

due to age, constitution and the like, the cells embedded with a polyethylene glycol or silicon capsule, a porous container and the like can also be transplanted to avoid rejection. The dose (amount to be transplanted) and administration frequency (number of times to be transplanted) of the medicament of the present invention can be appropriately determined according to the age, body weight, symptom and the like of the patients who receive administration.

[0048]

A medicament containing the dopaminergic neuron of the present invention can efficiently engraft in the body of patients by administration (transplantation) thereof, which in turn enables efficient production (release) of dopamine in the body of patients. Therefore, the medicament of the present invention is useful for the treatment of diseases caused by decreased production (release) of dopamine, for example, neurodegenerative diseases such as Parkinson's disease, Huntington chorea, Alzheimer's disease, epilepsy and schizophrenia and the like.

[0049]

3. Other use

Since the dopaminergic neuron of the present invention has phenotypic characteristics and functions similar to those of dopaminergic neuron in vivo, it is useful for screening for a drug compound, preferably a compound for the treatment of neurodegenerative diseases. For example, whether the test compound is useful as a medicament can be evaluated by adding the test compound alone or in combination with other medicament to the dopaminergic neuron of the present invention, and measuring morphological or functional change of the neuron. ~~As an example~~ Examples of the method for measuring the functional change, include measuring the amount of dopamine produced or released from the neuron ~~is measured~~. As used herein, the dopaminergic neuron is preferably a cell showing the same phenotype as the disease to be the treatment target, and particularly preferred is a dopaminergic neuron produced by

the like; flavors such as peppermint, Gaultheria adeno-thrix oil, cherry and the like; liquid carriers such as fats and oils, water for injection, vegetable oil (e.g., sesame oil, coconut oil, soybean oil), buffering agent (e.g., phosphate buffer, sodium acetate buffer) and the like; solubilizing agents (e.g., ethanol, propylene glycol, polyethylene glycol); non-ionic surfactants (e.g., polysorbate80™, HCO-50); solubilizing agents (e.g., benzyl benzoate, benzyl alcohol); soothing agents (e.g., benzalkonium chloride, procaine hydrochloride); stabilizers (e.g., human serum albumin, polyethylene glycol); preservatives (e.g., benzyl alcohol, phenol); and antioxidants.

Examples of the aforementioned water for injection include saline; and isotonic solutions containing glucose, D-sorbitol, D-mannitol, sodium chloride and the like.

Since a medicament (preferably, a therapeutic drug for neurodegenerative disease) obtained by the above-mentioned screening is safe and low toxic, it can be administered orally or parenterally to, for example, mammals (e.g., human, mouse, rat, rabbit, sheep, swine, bovine, horse, cat, dog, monkey, chimpanzee).

The dose and administration frequency of the medicament can be appropriately determined according to the action thereof, target disease, administration subject, administration route and the like.
[0051]

The dopaminergic neuron of the present invention can also be used for the toxicity evaluation of a compound. For example, whether the test compound has toxicity can be evaluated by adding the test compound alone or in combination with other medicament to the dopaminergic neuron of the present invention, and measuring morphological or functional change of the neuron. ~~As an example~~ Examples of the method for measuring the functional change, include measuring the amount of dopamine produced or released from the neuron-is-measured.

Examples of the test compound include peptide, protein, antibody, nonpeptidic compound, synthetic compound,

upstream) inhibitory action.

[0071]

[Experimental Example 3]

Expression variation of each differentiation marker in differentiation induction process

Based on the results of the above-mentioned Reference Examples, Examples and Experimental Examples, a dopaminergic neuron differentiation system consisting of the three steps shown in Fig. 9 was set, and expression of various differentiation markers in the differentiation induction process from undifferentiated iPS cell was examined.

[0072]

In step 1, the cells were cultured in Neuro/B27 added with 0.5 μ M LDN193189, 10 μ M SB431542, 0.5 μ M purmorphamine, 200 ng/ml SHH and 1 μ M CHIR99021 for 5 days. In step 2, the cells were cultured in Neuro/B27 added with 0.5 μ M LDN193189 and 1 μ M CHIR99021 for 8 days (total 13 days). In step 3, the cells were cultured in Neuro/B27 added with 0.1 mM ascorbic acid, 0.5 mM dbcAMP and 3 μ M PD0325901 for 32 days (total 45 days). After culture, time-course expression variation of various differentiation markers was measured by a method similar to Reference Example 3. The results of the expression analysis are shown in Fig. 10.

[0073]

As a control, the cells were cultured under conditions without addition of a differentiation inducing factor (- in Figure), conditions excluding only SHH (-SHH in Figure) or conditions excluding only purmorphamine (-Pur in Figure). Also, the group subjected to differentiation induction under the conditions shown in Fig. 9 from step 2 onward was studied.

[0074]

Under conditions added with all (All in Figure), the expression of floor plate cell marker FOXA2 was elevated up to day 7 of differentiation induction, and the expression level was maintained until completion of the differentiation