Modeling Mechanisms and Applications of Parkinson's Disease Animal Models

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6-OHDA model shows many biochemical and pathological similarities to human PD, and can closely simulate the DA decrease, loss of DA neurons, and some neurobehavioral defects. However, the main motor disorder observed in this model is lateral rotation, which is not fully consistent with the common clinical symptoms of human PD such as static tremor, weakened motor function, and muscle rigidity. Further, the pathological characteristic of Lewy Body (LB) formation is not observed. Despite these limitations, this model has been widely used, because of easy availability, low cost, and stable and lasting behavior changes, to confirm the efficacy of anti-PD complexes, evaluate the therapeutic effect of neurotropic factors, and more. This model has some additional advantages: the rotation behavior induced by 6-OHDA can be quantitatively evaluated, and this model is the only PD model in which quantitative detection on behavioral changes is possible. Moreover, complete or partial substantia nigra striatum bundle damage can be induced in the unilateral 6-OHDA model by adjusting the dose and site of administration individually or simultaneously, to simulate the pathological changes in patients with PD in the early, middle, and late stages. This offers a solution to the high mortality observed in the bilateral total brain injury model, and also allows the use of the undamaged hemisphere as a control in each model animal.

3-Nitrotyrosine (3-NT) model: The nitrite peroxide anion (ONOO-) is a prominent reactive nitrogen species involved in oxidative stress *in vivo*. It nitrifies free tyrosine residues or tyrosine in protein structures to produce 3-NT, which can cause protein denaturation, functional changes, and eventually cell damage [11-14]. At present, 3-NT is considered a relatively specific marker of oxidative stress. Several patients with neurodegenerative diseases including PD have shown elevated levels of 3-NT in the brain, suggesting that protein nitrification could play a role in PD neurodegeneration. Intra striatal injection of free 3-NT resulted in decreased TH-positive nerve endings, decreased DA neurons in the substantia nigra, and abnormal behavior in mice, suggesting that 3-NT can induce neurodegeneration in animal models. The 3-NT model is an acute model, and it is not clear whether the protein aggregation and emergence of intracellular inclusion bodies observed are related to PD. However, this model is an oxygen stress PD model, and could be valuable in exploring the pathogenesis and developing treatment methods against the stress-induced aspect of PD.

Biological toxicity models

Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) model: MPTP is converted to the Methyl-Phenyl Pyridine ion (MPP+) by Monoamine Oxidase (MAO)-B. MPP+ has a high affinity for the Dopamine Transporter (DAT), and can be transported by DAT to DA neurons, where it inhibits oxidative phosphorylation by selectively inhibiting the activity of mitochondrial complex I, causing mitochondrial dysfunction and Reactive Oxygen Species (ROS) accumulation, and eventually DA neuron degeneration by necrosis or apoptosis. MPTP treatment simulates PD in several species including mice, dogs, and primates. The toxicity of MPTP is different in different species; the highest sensitivity is observed in humans and primates, followed by mice. Rats and guinea pigs have a high tolerance to MPTP, and not preferred for use as MPTP PD models. MPTP can be administered by several methods including brain stereotaxic injection and systemic administration. The most commonly used systemic administration methods are subcutaneous, intravenous, abdominal, or intramuscular injection. Single intraperitoneal injection of MPTP only reduces TH expression without the loss of SNpc DA

neurons. Primate MPTP models can induce clear, persistent, and irreversible behavioral responses, pathological and biochemical changes, and responses to drugs (including adverse reactions) similar to those observed in patients with PD. They are therefore the most ideal PD animal model. However, their application is limited by the availability and cost of primates. Rodents have the advantage of availability, ease of maintenance, and low cost, and the behavioral changes after MPTP treatment have a short duration and can be completely reversed. Therefore, the mouse model is a classic model for exploring the molecular pathway of PD neuronal death and evaluating the efficacy of neuroprotective agents. The monkey model is mainly used to identify the behavioral aspects and symptoms of PD, and is typically used in the last stage of PD treatment research before testing in humans [14-23]. There have been reports on the production or absence of LBs-like inclusions in MPTP models. Low-dose MPTP is likely insufficient to promote the formation of LB's. The formation of inclusion bodies may be related to an increase in lactic acid levels in the brain of MPTP-treated mice, because it can activate AMP-activated protein kinase and promote the accumulation and phosphorylation of -synuclein.

