Treatment of Traumatic Brain Injury

Field of invention

The present invention relates to the use of 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine for manufacturing a pharmaceutical preparation for the treatment of traumatic brain injury (TBI).

Background of the Invention

EP-A1 0 296 721 disclosed a class of piperidine or 1,2,3,6-terahydropyridine compounds substituted in the 5-position with a five-membered heterocyclic group including a subclass of optionally substituted 5-tetrazolyl-1,2,3,6-tetrahydro-pyridine compounds. The compounds were disclosed to have high affinity to central cholinergic receptors, in particular high affinity for central muscarinic M_1 receptors, thus being useful in the treatment of Alzheimer's disease, senile dementia, and impaired learning and memory functions.

The structure-activity relationship of this subclass was described by Moltzen et al., *J. Med. Chem.* **1994**, *37*, 4085-4099. One of the compounds, *i.e.* 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine has been reported to be selective for muscarinic receptors with a several fold higher affinity for M_1 than for M_2 and M_3 receptors (*Subtypes of Muscarinic Receptors, The Sixth International Symposium*, Nov. 9-12, **1994**, *Fort Lauderdale*). Functionally, it has been described to behave as a partial agonist at M_1 receptors and an antagonist at M_2 and M_3 receptors. Furthermore, the only prominent *in vivo* effect reported was effect on spatial memory acquisition in young and aged rats, respectively.

TBI caused by physical or neurological conditions or various diseases is of increasing importance among the population and there is a great demand for effective and safe drugs for the treatment of such disorders and the sequelae thereof.

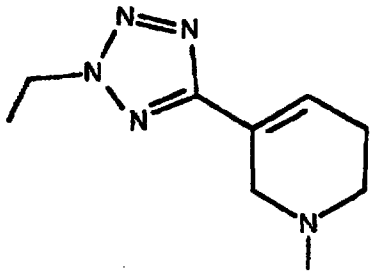
It has now surprisingly been found that the compound 5-(2-ethyl-*2H*-tetrazol-5-yl)-

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1,2,3,6-tetrahydro-1-methylpyridine shows beneficial effects in the treatment of TBI and the sequelae thereof.

Summary of the Invention

Accordingly, the present invention relates to the use of 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine or an acid addition salt thereof



for the manufacture of a pharmaceutical preparation for the treatment of traumatic brain injury or sequelae thereof.

The term traumatic brain injury (TBI) is in this specification meant to include all conditions associated with trauma to the brain or spinal cord e.g. caused by physical forces acting on the scull or spinal column, ischaemia, stroke, arrested breathing, cardiac arrest, cerebral thrombosis or embolism, neurological problems caused by AIDS, cerebral hemorrhage, encephalomyelitis, hydrocephalus, post-operative events, cerebral infections, concussions or elevated intracranial pressure.

The pharmaceutically acceptable acid addition salts of the compounds used in the invention are salts formed with non-toxic organic or inorganic acids. Exemplary of such organic salts are those with maleic, fumaric, benzoic, ascorbic, pamoic, succinic, oxalic, bis-methylenesalicylic, methanesulfonic, ethanedisulfonic, acetic, propionic, tartaric, salicylic, citric, gluconic, lactic, malic, mandelic, cinnamic, citraconic, aspartic, stearic, palmitic, itaconic, glycolic, p-amino-benzoic, glutamic, benzene sulfonic and theophylline acetic acids, as well as the 8-halotheophyllines, for example 8-bromo-theophylline. Exemplary of such inorganic salts are those with hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric and nitric acids.

The compound used according to the invention has now been found to be useful in the treatment of TBI. So, for example it improves cognitive performance following to moderate traumatic brain injury and it attenuates injury-reduced reductions of cholinergic neurones. Furthermore it has been found not to cause adverse cardiac or other side effects in doses believed to be clinically relevant.

In another aspect, the present invention provides a method for the prevention or treatment of TBI in man comprising the step of administering a therapeutically effective amount of 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine or acid addition salt thereof to a patient in need thereof.

