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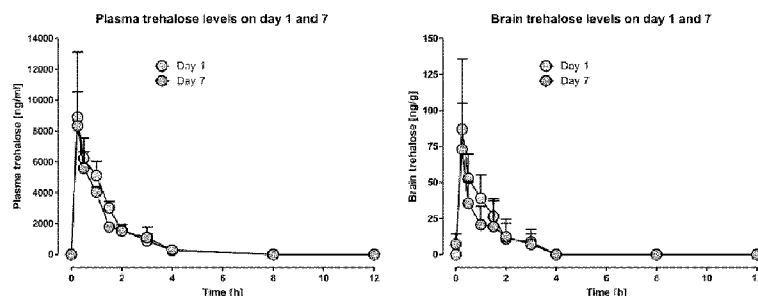


FIGURE 1

(57) Abstract: The present disclosure discloses trehalose for use in treatment of neurological disorders, wherein the trehalose is for a single daily administration with the daily dose between about 0.25 to about 12.5 g/kg/day. The daily dose may be about 2.67 g/kg/day. A method for treating a neurological disorders is disclosed which involves administering trehalose to a subject as a single daily administration with a daily dose between about 0.25 to about 12.5 g/kg/day and in an embodiment of this method the daily dose is about 2.67 g/kg/day. Also disclosed herein is the use of trehalose in the manufacture of a medicament for treatment of neurological disorders, wherein the trehalose is formulated as a single daily dose with the trehalose present in the medicament in an amount of between about 0.25 to about 12.5 g/kg/day. In an embodiment the daily dose is about 2.67 g/kg/day. The present disclosure provides a pharmaceutical composition for treating neurological disorders, comprising a daily dose of trehalose, and a pharmaceutically acceptable carrier wherein the daily dose of the trehalose is between about 0.25 to about 12.5 g/kg. The trehalose may be formulated as part of a foodstuff.

USE OF TREHALOSE FOR TREATMENT OF NEUROLOGICAL DISEASES

FIELD

5 The present disclosure relates to the use of trehalose for the treatment of neurological disorders, including but not limited to Parkinson's disease (PD).

BACKGROUND

10 Autophagy is a natural, conserved, process that allows the orderly degradation of cytoplasmic contents. There are three pathways of autophagy, macroautophagy, microautophagy and chaperone-mediated autophagy, of which macroautophagy is the main pathway. Autophagy plays several roles in cellular functioning including the breakdown and recycling of proteins, the degradation of infectious particles and the removal of damaged organelles, cell
15 membranes and proteins. In certain diseases, called proteinopathies, accumulation of structurally abnormal proteins disrupts normal cellular function. There is a wide range of proteinopathies including many neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis. Strategies aimed at limiting the accumulation of the abnormal
20 proteins, such as enhancing the removal of abnormal proteins, are being investigated as potential therapies for proteinopathies including neurodegenerative disorders. For reviews on autophagy and neurodegenerative disorders see Rubinsztein *et al*, J Exp Med., 2015, **212**, pp. 979-990; Kiriya and Nochi, Int J Mol Sci., 2015, **16**, pp. 26797-26812.

25 Trehalose is a disaccharide that represents one such strategy to reduce the accumulation of abnormal proteins. The exact mechanism of action of trehalose is not known, however, it possesses several properties that may be

useful in preventing neurodegeneration including stabilizing proteins, acting as a chemical chaperone for misfolded proteins and improving the clearance of abnormal proteins (for a review see Emanuele, *Curr Drug Targets*, 2014, **15**, pp 551-557). Trehalose reduces levels of α -synuclein (α SYN), the protein which is

5 misfolded in PD and drives the pathophysiology of the disease (for review see Wang and Hay, *Front Neurosci.*, 2015, **9**, pp 1-8), in rodent models of PD (Tanji *et al.*, *Biochem Biophys Res Commun.*, 2015, **465**, pp746-752; He *et al.*, *Mol Neurobiol.*, 2015, DOI 10.1007/s12035-015-9173-7; Wu *et al.*, *Neuroscience*, 2015, **284**, pp 900-911).

10 There are several published papers showing the benefit of trehalose in animal models of PD when the trehalose is dissolved in the animal drinking water such that it is constantly available to the animals. These are summarized in **Table 1** below.

Model	Trehalose administered	Effect	Reference
MPTP-lesioned mouse	2% trehalose in drinking water	Neuroprotection observed	Sarkar <i>et al.</i> , <i>Neurotoxicology</i> , 2014, 44 , 250-262.
Rotenone-lesioned rat	2% trehalose in drinking water	Neuroprotection observed	Wu <i>et al.</i> , <i>Neuroscience</i> , 2015, 284 , pp 900-911
AAV α -synuclein rat	2 and 5% in drinking water	Neuroprotection observed	He <i>et al.</i> , <i>Mol Neurobiol.</i> , 2015, DOI 10.1007/s12035-015-9173-7.
Chronic MPTP-lesioned mouse	1% in the drinking water	No behavioural effect. Some effect on dopamine levels	Ferguson <i>et al.</i> , <i>Behav Brain Res.</i> , 2015, 292 , pp 68-78.
Transgenic α -synuclein mouse	2% trehalose in drinking water	Increased autophagy	Tanji <i>et al.</i> , <i>Biochem Biophys Res Commun.</i> , 2015, 465 , pp746-752.

Table 1

These studies demonstrate the potential of trehalose as a treatment for PD. However, the most efficacious method of administering trehalose is unknown and uninvestigated with trehalose administered in the drinking water in all these studies. Similarly, whilst there are several papers in the literature
5 showing a positive effect of trehalose in animal models of other neurodegenerative proteinopathies (e.g. Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease) trehalose was administered in the drinking water.

Canadian Patent No. CA2608198 A1 (Lindquist *et al.*, 2006) discloses a
10 method of inhibiting α -synuclein-mediated cellular toxicity by contacting a cell expressing a toxicity-inducing amount or form of α -synuclein with an effective amount of an osmolyte, in which the osmolyte is trehalose.

As noted above, in all the previous work performed in animal models of PD, trehalose has been administered to animals as a solution in their drinking
15 water. Furthermore, no fully quantitative methods have been used to measure trehalose levels in either the plasma or brain in these studies.

Determining the best dosage regime and methodology for delivery of the trehalose to give the most efficacious treatment of neurological disorders would be very beneficial. With a rapidly aging population, and with the cost of health
20 care treatment increasing rapidly as a result, finding an economical and simple method of treating a wide variety of neurological disorders, including PD, would be very advantageous.

SUMMARY

The present disclosure provides a pharmaceutical kit comprising:

trehalose for treatment of neurological disorders, and

instructions for a single daily administration of the trehalose with

5 the daily dose being between about 0.25 to about 12.5 g/kg/day.

A preferred range of trehalose is between about 0.5 to about

10 g/kg/day. A more preferred range of trehalose is between about 0.75 to

about 7.5 g/kg/day. A more preferred amount is about 2.67 g/kg/day.

Disclosed herein is trehalose for use in the treatment of neurological

10 disorders, wherein the trehalose is administered as a single daily administration

with the daily dose between about 0.25 to about 12.5 g/kg/day. In an

embodiment the daily dose is about 2.67 g/kg/day.

Disclosed herein is a method for treating neurological disorders,
comprising:

15 administering trehalose to a subject as a single daily administration with

a daily dose between about 0.25 to about 12.5 g/kg/day. In an embodiment of

this method the daily dose is about 2.67 g/kg/day.

Also disclosed herein is the use of trehalose in the manufacture of a

medicament for treatment of neurological disorders, wherein the trehalose is

20 formulated as a single daily dose with the trehalose present in the medicament

in an amount of between about 0.25 to about 12.5 g/kg/day. In an embodiment

the daily dose is about 2.67 g/kg/day.

The present disclosure provides a pharmaceutical composition for

treating neurological disorders, comprising a daily dose of trehalose, and a

25 pharmaceutically acceptable carrier wherein the daily dose of the trehalose is

between about 0.25 to about 12.5 g/kg. In an embodiment the daily dose is about 2.67 g/kg/day.

