Abstract:
The present invention relates to novel pharmaceutical formulations for injections, infusion techniques, and topical administration in the form of an aqueous solution comprising succinic acid dicholine salt and a pharmaceutically acceptable buffer at a concentration effective to maintain the pH of the formulation at between about 4.0 to about 7.5. The present invention also relates to the use of these formulations for the preparation of a medicament for the treatment of neurodegenerative disorders.
PHARMACEUTICAL FORMULATION COMPRISING DICHOLINE SALT OF SUCCINIC ACID

Field of the Invention

The present invention relates to novel pharmaceutical formulations for injections, infusion techniques, and topical administration comprising succinic acid dicholine salt.

Background of the invention

Dicholine salt of succinic acid is a biologically active compound useful for the treatment of conditions of insulin resistance, including cerebral insulin resistance. Storozheva et al. BMC Pharmacol. 2008 Jan 23: 8(O: 1).


However, the use of succinic acid dicholine salt for preparation of formulation in form of aqueous solutions is limited by low stability of this substance in the aqueous solution under long-term storage. Aqueous solution of individual dicholine salt of succinic acid (5%) has pH about 8.2 to 8.4 and undergoes continuous undesirable decomposition under room temperature with release of trimethylamine and ethyleneglycol. Thus, there is the need in stabilization of aqueous formulations of succinic acid dicholine salt. Surprisingly, it has been found that the decomposition can be significantly slowed under pH below 7.5 and almost completely stopped in practical terms under pH 6.5 and less.

It is an object of the present invention to provide stable aqueous pharmaceutical formulations for injections, infusion techniques, and topical administration comprising succinic acid dicholine salt.

Detailed Description of the Invention

The present invention provides a pharmaceutical formulation in the form of an aqueous solution for injections, infusion techniques, and topical administration comprising succinic acid salt of formula (I)
and a pharmaceutically acceptable buffer at a concentration effective to maintain the pH of the formulation at between about 4.0 to about 7.5.

The term "injections" refers to any injection dependent route of administration of the formulation of the invention. Such routes include, but are not limited to, intramuscular, intravenous, subcutaneous, intradermal, intrasternal, intraarticular, epidural, and intrathecal injections.

The term "infusion techniques" refers to any infusion techniques used in medical practice. Such techniques include, but are not limited to, gravity infusion, pressure infusion, and continuous infusion.

The term "topical administration" refers to administration onto skin surface or ocular surface.

The term "pharmaceutically acceptable buffer" refers to a compound that is known to be safe for use in pharmaceutical formulations and that has the effect of controlling the pH of the formulation at the pH desired for the formulation. The buffer may be any suitable pharmaceutically acceptable buffer. Buffers for a variety of pH ranges are available (see Flynn, G.L. Journal of Parenteral Drug Association, 1980, 34, 139-162, Table V, page 156) which may be used to control the pH of pharmaceutical formulations. Preferred buffers include phosphate, acetate, citrate, succinate, TPJS, or histidine buffer. The most preferred buffer is succinate buffer.

The formulations of the invention are prepared by methods well-known from the art in accordance with accepted pharmaceutical procedures, for example, as described in Remington's Pharmaceutical Sciences, seventeenth edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa., Eighteenth edition (1990).

The content of succinic acid salt of formula (I) is in the range from 1 to 20 %, preferably 5 to 10 % by the weight of the formulation.

The preferred unit dose of the pharmaceutical formulation of the invention contains from 1 to 5 ml of salt of formula (I) solution.
The formulation of the invention may also include one or more optional ingredients, for example, preservatives, solubilizers, fillers, stabilizers, antimicrobial agents, albumin, isotonic agent, inorganic salts, and the like. Examples of the above are disclosed, for example, in Remington: The Science and Practice of Pharmacy, 763-764 (Alfonso R. Gennaro ed., 20th ed., Lippincott, Williams & Wilkins 2000). Such optional ingredients generally are used individually at levels from about 0.0005% to about 10.0%, preferably from about 0.005% to about 1.0% by weight of the formulation. The amount of any of these optional ingredients to use in the present formulations can be determined by one skilled in the art.