The compound used according to the invention and the pharmaceutically acceptable acid addition salts thereof may be administered in any suitable way, e.g. orally or parenterally, and the compounds may be presented in any suitable form for such administration, eg. in the form of tablets, capsules, powders, syrups or solutions or dispersions for injection.

An effective daily dose of the compound according to the invention or a pharmaceutically acceptable salt thereof is from 10 µg/kg to 10 mg/kg body weight, preferably 25 µg/day/kg body weight to 1.0 mg/day/kg body weight. Accordingly, a suitable daily dose is 500 µg to 600 mg/day, preferably 1.0 mg to 100 mg.

The compound used according to the invention may be obtained as described in EP-A1 0 296 721 and the acid addition salts thereof are easily prepared by methods well known in the art.

Pharmacology

The compound used according to the invention was tested by the following well recognized and reliable method:

Cognitive Function following to TBI

Rats were subjected to central fluid percussion traumatic brain injury as described by Dixon, C.E., et al., *J. Neurosurgery*, 67 (1987) 110- 119. The injured animals were treated s.c. daily on days 1-15 postinjury beginning 24 hours after injury with

either saline or 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine, 3.6 µmol/kg or 15 µmol/kg.

Righting reflex suppression after TBI was determined according to the method of Dixon et al. 1987, *supra.*

Body weight was recorded prior to injury and on Days 1-5 postinjury and rotarod performance was determined on Days 1-5 post injury according to the method of Hamm, R.J., *et al., J. Neurotrauma,* 11 (1994) 187-196. The rotarod test was used to measure motor performance following TBI.

Finally, mean latency (+S.E.M.) to find the goal platform in the Morris water maze was measured on days 11-15 postinjury. The results were analysed by an ANOVA. During the water maze testing all animals were injected 10 minutes prior to assessment in the water maze.

Shaminjured animals (animals prepared for injury but not delivered a fluid pulse) were included for comparison.

Qantification of ChAT neurones following to TBI

Rats were subjected to central fluid percussion TBI and treated as described above. They were injected s.c. with saline (n=5) or test compound (5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine, 15 µmol/kg) (n=5) . Shaminjured rats were injected (s.c.) with saline (n=4) or test compound 15 µmol/kg (n=4).

Righting reflex suppression was determined as described above.

Possible loss of cholinergic neurones following to TBI was determined by quantification of choline acethyltransferase (ChAT) immunoreactivity neurones in basal forebrain. On day 15 post-injury (2-4 hours following last injection) all animals were anesthetized with pentobarbital (90 mg/kg, i.p.) and perfused transaortically with 200-250 ml of isotonic saline followed by 500 ml of 4.0% paraformaldehyde/0.2% picric acid in 0.1 M phosphate buffer at a rate of 500 ml/30 min at room temperature. Following perfusion, brains were trimmed into two blocks and postfixed for 24 hours

in the same solution at 10 °C. Coronal 40 µm sections were collected on a vibratome through the forebrain nuclei and every fifth section was processed for ChAT immunoreactivity. Parallel sections were stained for Nissi substance with cresyl violet for quantitative assesment of any possible cholinergic and non-cholinergic neuronal loss in the basal forebrain nuclei.

Free floating forebrain sections were incubated in a final ChAT antibody concentration (1:50) of 1.0 µg/ml in a 0.01 M phosphate buffered saline (PBS) solution containing 0.1% triton X-100. The forebrain sections were trimmed and incubated in the primary antibody for 24 hours at room temperature in culture trays (4 sections/300 µL/well).

After 4 washes in PBS, the sections were incubated in the secondary antibody (horse antimouse IgG; Vector Laboratories, Burlingame, CA) for 1 hour at 37°C. After 3 washes in PBS, the sections were incubated with mouse avidin-biotin-peroxidase complex (ABC) (Vector) technique (Hsu et al *J. Histochem. Cytochem.* **29**, *1981*, 577-580) for 2 hours at 37°C. Following three washes with PBS and one in 0.1M Tris-buffered saline (TBS), free floating sections were processed by the gucose oxidase-diaminobezidine-nickel method described by Shu et al. *Neurosci. Lett.* **85** *1988*, 169-171. The reaction was stopped by transferring the sections into TBS. The sections were mounted on gelatin-chrome alum coated glass slides and allowed to dry overnight at room temperature, dehydrated in ascending concentrations of ethanol and xylene, and then coverslipped with Permount.