The present disclosure provides a “foodstuff”, “food supplement”, “beverage” or “beverage supplement” composition, where “foodstuff”, “food supplement”, “beverage” or “beverage supplement” have normal meanings for those terms and are not restricted to pharmaceutical preparations, for treating neurological disorders, comprising a daily dose of trehalose, and a dietary acceptable carrier wherein the daily dose of the trehalose is between about 0.25 to about 12.5 g/kg. In an embodiment of the daily dose is about 2.67 g/kg/day. Examples of “foodstuffs”, “food supplements”, “beverages” or “beverage supplements” include, but are not limited to, processed foods, ingredients added to prepared foodstuffs and beverages (e.g. cooking ingredients, sweeteners), energy bars, baked goods, protein-shakes, soft-drinks and alcoholic drinks.

The present disclosure provides a “Medical food” as defined in the Food and Drug Administration's 1988 Orphan Drug Act Amendments for treating neurological disorders, comprising a daily dose of trehalose, and a dietary acceptable carrier wherein the daily dose of the trehalose is between about 0.25 to about 12.5 g/kg. In an embodiment of the daily dose is about 2.67 g/kg/day. Medical foods are foods that are specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone.

Medical foods are distinct from the broader category of foods for special dietary use and from traditional foods that bear a health claim. In order to be considered a medical food the product must, at a minimum (i) be a food for oral

ingestion or tube feeding (nasogastric tube), (ii) be labeled for the dietary management of a specific medical disorder, disease or condition for which there are distinctive nutritional requirements, and (iii) be intended to be used under medical supervision.

5 The neurological disorders are any one or combination of PD, synucleinopathies and proteinopathies. The synucleinopathies are any one of PD with dementia, dementia with Lewy bodies, MSA, essential tremor, Gaucher disease and other lysosomal storage disorders, and neurodegeneration with brain iron accumulation. The proteinopathies include Alzheimer's disease, cerebral β -amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, 10 prion diseases, tauopathies, frontotemporal lobar degeneration, FTL D-FUS, amyotrophic lateral sclerosis (ALS), Huntington's disease and other triplet repeat disorders, familial British dementia, familial Danish dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL, Alexander disease, 15 seipinopathies, familial amyloidotic neuropathy, serpinopathies and retinitis pigmentosa with rhodopsin mutations.

 Also disclosed herein is the use of trehalose in the manufacture of a medicament for treatment of neurological disorders, wherein the trehalose is formulated as a single daily dose with the trehalose present in the medicament 20 in an amount of between about 0.25 to about 12.5 g/kg/day.

 The present disclosure provides a pharmaceutical composition for treating neurological disorders, comprising a daily dose of trehalose, and a pharmaceutically acceptable carrier wherein the daily dose of the trehalose is between about 0.25 to about 12.5 g/kg.

A further understanding of the functional and advantageous aspects of the invention can be realized by reference to the following detailed description.

BRIEF DESCRIPTION OF DRAWINGS

5 The following is a description of the use of trehalose for treatment of neurological diseases, reference being had to the accompanying drawings, in which:

Figure 1 shows the timecourse of trehalose exposure in the plasma and brain of rats following 1 and 7 days oral administration of trehalose (2.67
10 g/kg/day, p.o. administered as a single bolus dose).

Figure 2 shows the timecourse of trehalose exposure in the plasma of macaques following 1 and 7 days oral administration of trehalose (2.67 g/kg/day and 5.34 g/kg/day, p.o. administered as a single bolus dose).

Figure 3 shows the comparative timecourse of trehalose exposure in the
15 plasma of rats and macaques following 1 and 7 days oral administration of trehalose (2.67 g/kg/day, p.o. administered as a single bolus dose).

Figure 4 shows the correlation between trehalose levels in the CSF and brain of macaques following 17 weeks of oral administration of trehalose (2.67 g/kg/day, p.o. administered as a single bolus dose).

20

DETAILED DESCRIPTION

 Various embodiments and aspects of the disclosure will be described with reference to details discussed below. The following description and drawings are illustrative of the disclosure and are not to be construed as limiting
25 the disclosure. Numerous specific details are described to provide a thorough

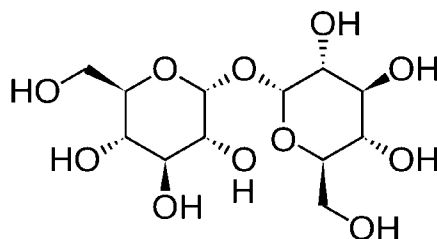
understanding of various embodiments of the present disclosure. However, in certain instances, well-known or conventional details are not described in order to provide a concise discussion of embodiments of the present disclosure.

As used herein, the terms “comprises” and “comprising” are to be construed as being inclusive and open ended, and not exclusive. Specifically, when used in the specification and claims, the terms “comprises” and “comprising” and variations thereof mean the specified features, steps or components are included. These terms are not to be interpreted to exclude the presence of other features, steps or components.

As used herein, the term “exemplary” means “serving as an example, instance, or illustration,” and should not be construed as preferred or advantageous over other configurations disclosed herein.

As used herein, the terms “about” and “approximately” are meant to cover variations that may exist in the upper and lower limits of the ranges of values, such as variations in properties, parameters, and dosage ranges to give a few examples.

As use herein, the word “trehalose” refers to the molecule shown in **Formula 1** below.



FORMULA 1

Trehalose is also known by other names including “ α,α -trehalose”;

“ α -D-glucopyranosyl-(1 $\rightarrow$ 1)- α -D-glucopyranoside”. Its IUPAC name is “(2R,3S,4S,5R,6R)-2-(Hydroxymethyl)-6-[(2R,3R,4S,5S,6R)-3,4, 5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxane-3,4,5-triol”. The CAS no. of trehalose is 99-20-7 (anhydrous) 6138-23-4 (dehydrate).

5 The present disclosure will be illustrated using the following non-limiting examples/studies.

STUDIES

10 In the following examples these abbreviations are used: h = hours; min = minutes; p.o. = *per oro* (by mouth); t.i.d. = *ter in die* (3 times daily); s.e. = standard error; EV = empty vector; α SYN = alpha synuclein; TH^{+ve} = tyrosine hydroxylase positive; w/v = weight/volume; AP = anterior/posterior; DV = dorsal/ventral; AAV1/2 = adeno-associated virus 1/2; LC-MS/MS = liquid chromatography tandem mass spectrometry; BLQ = below limit of
15 quantification; C_{max} = The peak concentration of a drug after administration; T_{max} = time to reach maximum concentration; AUC = area under the curve; t_{1/2} = elimination half-life; CSF = cerebrospinal fluid.

20 Example 1

 The following example shows that trehalose is more efficacious at reducing parkinsonian symptoms in a rodent model of PD when trehalose is administered as a single, oral administration (2.67 g/kg/day) compared to when the same amount of trehalose is administered as three separate doses

administered 8 h apart (0.89 g/kg/t.i.d.) or provided *ad libitum* in the drinking water (2% trehalose w/v in sterile water).

Thirty female, Sprague-Dawley rats (280-325 g) split into 5 groups (N=4-8 rats/group) and received a unilateral injection in to the substantia nigra (at coordinates AP -5.2 mm; ML -2 mm relative to Bregma, DV -7.5 mm relative to skull over SN) of AAV1/2 delivering A53T alpha-synuclein (AAV1/2 α SYN) or empty vector (control). The A53T aSyn expressed is of the human sequence. Its control is an empty AAV1/2 vector of the same serotype and viral construction. Both AAV1/2 vectors were diluted in sterile phosphate buffered saline and administered at a volume of 2 μ l. The concentration of AAV1/2 used was 1.7×10^{12} genomic particles/ml, which produces significant behavioural and dopaminergic nigrostriatal deficits between 3 and 6 weeks following surgical delivery (Koprach *et al.*, PLoS One, 2011, DOI 10.1371/journal.pone.0017698).

Commencing on the day of surgery and continuing for 6 weeks, rats received either vehicle (sterile drinking water) or trehalose (2.67 g/kg/day) administered either in the drinking water (2% w/v), as three separate doses administered 8 h apart (0.89 g/kg/t.i.d., p.o.) or as a single administration (2.67 g/kg/day, p.o).

