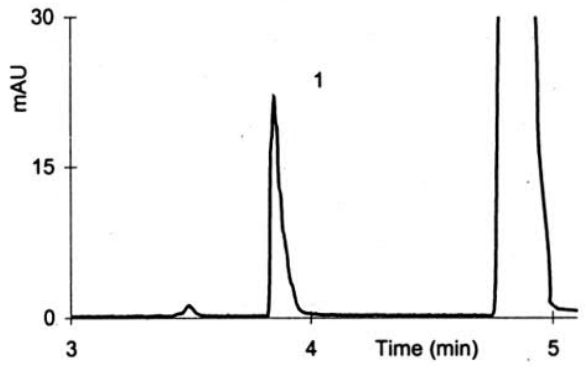

Fig. 3 Electropherogram for galanthamine determination in urine. CZE conditions: 12 kV as separation voltage, 100 mM tetraborate buffer (pH 8) as BGE. Electrokinetic injection (15 s, 10 kV).

2) Urine

A ten-fold dilution of urine samples was used for galanthamine determination. Figure 3 shows that the nearest urine components are slower than galanthamine and cause no interference during the determination.

In used conditions, the limit of detection for the determination of galanthamine was estimated as 0.4 ppm (Table I).

Table I

Results obtained for galanthamine determination in various samples

Sample Type	LOD (ppb)[a]	r^2
Ampoule	40	0.9976
Urine	400	0.9982
Serum	35	0.9984
Serum - SPE	5.2	0.9976

[a] LOD = limit of detection (S/N = 3)

3) Serum

Serum was deprotenized before CZE analysis. It was found that using 10 mM magnesium chloride it was possible to obtain a better separation of galanthamine from the matrix components (Fig. 4). After optimization of the method, the detection limit was estimated to be equal to 35 ppb. In order to decrease detection limit, solid phase extraction was applied for the serum using methanol. A 7 times preconcentration was achieved and thus detection limit of 5.2 ppb was reached.

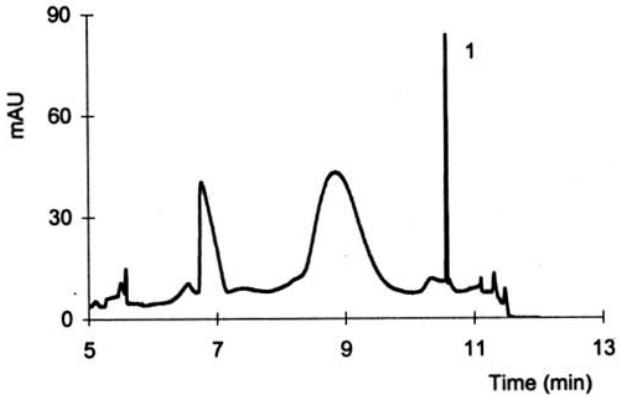

Fig. 4 Electropherogram for galanthamine determination in serum. CZE conditions: 12 kV as separation voltage, 50 mM tetraborate buffer (pH 8) as BGE and electrokinetic injection (22 s, 10 kV).

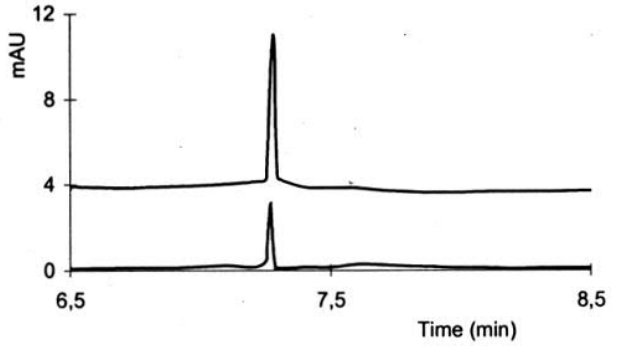

Fig. 5 Electropherograms for galanthamine determination in serum sample after pre-concentration with SPE. CZE conditions: 15 kV as separation voltage, 500 mM tetraborate buffer (pH 8) as BGE and electrokinetic injection (30 s, 10 kV).
A .....35 ppb B .....100 ppb

Conclusions

CZE methods for the determination of galanthamine in pharmaceutical preparations and biological fluids such as urine and serum were developed. Urine and ampoule samples do not need labouring pre-treatment before analysis. Using solid phase extraction, the detection limit can be improved more than 7 times less, as proved for serum samples.

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