The ChAT immunoreactive neurones in the medial septal nucleus (MSN), the vertical limb nucleus of the diagonal band (VDB) and the nucleus basalis magnocellularis (NMB) were counted using a Microcomputer Imaging Device system (MCID) (Imaging Research Inc., Ontario, Canada). The boundary between MSN and VDB was defined as the anterior commisure. The NMB was defined as the imunolabelled neurons located in the globulus pallidus and the adjoining part of the internal capsule. Celle counts were made from each animal obtained at 0.2 mm intervals through the forebrain nuclei. Cell numbers are reported as group means per 10,000 μm^2 for each forebrain nucleus.

Neurons of the MSN, VDB and NMB from parallel sections stained for cresyl violet were also counted on the MCID. Because of the large number of the total cholinergic and non-cholinergic neurons that stain for cresyl violet throughout these regions, a bilatetral sample of beurons was couted in each nucleus. A grid with predetermined areas was used to count cells for each nucleus, For MSN and VDB nuclei, three 2,000 μm^2 regions were counted on each side (thus a total area of 12,000 μm^2 was sampled for each nucleus in each of the four section counted). For the NMB, one 12,000 μm^2 region was counted on each side (total area=24,000 μm^2 in each of the four section counted). Only large cells (>20 µm diameter) with visible somatic Nissi bodies and with a clear neuronal type nucleus were quantified.

Results

There were no significant differences between any of the injured animals on righting reflex suppression after TBI. This indicates that within each experiment the injury groups received an equivalent severity of injury. Sham-injured animals righted significantly faster than any of the TBI animals ($p < 0.0001$ for each comparison). Suppression of righting in the sham-injured group is due to the gas anesthesia used prior to injury.

There was no significant difference between any of the injured groups on loss of body weight following TBI, again indicating an equivalent injury severity between injured groups.

There were no significant differences on rotarod performance on Days 1-5 post injury between any of the injured groups. Because test compound was administered on Days 1-15 postinjury, these tests also indicate that the drug had no effect on body weight or rotarod performance following injury.

ANOVA indicated that the injured animals injected (s.c.) daily with test compound performed better in Morris water maze than injured saline treated animals. Injured animals treated with test compound 15 µmol/kg significantly improved performance with $p < 0.01$.

TBI caused a significant reduction in the number of ChAT-IR neurons in VDB and NMB in rats treated with saline and test compound, respectively. However, the compound of the invention significally attenuated injury reduced reductions in ChAT-IR (32% reduction as compared to injured saline treated group in VDB and 51% in NMB). The parallel cresyl violet stained sections showed no decrease in cell numbers in the MSN, VDB or NMB indicating that the loss in ChAT-IR neurons does not result from cell death.

Formulation Examples

The pharmaceutical formulations of the invention may be prepared by conventional methods in the art.

For example: Tablets may be prepared by mixing the active ingredient with ordinary adjuvants and/or diluents and subsequently compressing the mixture in a conventional tabletting machine. Examples of adjuvants or diluents comprise: corn starch, lactose, talcum, magnesium stearate, gelatine, lactose, gums, and the like. Any other adjuvant or additive colourings, aroma, preservatives etc. may be used provided that they are compatible with the active ingredients.

Solutions for injections may be prepared by solving the active ingredient and possible additives in a part of the vehicle, preferably sterile water, adjusting the solution to the desired volume, sterilization of the solution and filling in suitable ampules or vials. Any suitable additive conventionally used in the art may be added, such as tonicity agents, preservatives, antioxidants, etc.