Animal behavior was assessed pre-surgery and at 3 and 6 weeks post-surgery. Behaviour was assessed by the cylinder test to assess forelimb asymmetry with asymmetry indicating an imbalance in striatal dopaminergic function between the side injected with AAV1/2 α SYN and the contralateral side. Compounds that normalize the forelimb asymmetry are potentially useful for treating PD. After 6 weeks of trehalose treatment, the animals were killed

approximately 30 mins after the last administration of trehalose. Striatal tissue from both hemispheres was collected and analysed for dopamine levels. Substantia nigra tissue was collected and analysed for the number of TH^{+ve} cells and the amount of A53T α SYN measured. Plasma and brain (cerebellum) samples were collected and trehalose levels analysed by LC-MS/MS.

Rats receiving A53T α SYN exhibited an increased asymmetry, and indication of parkinsonism, as measured by the cylinder test on both Day 21 and Day 42 compared to rats receiving EV (**Table 2**). Administration of trehalose as a single daily oral administration (2.67 g/kg/day, p.o) for 21 or 42 days reduced the asymmetry compared to mice receiving A53T α SYN alone to a level similar to rats receiving EV (**Table 2**). When the same daily dose of trehalose (2.67 g/kg/day) was administered as 3 doses, 8 h apart (0.89 g/kg/t.i.d., p.o) or provided *ad libitum* as a 2% w/v solution in the drinking water, there was no significant change in the level of asymmetry on either day 21 or day 42 (**Table 2**).

Table 2 Effect of A53T α SYN and trehalose on forelimb asymmetry in rats

Group	% asymmetry (mean $\pm$ s.e.mean)	
	Day 21	Day 42
Empty vector control	7.7 $\pm$ 9.4	14.9 $\pm$ 7.4
A53T α SYN	51.0 $\pm$ 14.8*	52.4 $\pm$ 20.6
A53T α SYN + 2% trehalose in drinking water	48.1 $\pm$ 7.0*	51.9 $\pm$ 11.9
A53T α SYN + trehalose (0.89 g/kg, 3 times per day)	38.9 $\pm$ 16.3	67.9 $\pm$ 11.1*
A53T α SYN + trehalose (2.67 g/kg, once per day)	12.3 $\pm$ 16.9	3.8 $\pm$ 14.5 [#]

Mean $\pm$ s.e.mean. *= P <0.05 vs. EV control, #= P <0.05 vs. A53T α SYN. One-way ANOVA followed by Fisher's LSD *post-hoc* test.

Rats receiving A53T α SYN also exhibited a significantly lower striatal dopamine level compared to rats receiving EV (**Table 3**). Administration of trehalose as a single daily oral administration (2.67 g/kg/day) partially, but significantly, restored striatal dopamine levels (**Table 3**). Administration of the

same daily dose of trehalose (2.67 g/kg/day) as either 3 doses, 8 h apart (0.89 g/kg/day, t.i.d., p.o) or provided *ad libitum* as a 2% w/v solution in the drinking water, did not significantly alter striatal dopamine levels (**Table 3**).

5 **Table 3 Effect of A53T α SYN and trehalose on striatal dopamine levels in rats**

Group	Dopamine (ng/mg protein)
Empty vector control	123.8 $\pm$ 6.8
A53T Asyn	42.4 $\pm$ 5.8*
A53T α SYN + 2% trehalose in drinking water	60.2 $\pm$ 11.5*
A53T α SYN + trehalose (0.89 g/kg, 3 times per day)	47.3 $\pm$ 7.5*
A53T α SYN + trehalose (2.67 g/kg, once per day)	65.4 $\pm$ 7.5*#

Mean $\pm$ s.e.mean. *= P <0.05 vs. EV control, #= P <0.05 vs. A53T α SYN. One-way ANOVA followed by Fisher's LSD *post-hoc* test.

10 As expected the amount of A53T α SYN per TH^{+ve} neuron increased in rats receiving A53T α SYN compared to rats receiving EV (**Table 4**). Administration of trehalose as a single daily oral administration (2.67 g/kg/day, p.o) reduced the amount of A53T α SYN per TH^{+ve} neuron compared to rats receiving A53T α SYN alone (**Table 4**). Administration of the same daily dose of trehalose provided *ad libitum* as a 2% w/v solution in the drinking water, did not significantly alter the amount of A53T α SYN per TH^{+ve} neuron compared to rats receiving A53T α SYN alone (**Table 4**).

20 **Table 4 Effect of A53T α SYN and trehalose on A53T α SYN expression per TH neuron in rats**

Group	aSYN/TH ratio
Empty vector control	0.00000 $\pm$ 0.00000
A53T α SYN	0.00027 $\pm$ 0.00010*
A53T α SYN + 2% trehalose in drinking water	0.00023 $\pm$ 0.00007*
A53T α SYN + trehalose (2.67 g/kg, once per day)	0.00016 $\pm$ 0.00008

Mean $\pm$ s.e.mean. *= P <0.05 vs. EV control. One-way ANOVA followed by Fisher's LSD *post-hoc* test.

Together, these data demonstrate that administering trehalose as a single bolus dose is more efficacious at removing α SYN, maintaining striatal

dopamine levels and reducing behavioural impairments compared to the same dose of trehalose administered as 3 separate doses 8 h apart or administered over a 24 h period in the drinking water.

When the animals were killed 30 mins post trehalose administration plasma and brain samples were collected (rats in the 0.89 g/kg/day group only received one dose of trehalose (0.89 g/kg) on this day). This time corresponds to approximately the T_{max} of orally administered trehalose. Trehalose plasma levels were below the limit of quantification (BLQ) in rats receiving trehalose in the drinking water (**Table 5**). Trehalose was measurable in the plasma 30 mins after receiving trehalose (0.89 or 2.67 g/kg) by oral gavage (**Table 5**). A 3-fold increase in the trehalose dose (0.89 g/kg to 2.67 g/kg) lead to an ~6-fold increase in the trehalose plasma level, i.e. that increasing the dose produced a greater than dose-proportional increase in trehalose exposure. Trehalose levels were below the limit of detection in the brain of rats receiving trehalose either in the drinking water or receiving trehalose (0.89 g/kg) by oral gavage (**Table 5**). Trehalose was measurable in the brains of rats receiving trehalose (2.67 g/kg) by oral gavage (**Table 5**).

Table 5 Plasma and brain trehalose levels after trehalose was administered in drinking water of via oral gavage

Group	Trehalose administered	Plasma trehalose level (ng/ml)	Brain trehalose level (ng/g)
1	2% in drinking water	BLQ	BLQ
2	0.89 g/kg 3 times daily	1131 ± 178 ng/ml	BLQ
3	2.67 g/kg once daily	6383 ± 890 ng/ml	52.8 ± 16.9 ng/ml

Mean ± s.e.mean. BLQ, below level of quantification

These data demonstrate that increasing the dose of trehalose produces a greater than dose-proportional increase in systemic exposure. This increased

systemic exposure also allows detectable levels of trehalose to occur in the brain. The brain is the target organ of trehalose as a treatment for PD and these results provide an explanation as to why a single bolus administration of trehalose is more efficacious compared to the same dose of trehalose administered as three separate doses 8 h apart or administered over a 24 h period in the drinking water (**Tables 2-4**).

Example 2

The following example shows the plasma and brain pharmacokinetics of trehalose on day 1 and day 7 in Sprague Dawley rats following oral administration of trehalose (2.67 g/kg/day) for 7 days. It shows that the plasma and brain samples taken for bioanalysis in Example 1 (30 minutes post-dose) was close to the T_{max} . It also shows that plasma levels and brain levels of trehalose quickly drop and are no longer detectable 8 h post-dose. These results thus support and expand upon the bioanalytical data presented in **Example 1**.

One hundred Sprague-Dawley rats (200-260 g), female (60) and male (40), received trehalose (2.67 g/kg/day, oral gavage in sterile water) for 1 or 7 days. Groups of 5 rats (3 female, 2 male) were sacrificed at pre-dose and 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 8 h and 12 h post-dose on days 1 and 7 and plasma and brain samples collected. Plasma and brain samples were analysed for trehalose levels using a validated LC-MS/MS method and plasma exposure timecourses (**Figure 1**) and pharmacokinetic parameters were calculated (**Table 6**). Brain levels of trehalose were approximately 1% of plasma levels at all time-points (**Figure 1**).