Any of formulations of the invention are preferably sterilized by any means known in the art. In some embodiments, sterilization or sterilizing involves subjecting any formulation herein to a set of sterilization exposure conditions over a period of time such that a surviving microbial population is reduced. Sterilization can be achieved by various methods known in the art. Compositions and formulations of the present invention may be sterilized by several sterilization techniques including lyophilization, steam sterilization, dry heat sterilization, chemical "cold" sterilization, filtration sterilization, radiation sterilization, or a combination thereof. In one embodiment, a composition or formulation herein is sterilized by lyophilization methods or by non-lyophilization methods.

Further, the present invention provides the use of the pharmaceutical formulation of the invention for the preparation of a medicament for the treatment of neurodegenerative disorders. Such disorders include, but are not limited to Alzheimer's disease, Parkinson's disease, Huntington's disease, cerebral ischemia and neurological damage due to stroke, diabetic polyneuropathy, and amyotrophic lateral sclerosis.

Further, the present invention provides a method of treatment of a neurodegenerative disorder selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, cerebral ischemia and neurological damage due to stroke, diabetic polyneuropathy, and amyotrophic lateral sclerosis, which method comprises administering parenterally to the subject in need thereof the effective amount of the pharmaceutical formulation of the invention.
In the method of the invention, the formulation of the invention can be administered to a subject in need thereof by injection dependent routes, including intramuscular, intravenous, subcutaneous, intrasternal, epidural, and intrathecal injections; infusion techniques; and topical administration.

The term "effective amount" refers to a nontoxic but sufficient amount of the formulation to provide the desired therapeutic effect.

As used herein, the term "treatment" means treating, controlling, preventing and/or reducing one or more clinical signs (i.e., symptoms) of the disease in a subject in need thereof.

The following examples are presented to demonstrate the invention. The examples are illustrative only and are not intended to limit the scope of the invention in any way.

**Example 1.**

This example demonstrates injection formulation comprising succinic acid salt of formula (I).

An aqueous solution was prepared so as to adjust the concentration of the succinic acid salt of formula (I) at 50 mg/ml and the concentration of the succinate buffer solution at 20 mM, whereupon the pH thereof was adjusted at 6.5. Ampoule products of aqueous injection preparation containing the succinic acid salt of formula (I) were prepared by filling each 2 ml of the above-obtained aqueous solution into ampoules of 2 ml capacity and sealing the ampoule. The "proportion of gas space" in the "portion subject to shaking" for this product was about 35% by volume.

**Example 2.**

This example demonstrates enhanced stability of buffered vs. non-buffered injection formulation comprising succinic acid salt of formula (I).

Non-buffered aqueous solution of was prepared to adjust the concentration of the succinic acid salt of formula (I) at 50 mg/ml, whereupon the pH thereof was at 8.2. Ampoule products of aqueous injection preparation were prepared by filling each 2 ml of the above-obtained aqueous solution into ampoules of 2 ml capacity and sealing the ampoule. The "proportion of gas space" in the "portion subject to shaking" for this product was about 35% by volume. To compare stability of the buffered formulation of the Example 1 (n=10) and the non-buffered formulation of
the Example 2 (n=10), both formulations were stored in darkness at 20°C for 6 month. After the storage, ampoules were opened and analyzed on the presence of trimethylamine, the product of decomposition of the succinic acid salt of formula (I). The samples added to a 1:4 solution of 1.0 N NaOH/ethanol and analyzed by gas chromatography using a flame ionization detector. Data are presented in Table 1 as mean ± SEM of trimethylamine levels after the storage.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Trimethylamine, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate salt of formula (I) + buffer</td>
<td>6.5</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>Succinate salt of formula (I)</td>
<td>8.2</td>
<td>954 ± 120</td>
</tr>
</tbody>
</table>

Example 3.

This example demonstrates efficacy of formulations of the invention for the treatment neurodegenerative disorders.

The formulation of the Example 1 was used for the experiment after storage in darkness at 20°C for 6 month.