Typical examples of recipes for the formulations of the invention are as follows (amounts of active ingredient calculated as the free base):

1) Tablets:

5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methyl-pyridine	20 mg
Lactose	60 mg
Maize starch	30 mg
Hydroxypropylcellulose	2.4 mg
Microcrystalline cellulose	19.2 mg

Croscarmellose Sodium Type A	2.4 mg
Magnesium stearate	0.84 mg

2) Tablets:

5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methyl-pyridine	10 mg
Lactose	46.9 mg
Maize starch	23.5 mg
Povidone	1.8 mg
Microcrystalline cellulose	14.4 mg
Croscarmellose Sodium Type A	1.8 mg
Magnesium stearate	0.63 mg

3) Syrup:

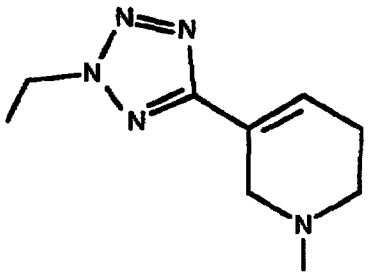
5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methyl pyridine	5.0 mg
Sorbitol	500 mg
Hydroxypropylcellulose	15 mg
Glycerol	50 mg
Methyl-paraben	1 mg
Propyl-paraben	0.1 mg
Ethanol	0.005 ml
Flavour	0.05 mg
Saccharin natrium	0.5 mg
Water	ad 1 ml

4) Solution:

5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methyl-pyridine	1.0 mg
Sorbitol	5.1 mg
Acetic acid	0.08 mg
Water for injection	ad 1 ml

CLAIMS

1. Use of 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine



or a pharmaceutically acceptable acid addition salt thereof for the manufacture of a pharmaceutical preparation for the treatment of traumatic brain injury.

2. Use according to any of claim 1, **characterised in** that the pharmaceutical preparation manufactured comprises 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine or a pharmaceutically acceptable acid addition salt thereof in a unit dosis form.

3. Use according to Claim 1, **characterised in** that the pharmaceutical preparation manufactured comprises 5-(2-ethyl-*2H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine or a pharmaceutically acceptable acid addition salt thereof in an amount of 500 µg to 600 mg/day, preferably 1.0 mg to 100 mg/day.

4. Use according to any of claims 1-3, **characterised in** that the pharmaceutical preparation manufactured is for the treatment of traumatic brain injury caused by physical forces acting on the scull or spinal column, ischaemia, stroke, arrested breathing, cardiac arrest, cerebral thrombosis or embolsm, neurological problem caused by AIDS, cerebral hemorrhage, encephalomyelitis, hydrocephalus, post-operative events, cerebral infections, concussions or elevated intracranial pressure and or for the treatment of the sequelae of such a condition.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/44 — A1

(11) International Publication Number: WO 97/17074

(43) International Publication Date: 15 May 1997 (15.05.97)

(21) International Application Number: PCT/DK96/00458

(22) International Filing Date: 5 November 1996 (05.11.96)

(30) Priority Data:
1232/95 6 November 1995 (06.11.95) DK

(71) Applicant *(for all designated States except US)*: H. LUNDBECK A/S [DK/DK]; 9, Ottiliavej, DK-2500 Copenhagen-Valby (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: PIKE, Brian, R. [US/US]; Division of Neurosurgery, University of Texas Health Center - Houston, Suite 7.148, 6431 Fannin Street, Houston, TX 77030 (US).

(74) Agent: MEIDAHL PETERSEN, John; H. Lundbeck A/S, Patent Dept., 9, Ottiliavej, DK-2500 Copenhagen-Valby (DK).

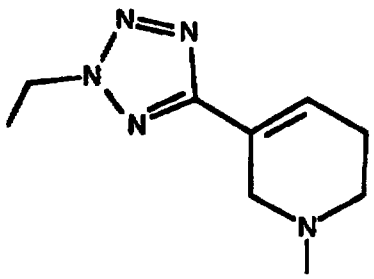
(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: TREATMENT OF TRAUMATIC BRAIN INJURY

(57) Abstract

The compound 5-(2-ethyl-2*H*-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine of formula (I) improves cognitive performance and attenuates injury-reduced reductions of cholinergic neurones in traumatic brain injury models and is useful for the manufacture of a pharmaceutical preparation for the treatment of traumatic brain injury.



(I)