Table 6 Plasma and brain pharmacokinetics of trehalose following 1 and 7 days oral administration of trehalose (2.67 g/kg)

PK parameter	Plasma		Brain	
	Day 1	Day 7	Day 1	Day 7
T _{max} (h)	0.25	0.25	0.25	0.25
C _{max} (ng/ml)	8900	8336	87.0	73.0
AUC _{0-12 h} (h.ng/ml)	10851	9494	86.8	65.3
AUC _{0-inf} (h.ng/ml)	11136	9876	97.0	76.2
t _{1/2} (h)	0.76	0.89	0.81	1.06

Example 3

5 The following example shows the plasma and brain pharmacokinetics of trehalose on day 1 and day 7 in female macaques following oral administration of trehalose (2.67 and 5.34 g/kg/day) for 7 days. It shows that in macaques, as in rats, increasing the dose of trehalose leads to a greater than dose-proportional increase in plasma trehalose level. As this occurs in two

10 mammalian species, it is likely that it will also occur in a third mammalian species (humans). Therefore, in humans, administration of trehalose as a single daily oral dose is likely to produce higher systemic exposure and brain levels of trehalose than the same daily dose administered as multiple doses over 24 h and thus provide greater therapeutic benefit.

15 Three female macaques (3.81-4.67 kg) received trehalose (2.67 and 5.34 g/kg/day, oral gavage in sterile water) for 7 days. Plasma samples were collected pre-dose and 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 12 h and 24 h post-dose on days 1 and 7 and analysed for trehalose levels using a validated LC-MS/MS method and plasma exposure time courses (**Figure 2**) and

20 pharmacokinetic parameters were calculated (**Table 7**).

Table 7 Plasma pharmacokinetics of trehalose following 1 and 7 days oral administration of trehalose (2.67 and 5.34 g/kg)

PK parameter	2.67 g/kg/day		5.34 g/kg/day	
	Day 1	Day 7	Day 1	Day 7
T _{max} (h)	0.83	0.67	0.67	0.83

C _{max} (ng/ml)	10918	9578	36962	22444
AUC _{0-24 h} (h.ng/ml)	26657	26023	78899	56483
AUC _{0-inf} (h.ng/ml)	27445	27363	80040	57172
t _{1/2} (h)	1.1	1.5	1.8	2.3

In a subsequent step, the same 3 macaques were administered trehalose (2.67 g/kg) for two days. The macaques were then killed 1 h post-administration on day 2 and terminal brain and CSF samples collected. The brain and CSF trehalose levels were 81.3 ± 13.8 ng/g and 562 ± 346.5 ng/ml respectively. Similar to rats, trehalose levels in macaque brain tissue were ~1% of the plasma level at the corresponding timepoint.

Example 4

The following example shows that trehalose can reduce dysfunction of the dopaminergic system in a non-human primate model of PD when trehalose is administered as a single, oral administration (2.67 g/kg/day). Moreover, the dose of trehalose used provided exposure levels similar to the exposure that was demonstrated to be efficacious in a rodent model of PD (see **Examples 1 and 3**).

Twenty-five (25), female cynomolgus monkeys (*Macaca fascicularis*, 8.0 – 9.3 years of age, 3.2–4.4 kg) were split into 3 groups (n=8-9/ group) and received stereotaxic injection of AAV vectors, either a vector expressing mutant A53T human alpha-synuclein (AAV1/2-A53T-alpha-synuclein) or an empty vector (AAV1/2-EV), Genedetect, Auckland, New Zealand) into the substantia nigra. The A53T aSyn expressed is of the human sequence the AAV1/2-EV is a control vector of the same serotype and viral construction. Precise stereotaxic coordinates for all surgeries were calculated prior to surgery from individual

monkey MRI scans. Injections containing the AAV1/2-A53T-alpha-synuclein or empty vector were made at a speed of 0.5 µl/min and a volume of 7 µl (at a viral titre of 1.7×10^{12} active particles per ml) into 4 sites of each hemisphere of the substantia nigra.

- 5 Commencing on the day of surgery and continuing for 17 weeks, macaques received either vehicle (sterile drinking water) or trehalose (2.67 g/kg/day) administered as a single daily administration (2.67 g/kg/day, p.o). The macaques were then killed and striatal tissue from both hemispheres was collected and analysed for dopamine and dopamine transporter (DAT) levels.
- 10 Substantia nigra tissue was collected and analysed for the number of TH⁺ cells and the amount of αSYN measured. Plasma, CSF and brain (cerebellum) samples were collected and trehalose levels analysed by LC-MS/MS. Macaques receiving A53T exhibited a significantly lower striatal dopamine and DAT levels compared to macaques receiving EV (**Table 8**). Administration of
- 15 trehalose as a single daily oral administration (2.67 g/kg/day) partially, but significantly, restored striatal dopamine levels (**Table 8**). Similarly, trehalose partially restored DAT levels (**Table 8**).

Table 8 Effect of A53T αSYN and trehalose on striatal dopamine and DAT levels in macaques

Group	Striatal dopamine (ng/mg protein)	Striatal DAT (nCi/mg tissue)
Empty vector control	160.0 ± 7.2	493.7 ± 52.5
A53T αSYN	78.9 ± 13.1***	273.7 ± 35.7**
A53T αSYN + trehalose (2.67 g/kg/day)	110.0 ± 8.3** #	410.7 ± 47.9

Mean ± s.e.mean. ** / *** = P<0.01 or P<0.001 cf. EV / vehicle. # = P<0.05 cf. αSyn / vehicle, 1-way ANOVA with Holm-Sidak multiple comparisons test.

Macaques receiving A53T also exhibited fewer TH^{+ve} neurons in the substantia nigra and an increased expression of α SYN in the striatum compared to macaques receiving EV (**Table 9**). Administration of trehalose as a single daily oral administration (2.67 g/kg/day, p.o) only slightly prevented the loss of TH^{+ve} neurons and increase in α SYN (**Table 9**).

Table 9 Effect of A53T α SYN and trehalose on TH^{+ve} neurons in the substantia nigra and α SYN expression in the striatum levels in macaques

Group	No. of TH+ve neurons	Striatal α SYN (ng/mg protein)
Empty vector control	95678 $\pm$ 7799	36394 $\pm$ 2362
A53T α SYN	59513 $\pm$ 7720*	55455 $\pm$ 5120*
A53T α SYN + trehalose (2.67 g/kg/day)	69084 $\pm$ 7628*	54604 $\pm$ 4486*

Mean $\pm$ s.e.mean. * = P<0.01 cf. EV / vehicle. 1-way ANOVA with Holm-Sidak multiple comparisons test.

10

Together, these data demonstrate that trehalose can partially prevent α SYN-mediated dopaminergic dysfunction though this effect is more pronounced at the nerve terminals than at the cell bodies, as demonstrated by a larger effect on dopamine and DAT compared to the effect on the number of TH^{+ve} neurons.

15

Single plasma, brain and CSF samples were collected 1 h after the final administration of trehalose. One-hour post-dose corresponds to approximately the T_{max} of orally administered trehalose in macaques. The plasma, brain and CSF trehalose levels were 2449 $\pm$ 884 ng/ml, 129 $\pm$ 35 ng/g and 33 $\pm$ 9 ng/ml respectively. The trehalose level in the CSF significantly correlated with the level in the brain demonstrating that trehalose levels in the CSF can be used to predict trehalose levels in the brain (**Figure 4**).

20

Results

The results for the behavioural assessment in rats on days 21 and 42 are shown in **Table 2**. The α SYN (PD model) group showed an increased asymmetry compared to the empty vector (control group). The degree of asymmetry is indicative of an imbalance in striatal dopaminergic function between the side the viral vector that produces α SYN was administered and the contralateral side. Compounds that normalize the forelimb asymmetry are potentially useful for treating PD. Treatment with trehalose as a single, oral administration (2.67g/kg/day as a single dose group) reduced the degree of asymmetry compared to mice receiving A53T α SYN alone, indicating that trehalose given as a single daily administration was normalising behaviour. By contrast, the same daily dose of trehalose administered either as 2% in the drinking water or when given as three (3) separate doses, 8 h apart did not produce a beneficial effect on behaviour.