A cognitive deficit relevant to human Alzheimer's disease was induced by injection of beta-amyloid peptide 25-35 (beta-amyloid) into nucleus basalis magnocellularis (NBM) of rat brains as described by Harkany T et al. in Behav Brain Res. 1998 90(2):133-45. Beta-amyloid was administered bilaterally into NBM of male Wistar rats in dose of 2 µg per each side. On day 16th after the amyloid injection, rats received intramuscularly 25 mg/kg of the succinic acid salt of formula (I) in form of buffered formulation of Example 1 for 7 days singly a day. Control rats received saline intramuscularly. On day next to the last day of the treatment, passive avoidance performance in rats was tested for two consecutive days. A two-compartment, step-through, passive avoidance apparatus consisting of illuminated (25 x 40 x 25 cm) and dark (25 x 40 x 25 cm) compartments attached to an electrified grid floor and separated by a guillotine door (8 x 8 cm) was used.

In the acquisition trial, the rat was placed in the illuminated compartment in a position its tail directed to the closed door for 2 min to habituate to the apparatus. The guillotine door was opened and time to enter to dark compartment was recorded. When the rat entered to dark compartment completely (four foots in dark compartment), the guillotine door was closed and the rat was delivered an
electrical shock of 0.8 mA for 3 sec through the grid floor. After the shock, the rat was immediately placed in home cage. In the retention trial, conducted 24 h after the acquisition trial, the rat was placed in the illuminated compartment and the retention latency to enter into the dark compartment was recorded until 180 s had elapsed. The latency was accepted for 180 s, if the rat did not enter the dark compartment for 180 s. Data are presented in Table 2 as retention latency mean ± SD (n=8).

<table>
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<th>Groups</th>
<th>Latency, s</th>
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<tr>
<td>Control</td>
<td>39 ± 12</td>
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<tr>
<td>Succinic acid salt of formula (I)</td>
<td>98 ± 21*</td>
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</table>

*Differs significantly of control (PO. 0.05).

Thus, intramuscular administration of formulation of Example 1 after long-term storage is effective for the treatment symptoms of neurodegenerative disorders.
What is claimed is:

1. A pharmaceutical formulation in the form of an aqueous solution for injections, infusion techniques, and topical administration comprising succinic acid salt of formula (I)

\[
\begin{align*}
\text{CH}_3 & \quad + \quad \text{N} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{OH} \\
\text{CH}_3 & \quad \text{CH}_2 \quad \text{COO}^- \\
\text{CH}_3 & \quad \text{CH}_2 \quad \text{COO}^-
\end{align*}
\]

and a pharmaceutically acceptable buffer at a concentration effective to maintain the pH of the formulation at between about 4.0 to about 7.5.

2. The formulation as claimed in claim 1, wherein said aqueous solution comprises a buffer selected from the group consisting of phosphate, acetate, citrate, succinate, TRIS, or histidine.

3. Use of the pharmaceutical formulation as claimed in claim 1 for the preparation of a medicament for the treatment of a neurodegenerative disorder selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, cerebral ischemia and neurological damage due to stroke, diabetic polyneuropathy, and amyotrophic lateral sclerosis.

4. A method of treatment of a neurodegenerative disorder selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, cerebral ischemia and neurological damage due to stroke, diabetic polyneuropathy, and amyotrophic lateral sclerosis, which method comprises administering parenterally to the subject in need thereof the effective amount of the pharmaceutical formulation as claimed in claim 1.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the International search (name of database and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS, CHEMABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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Further documents are listed in the continuation of Box C. See patent family annex.

Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the International filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the International filing date but later than the priority date claimed

Date of the actual completion of the International search 10 November 2008

Date of mailing of the international search report 18/11/2008

Name and mailing address of the ISA/European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax (+31-70) 340-3016

Authorized officer Terenzi, Carl a
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<th>Relevant to claim No.</th>
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<td>STOROZHEVA ZINAIDA I ET AL: &quot;Dicholine salt of succinic acid, a neuronal insulin sensitizer, ameliorates cognitive deficits in rodent models of normal aging, chronic cerebral hypoperfusion, and beta-amyloid peptide-(25a 35)-induced amnesia&quot; BMC PHARMACOLOGY, BIOMED CENTRAL, LONDON, GB, vol. 8, no. 1, 23 January 2008 (2008-01-23), page 1, XP02103797 ISSN: 1471-2210 abstract</td>
<td>3,4</td>
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<tr>
<td>Patent document cited in search report</td>
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