Dopamine is the neurotransmitter that is decreased both in PD and in animal models of PD. Increasing striatal dopamine levels is a treatment for PD and also reverses the parkinsonism seen in this rat model of PD. Therefore, if trehalose (single administration) increases dopamine levels it provides a rationale for how it is improving behaviour.

As can be seen from **Table 3**, when trehalose is given as a once daily, single administration it significantly increases dopamine levels compared to animals receiving α SYN alone. Interestingly, when the same total dose of trehalose is administered either in the drinking water or divided into three (3) times daily administrations, the increase in dopamine levels is smaller and no longer significantly different from α SYN alone.

These results support the behavioural data; trehalose given once a day increases dopamine levels as well as reducing the degree of asymmetry. In addition, they provide a rationale why trehalose administered in other ways (2% in the drinking water or three (3) times daily) did not improve behaviour (they
5 did not increase dopamine levels).

Referring to **Table 4**, when the amount of α SYN per TH^{+ve} neuron was measured there was a clear increase in the α -synuclein per neuron in the PD model group compared to the empty vector control group. Treatment with trehalose once daily reduced the amount of α -synuclein per neuron by ~40%.
10 Trehalose in the drinking water produced only a small reduction in the amount of α -synuclein per neuron.

This result supports the hypothesis that trehalose reduces the amount of α -synuclein. It also further supports the idea that administering all the trehalose in one dose is more efficacious than administering the same amount of
15 trehalose over a 24 hour period.

Pharmacokinetics of trehalose in rats

To better understand why administering the same amount of trehalose by different routes led to differences in efficacy, studies were conducted to measure blood and brain levels of trehalose following administration of
20 trehalose by 2% in the drinking water, three (3) times daily (0.89 g/kg each dose) and once daily (2.67 g/kg/day). Plasma samples were collected at 30 min post dose (around the T_{max}) so that rats either received whatever trehalose they had drunk in the water in the previous 24 h, 0.89 g/kg or 2.67 g/kg. The results are shown in **Table 5**.

There are several observations of note in the results, which are listed below.

1. That trehalose in the drinking water did not produce any measurable trehalose levels in the plasma and brain. Although we do not know when the rats last drank relative to when the blood was collected it provides an explanation of why trehalose administered in the drinking water was not effective.
2. That trehalose (0.89 g/kg 3x/day) produced measurable levels of trehalose in the plasma but did not produce measurable brain trehalose levels.
3. That trehalose (2.67 g/kg 1x/day) produced measurable levels of trehalose in the plasma and the brain.
4. That the peak plasma trehalose level following 2.67 g/kg was 5.6-fold higher than the plasma trehalose level following 0.89 g/kg. That is, a 3-fold increase in dose led to a 5.6-fold increase in systemic trehalose exposure.

Full pharmacokinetic study in rats

A full pharmacokinetic study was performed in rats following 1 and 7 days administration of trehalose (2.67 g/kg/day given as a single administration) and the results are summarized in **Figure 1** and **Table 6**.

As can be seen, trehalose is rapidly absorbed into, and rapidly eliminated from, the plasma following oral administration. No trehalose is found in the plasma or brain 8 h post-dose. This is important as in the efficacy study the group receiving 3 administrations of trehalose were given 8 h apart and so plasma trehalose levels had declined to below the limit of detection between

each subsequent trehalose administration. Brain trehalose levels represent approximately 1% of plasma levels and brain levels follow a very similar time-course to plasma levels.

Pharmacokinetics of trehalose in non-human primates

5 A full pharmacokinetic study was performed in macaques following 1 and 7 days administration of trehalose (2.67 or 5.34 g/kg/day given as a single administration) and the results are summarized in **Figure 2** and **Table 7**.

 Similar to rats, trehalose is rapidly absorbed into, and rapidly eliminated from, the plasma following oral administration to macaques. No trehalose was
10 measurable in the plasma ~8 h post-dose. Also similar is that a doubling of the dose of trehalose led to an approximately 3-fold increase in plasma trehalose levels (as measured by C_{max} or AUC) – again showing that increasing the dose produces a greater than expected increase in plasma levels.

 Brain and CSF levels of trehalose were also measured following 2 days
15 administration of trehalose (2.67 g/kg). Brain and CSF samples were collected 1 h post-dose (approximately the plasma T_{max}) and brain and CSF trehalose levels were 81.3 ± 13.8 ng/g and 562 ± 346.5 ng/ml respectively. Therefore, the brain trehalose levels represent approximately 1% of plasma levels, which is similar to rats.

20 Efficacy of trehalose in non-human primates

 As can be seen from **Table 8** when trehalose is administered once daily to non-human primates it significantly increases dopamine levels compared to animals receiving α SYN alone. This result is very similar to the result obtained in rats (compare **Tables 3** and **8**).

This result, along with the finding that the same dose of trehalose on a g/kg basis provides a similar plasma trehalose exposure, supports the hypothesis that a dose of trehalose that provides a similar trehalose exposure in rats and non-human primates also significantly reduces α SYN-induced striatal dopamine loss in both species. Together, these results suggest that an equivalent dose, on a g/kg basis, of trehalose produces similar trehalose exposures in the plasma and brain of rats and non-human primates and that this exposure also produces similar efficacious effects in rats and non-human primates. As these effects are preserved between rats and non-human primates it is also likely that they will translate to humans with Parkinson's disease and this allows us to estimate a dose of trehalose that is likely to be efficacious in treating Parkinson's disease.

Extrapolating from animal data to a human dose

On a dose per body weight basis, trehalose produced very similar plasma and brain levels in rats and macaques (see **Figure 3** and **Tables 6 and 7**) when dosed with 2.67 g/kg/day. We also know that a trehalose dose of 2.67 g/kg/day was efficacious in the rat and non-human primate. Therefore, it is contemplated that an efficacious dose for humans includes 2.67 g/kg which is estimated to produce a trehalose plasma C_{max} of ~10000 ng/ml. From the rat data it is also clear that a dose of 0.89 g/kg will be ineffective.

Therefore, if we assume the average human weight is 80 kg then based on the results disclosed herein, it is estimated that 214 g/day would be an efficacious dose. Similarly, it is contemplated that a dose below 71 g/day will not be efficacious in human. **Table 10** below shows the required daily dosages for the various weight ranges.

Body weight (kg)	Required daily trehalose (g)
Up to 50	130
51-60	160
61-70	190
71-80	210
81-90	240
91-100	270
101-110	300
Over 110	330

Table 10

The trehalose may be formulated as a pharmaceutical composition for treating neurological disorders, comprising a daily dose of trehalose, and a pharmaceutically acceptable composition wherein the daily dose of the trehalose is between about 0.25 to about 12.5 g/kg. Examples of pharmaceutical acceptable compositions include carriers, diluents, adjuvants, excipients or vehicles, preserving agents, fillers, disintegrating agents, buffering agents, penetration enhancers, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavouring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents. Suitable dosage forms include, for example, tablets, dragees, powders, elixirs, syrups, liquid preparations including suspensions and emulsions, lozenges, granules and capsules. Techniques and formulations generally may be found in Remington, Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition.

The trehalose may be incorporated into a “foodstuff”, “food supplement”, “beverage” or “beverage supplement” composition, where “foodstuff”, “food

supplement”, “beverage” or “beverage supplement” have normal meanings for those terms and are not restricted to pharmaceutical preparations, for treating neurological disorders, comprising a daily dose of trehalose, and a dietary acceptable carrier wherein the daily dose of the trehalose is between about 0.25 to about 12.5 g/kg. In an embodiment of the daily dose is about 2.67 g/kg/day.

The present disclosure provides a pharmaceutical kit comprising:
trehalose for treatment of neurological disorders, and
instructions for a single daily administration of the trehalose with
the daily dose being between about 0.25 to about 12.5 g/kg/day.

In some embodiments of the pharmaceutical kit the daily dose is between about 0.5 to about 10 g/kg/day.

In some embodiments of the pharmaceutical kit the daily dose is between about 0.75 to about 7.5 g/kg/day.

In some embodiments of the pharmaceutical kit the daily dose is one of between about 1 to about 5 g/kg/day and about 1.25 to about 3.75 g/kg/day.

In an embodiment of the pharmaceutical kit the daily dose is about 2.67 g/kg/day.

The pharmaceutical kit may include the following instructions:

Body weight (kg)	Required daily trehalose (g)
Up to 50	130
51-60	160

61-70	190
71-80	210
81-90	240
91-100	270
101-110	300
Over 110	330

Translatability to other diseases

While the present studies have been focused on PD, it will be appreciated that for PD the studies have shown that trehalose administered as a single

5 bolus dose is more efficacious than when administered throughout the day. It is therefore contemplated that these results may be applicable to diseases other than PD, especially those diseases where it has already been demonstrated that trehalose administered in the drinking water is efficacious (e.g. Alzheimer's disease, tauopathies, amyotrophic lateral sclerosis and Huntington's disease). It

10 is contemplated that there are two (2) other 'groups' of diseases, in addition to PD, that may exhibit the same results as found with PD, namely synucleinopathies (diseases that have misfolded α -synuclein), and proteinopathies (diseases that have a misfolded protein other than α -synuclein). PD is a synucleinopathy and synucleinopathies are a subset of proteinopathies.

15 In summary these diseases may include:

1. Parkinson's disease,
2. Synucleinopathies including (but not limited to), Parkinson's disease with dementia, dementia with Lewy bodies, MSA, essential tremor, Gaucher disease and other lysosomal storage disorders, neurodegeneration with

20 brain iron accumulation, and

3. Other proteinopathies including (but not limited to), Alzheimer's disease, Cerebral β -amyloid angiopathy, Retinal ganglion cell degeneration in glaucoma, Prion diseases, Tauopathies, Frontotemporal lobar degeneration, FTLD–FUS, Amyotrophic lateral sclerosis (ALS),
- 5 Huntington's disease and other triplet repeat disorders, Familial British dementia, Familial Danish dementia, Hereditary cerebral hemorrhage with amyloidosis, CADASIL, Alexander disease, Serpinopathies, Familial amyloidotic neuropathy, Senile systemic amyloidosis, Serpinopathies and Retinitis pigmentosa with rhodopsin mutations.

10 **Conclusion**

The present inventors have conducted studies to investigate the potential of trehalose to be developed as a treatment for various neurological disorders, including but not limited to Parkinson's disease. As part of these studies the inventors:

- 15 1) developed a quantitative method for measuring trehalose levels in plasma and brain tissues;
- 2) investigated the efficacy of three different ways of administering the same dose of trehalose (in drinking water, administered as a single dose or administered as 3 doses eight hours
- 20 apart).
- 3) investigated the plasma and brain pharmacokinetics of trehalose following oral administration of trehalose.

In summary the inventors have demonstrated the following.

1) When trehalose was given as a single administration it was much more efficacious than when the same amount of trehalose was administered in the drinking water or as 3 administrations, 8 hours apart.

2) That blood levels of trehalose are not dose linear. Increasing the dose 3-fold led to a 6 fold increase in trehalose plasma level in rats and a doubling of the dose led to a greater than 3-fold plasma exposure in macaques.

3) That trehalose only reached the brain in detectable amounts when the dose of trehalose was administered as a single bolus dose.

Taken together, these results demonstrate the very surprising result that trehalose is more efficacious when given as a single bolus dose. Without being bound by any hypothesis, the inventors contemplate that a reason for this enhanced efficacy may be that dosing all the trehalose in one administration leads to a greater systemic drug exposure than would be expected if the drug exhibited linear kinetics. The reason why this occurs is unknown but the inventors contemplate that it might be due to higher doses of trehalose saturating the systems that eliminate trehalose from the body.

The foregoing description of the preferred embodiments of the invention has been presented to illustrate the principles of the invention and not to limit the invention to the particular embodiment illustrated. It is intended that the scope of the invention be defined by all of the embodiments encompassed within the following claims and their equivalents.

THEREFORE WHAT IS CLAIMED IS:

1. A pharmaceutical kit comprising:
trehalose for treatment of neurological disorders, and
instructions for a single daily administration of the trehalose with
the daily dose being between about 0.25 to about 12.5 g/kg/day.
2. The pharmaceutical kit according to claim 1 wherein the daily dose is
between about 0.5 to about 10 g/kg/day.
3. The pharmaceutical kit according to claim 1 wherein the daily dose is
between about 0.75 to about 7.5 g/kg/day.
4. The pharmaceutical kit according to claim 1 the daily dose is one of
between about 1 to about 5 g/kg/day and about 1.25 to about 3.75 g/kg/day.
5. The pharmaceutical kit according to claim 1 wherein the daily dose is
about 2.67 g/kg/day.
6. The pharmaceutical kit according to one of claims 1 to 5, comprising the
following instructions:

Body weight (kg)	Required daily trehalose (g)
Up to 50	130
51-60	160
61-70	190
71-80	210
81-90	240
91-100	270
101-110	300
Over 110	330

7. The pharmaceutical kit according to any one of claims 1 to 6 wherein the neurological disorders are any one or combination of Parkinson's disease, synucleinopathies and proteinopathies.

8. The pharmaceutical kit according to claim 7, wherein the neurological disorder is Parkinson's disease.

9. The pharmaceutical kit according to claim 7 wherein the synucleinopathy is any one of Parkinson's disease with dementia, dementia with Lewy bodies, MSA, essential tremor, Gaucher disease and other lysosomal storage disorders, and neurodegeneration with brain iron accumulation.

10. The pharmaceutical kit according to claim 7 wherein the proteinopathies include Alzheimer's disease, cerebral β -amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, prion diseases, tauopathies, frontotemporal lobar degeneration, FTL D-FUS, amyotrophic lateral sclerosis (ALS), Huntington's disease and other triplet repeat disorders, familial British dementia, familial Danish dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL, Alexander disease, serpinopathies, familial amyloidotic neuropathy, serpinopathies and retinitis pigmentosa with rhodopsin mutations.

11. A dosage unit for treatment of neurological disorders, comprising trehalose in the following amount for a single daily administration:

Body weight (kg)	Amount of trehalose (g)
Up to 50	130
51-60	160
61-70	190
71-80	210
81-90	240
91-100	270
101-110	300
Over 110	330

12. The dosage unit according to claim 11 wherein said amount of the trehalose corresponds to the daily dose of between about 0.25 to about 12.5 g/kg/day.
13. The dosage unit according to claim 12 wherein the daily dose is between about 0.5 to about 10, preferably, about 0.75 to about 7.5 g/kg/day.
14. The dosage unit according to claim 12 wherein the daily dose is between about 1 to about 5 g/kg/day.
15. The dosage unit according to claim 12 wherein the daily dose is between about 1.25 to about 3.75 g/kg/day.
16. The dosage unit according to claim 12 wherein the daily dose is about 2.67 g/kg/day.
17. The dosage unit according to any one of claims 11 to 16 wherein the neurological disorders are any one or combination of Parkinson's disease, synucleinopathies and proteinopathies.
18. The dosage unit according to claim 17 wherein the neurological disorders is Parkinson's disease.
19. The dosage unit according to claim 17 wherein the synucleinopathy is any one of Parkinson's disease with dementia, dementia with Lewy bodies, MSA, essential tremor, Gaucher disease and other lysosomal storage disorders, and neurodegeneration with brain iron accumulation.
20. The dosage unit according to claim 17 wherein the proteinopathies include Alzheimer's disease, cerebral β -amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, prion diseases, tauopathies, frontotemporal lobar degeneration, FTL β -FUS, amyotrophic lateral sclerosis (ALS), Huntington's disease and other triplet repeat disorders, familial British dementia, familial Danish dementia, hereditary cerebral hemorrhage with

amyloidosis, CADASIL, Alexander disease, seipinopathies, familial amyloidotic neuropathy, serpinopathies and retinitis pigmentosa with rhodopsin mutations.

21. A composition for treating neurological disorders, comprising a daily dose of trehalose, and a physiologically acceptable carrier wherein the daily dose of the trehalose is between about 0.25 to about 12.5 g/kg.

22. The composition according to claim 21, wherein the carrier is a pharmaceutically acceptable carrier.

23. The composition according to claim 21, wherein the carrier is foodstuff, foodstuff supplement, beverage or beverage supplement.

24. The composition according to claim 23, wherein the carrier is a calorific material comprising fats, oils, carbohydrates, proteins, or sources of minerals, vitamins or fibre, or any combination thereof.

25. The composition according to claim 23, wherein the carrier is any one of dairy, cereal, vegetable, meat, fish, poultry, fruit based foodstuffs, processed foods, cooking ingredients and sweeteners.

26. The composition according to claim 23, wherein the carrier is any one of water, carbonated beverages, uncarbonated beverages, fruit juices, infusion drinks, coffee, teas, protein-shakes, soft-drinks or alcoholic drinks.

27. The composition according to any one of claims 21 to 26 wherein the daily dose is between about 0.5 to about 10 g/kg/day.

28. The composition according to claim 21 wherein the daily dose is between about 0.75 to about 7.5 g/kg/day.

29. The composition according to claim 21 wherein the daily dose is between about 1 to about 5 g/kg/day.

30. The composition according to claim 21 wherein the daily dose is between about 1.25 to about 3.75 g/kg/day.
31. The composition according to claim 21 wherein the daily dose is about 2.67 g/kg/day.
32. The composition according to any one of claims 21 to 31 wherein the neurological disorders are any one or combination of Parkinson's disease, synucleinopathies and proteinopathies.
33. The composition according to claim 32 wherein the neurological disorder is Parkinson's disease.
34. The composition according to claim 32 wherein the synucleinopathy is any one of Parkinson's disease with dementia, dementia with Lewy bodies, MSA, essential tremor, Gaucher disease and other lysosomal storage disorders, and neurodegeneration with brain iron accumulation.
35. The composition according to claim 32 wherein the proteinopathies include Alzheimer's disease, cerebral β -amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, prion diseases, tauopathies, frontotemporal lobar degeneration, FTL D-FUS, amyotrophic lateral sclerosis (ALS), Huntington's disease and other triplet repeat disorders, familial British dementia, familial Danish dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL, Alexander disease, serpinopathies, familial amyloidotic neuropathy, serpinopathies and retinitis pigmentosa with rhodopsin mutations.
36. A composition for treating neurological disorders, comprising a daily dose of trehalose in the following amount and a physiologically acceptable carrier:

Body weight (kg)	Amount of trehalose (g)
Up to 50	130
51-60	160
61-70	190
71-80	210
81-90	240
91-100	270
101-110	300
Over 110	330

37. The composition according to claim 36, wherein the carrier is a pharmaceutically acceptable carrier.

38. The composition according to claim 36, wherein the carrier is foodstuff, foodstuff supplement, beverage or beverage supplement.

39. The composition according to claim 38, wherein the carrier is a calorific material comprising fats, oils, carbohydrates, proteins, or sources of minerals, vitamins or fibre, or any combination thereof.

40. The composition according to claim 38, wherein the carrier is any one of dairy, cereal, vegetable, meat, fish, poultry, fruit based foodstuffs, processed foods, cooking ingredients and sweeteners.

41. The composition according to claim 38, wherein the carrier is any one of water, carbonated beverages, uncarbonated beverages, fruit juices, infusion drinks, coffee, teas, protein-shakes, soft-drinks or alcoholic drinks.

42. The composition according to any one of claims 36 to 41 wherein the neurological disorders are any one or combination of Parkinson's disease, synucleinopathies and proteinopathies.

43. The composition according to claim 42 wherein the neurological disorder is Parkinson's disease.

44. The composition according to claim 42 wherein the synucleinopathy is any one of Parkinson's disease with dementia, dementia with Lewy bodies, MSA, essential tremor, Gaucher disease and other lysosomal storage disorders, and neurodegeneration with brain iron accumulation.

45. The composition according to claim 42 wherein the proteinopathies include Alzheimer's disease, cerebral β -amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, prion diseases, tauopathies, frontotemporal lobar degeneration, FTL D-FUS, amyotrophic lateral sclerosis (ALS), Huntington's disease and other triplet repeat disorders, familial British dementia, familial Danish dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL, Alexander disease, serpinopathies, familial amyloidotic neuropathy, serpinopathies and retinitis pigmentosa with rhodopsin mutations.

46. A method for treating neurological disorders, comprising:
administering trehalose to a subject as a single daily administration with a daily dose between about 0.25 to about 12.5 g/kg/day.

47. The method according to claim 46 including administering trehalose to a subject as a single daily administration with a daily dose between about 0.5 to about 10 g/kg/day.

48. The method according to claim 46 including administering trehalose to a subject as a single daily administration with a daily dose between about 0.75 to about 7.5 g/kg/day.

49. The method according to claim 46 including administering trehalose to a subject as a single daily administration with a daily dose between about 1 to about 5 g/kg/day.

50. The method according to claim 46 including administering trehalose to a subject as a single daily administration with a daily dose between about 1.25 to about 3.75 g/kg/day.

51. The method according to claim 46 including administering trehalose to a subject as a single daily administration with a daily dose of about 2.67 g/kg/day.

52. The method for treatment of neurological disorders according to any one of claims 46 to 51 wherein the neurological disorders are any one or combination of Parkinson's disease, synucleinopathies and proteinopathies.

53. The method for treatment of neurological disorders according to claim 52 wherein the neurological disorder is Parkinson's disease.

54. The method for treatment of neurological disorders according to claim 52 wherein the synucleinopathy is any one of Parkinson's disease with dementia, dementia with Lewy bodies, MSA, essential tremor, Gaucher disease and other lysosomal storage disorders, and neurodegeneration with brain iron accumulation.

55. The method for treatment of neurological disorders according to claim 52 wherein the proteinopathies include Alzheimer's disease, cerebral β -amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, prion diseases, tauopathies, frontotemporal lobar degeneration, FTLD-FUS, amyotrophic lateral sclerosis (ALS), Huntington's disease and other triplet repeat disorders, familial British dementia, familial Danish dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL, Alexander disease, seipinopathies, familial amyloidotic neuropathy, serpinopathies and retinitis pigmentosa with rhodopsin mutations.

1/2

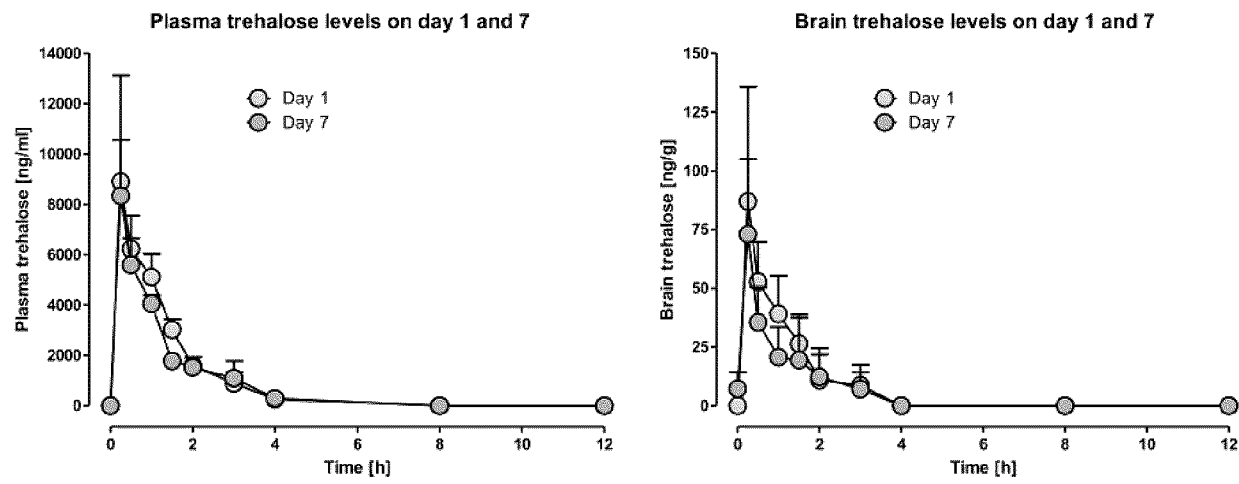


FIGURE 1

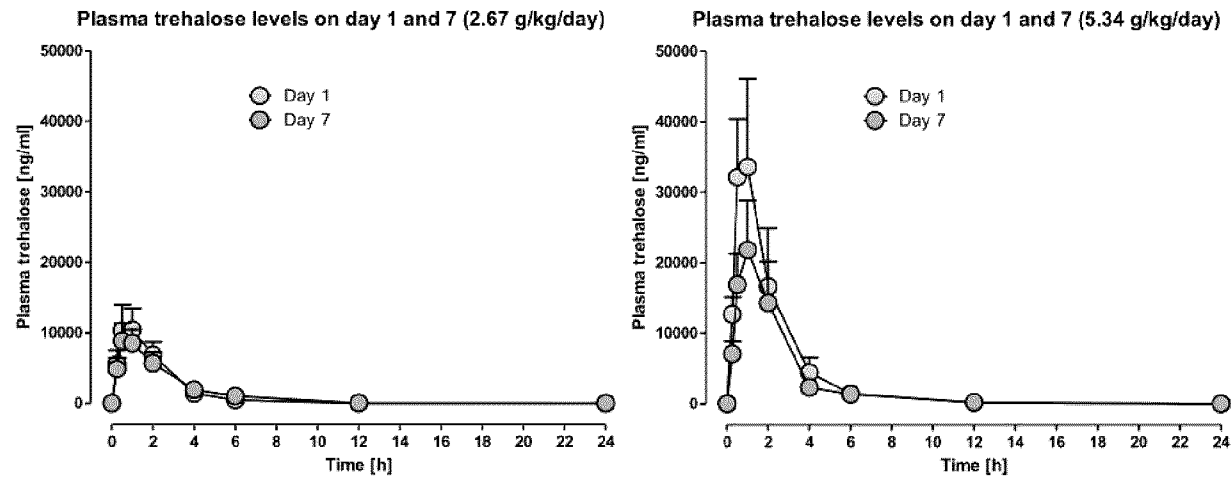


FIGURE 2

2/2

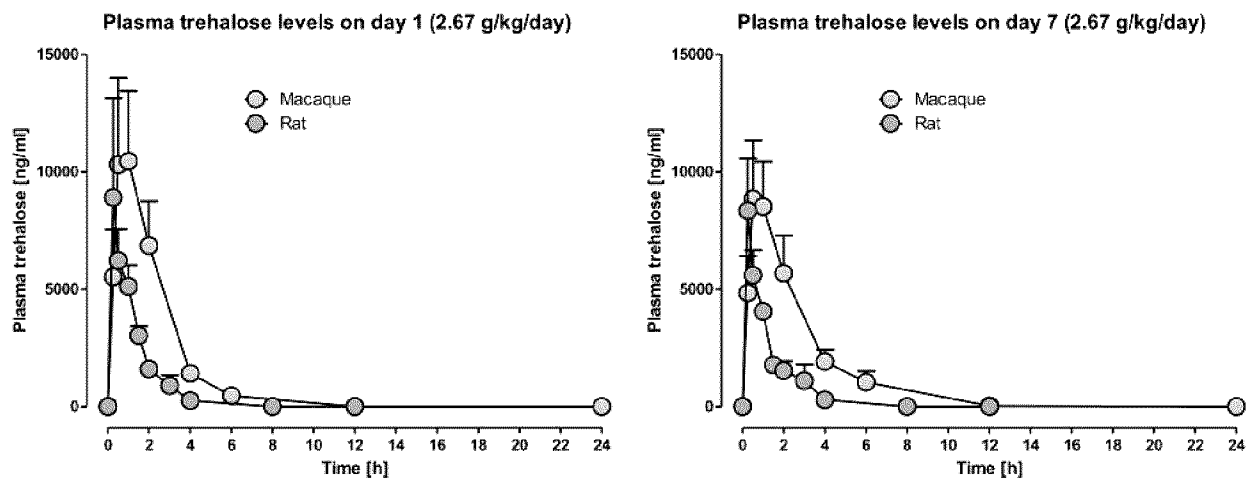


FIGURE 3

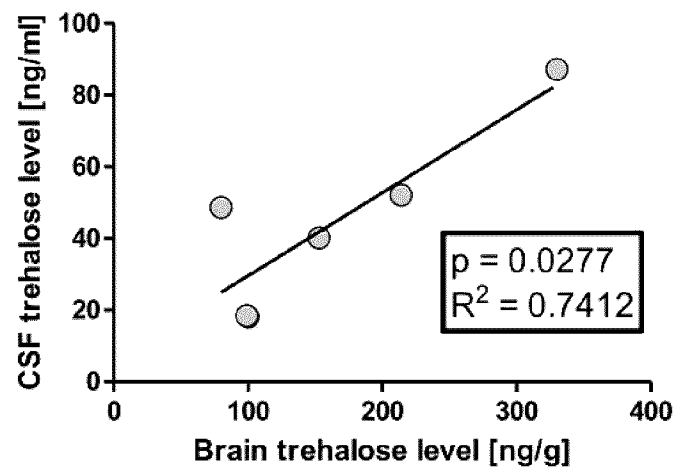


FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050123

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 31/7016** (2006.01), **A61P 25/00** (2006.01), **A61P 25/16** (2006.01), **C07H 3/04** (2006.01)

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)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

STN (CAPlus, Patent Pak)

Google Scholar (Trehalose neurological disorders, trehalose neurodegenerative diseases)

Google patent (trehalose neurological disorders, trehalose neurodegenerative diseases)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/181.333 (Megiddo D.) 13 November 2014 (13-11-2014)	1-3, 12-13, 21-28
A	Pages 4-6	1-55
X	WO 2014/065.606 (Ok Kim, M.) 1 May 2014 (01-05-2014) Whole document	1-3, 12-13, 21-28
A	WO 2015/008.372 (Ok Kim, M.) 23 July 2015 (23-07-2015) Whole document	1-55
A	ROSS et al., "Protein aggregation and neurodegenerative disease", <i>Nature Medicine</i> , July 2004 (07-2004), Vol. 10, pp. S10-S17,	1-55

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"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)	"&" document member of the same patent family
"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
14 May 2017 (14-05-2017)Date of mailing of the international search report
19 May 2017 (19-05-2017)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050123**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 46-55
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 46-55 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 46-55.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CA2017/050123

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2014181333A2	13 November 2014 (13-11-2014)	WO2014181333A2 WO2014181333A3 AU2014264228A1 CA2911399A1 CR20150637A EA201501054A1 EP2994145A2 HK1217300A1 JP2016518403A KR20160009617A MD20150120A2 MX2015015355A PE00122016A1 SG11201509030TA US2014336145A1 US9084720B2 US2015196575A1 US9125924B2 US2016022716A1 US9572825B2 US2016101122A1 US2016120798A1 US2017020905A1	13 November 2014 (13-11-2014) 08 January 2015 (08-01-2015) 12 November 2015 (12-11-2015) 13 November 2014 (13-11-2014) 05 April 2016 (05-04-2016) 29 April 2016 (29-04-2016) 16 March 2016 (16-03-2016) 06 January 2017 (06-01-2017) 23 June 2016 (23-06-2016) 26 January 2016 (26-01-2016) 31 March 2016 (31-03-2016) 18 July 2016 (18-07-2016) 14 February 2016 (14-02-2016) 27 November 2015 (27-11-2015) 13 November 2014 (13-11-2014) 21 July 2015 (21-07-2015) 16 July 2015 (16-07-2015) 08 September 2015 (08-09-2015) 28 January 2016 (28-01-2016) 21 February 2017 (21-02-2017) 14 April 2016 (14-04-2016) 05 May 2016 (05-05-2016) 26 January 2017 (26-01-2017)
WO2014065606A1	01 May 2014 (01-05-2014)	WO2014065606A1 KR20140052620A KR101473966B1	01 May 2014 (01-05-2014) 07 May 2014 (07-05-2014) 16 December 2014 (16-12-2014)
WO2015108372A1	23 July 2015 (23-07-2015)	WO2015108372A1 KR20150086429A	23 July 2015 (23-07-2015) 28 July 2015 (28-07-2015)