The advantage of the MPTP model is that it can accurately simulate PD-related movement disorders, and the neurons of substantia nigra and striatum are highly sensitive to MPTP. This model also reflects the inhibition of mitochondrial respiration during PD pathogenesis. The disadvantages include the high mortality, and the variations produced by different drug dosages and methods on the modeling results. It is also difficult to observe the production of LBs, and the behavioral dysfunction and substantia nigral lesion of the mouse model can be reversed quickly, making it difficult to simulate the characteristics of PD as a chronic neurodegenerative disease. Drinkut et al., used the PD model of MPTP mice with deficiency of the tyrosine kinase receptor Ret and overexpression of Brain-Derived Neurotrophic Factor (BDNF) in the striatum. Immunohistochemistry (IHC) and Enzyme-Linked Immunosorbent Assay (ELISA) were used to detect the number of TH and Nissl-positive substantia nigra neurons. Quantification of DA fiber density and determination of DA content in the striatum was performed by HPLC [20]. This confirmed that the lack of Ret completely offsets the neuroprotective and regenerative effects of BDNF on the DA energy system in the midbrain in the mouse PD model. Thus, Ret signaling is likely necessary for BDNF to prevent and compensate for the degradation of the DA system and Ret activation is the main mechanism underlying the effects of BDNF in the treatment of PD.

Rotenone model: The agricultural insecticide rotenone is a highly lipo-soluble neurotoxin that can easily penetrate biological barriers including the BBB. It can selectively destroy substantia nigra striatum DA neurons by inhibiting the activity of mitochondrial respiratory chain complex I and disrupting the mitochondrial respiratory chain, resulting in ROS production and mitochondrial dysfunction.

The Rotenone model reproduces several anatomical, neurochemical, neuropathological, and behavioral characteristics of human PD, including the accumulation of

induction of -synuclein. inclusion bodies similar to LB's in surviving DA neurons and the simulation of neuropathological features of LB's in substantia nigra neurons, which are lacking in the 6-OHDA and MPTP models. Further, rotenone is easier to administer than 6-OHDA owing to its lipophilicity, and can be administered by gavage, subcutaneous injection, intravenous injection, and intraperitoneal injection. Rotenone exposure is considered a health hazard; therefore, chronic subcutaneous injection with an osmotic pump is the most common drug delivery scheme. Different animals show different relativities to rotenone, which makes the amount, location, and degree of DA melanocytic striatum damage in the rotenone model variable, with poor reproducibility. In addition, rotenone exposure can cause multiple organ damage, and high animal mortality.

Betarbet et al., established the rotenone PD model in 2000. They implanted Alzet micro osmotic pumps subcutaneously into the back of rats, and performed low-dose intravenous injections of rotenone (3 mg/kg/day) for 33 days [21]. They observed selective degeneration of DA neurons in the striatum, and the rats exhibited characteristic PD features including dyskinesia, flexion posture, gait instability sometimes with rigidity, and tremors, and LB-like -nielcunys-positive inclusion bodies. However, rotenone (3 mg/kg/day) administered subcutaneously to rats for 28 days caused no damage to DA neurons and caused extensive toxicity to peripheral organ. Intracerebral injection of rotenone could be used to facilitate the expression and aggregation of DA neurons and -synuclein in the SNpc and progressive neuronal, without related peripheral toxicity.

Miyazaki et al., chronically exposed C57BL/6J mice to low-dose rotenone (2.5 mg/kg/day) for four weeks by subcutaneous implantation of an osmotic minipump to generate a rotenone mouse PD model [22]. The model mice showed dyskinesia and gastrointestinal dysfunction. The dyskinesia was evaluated by open field, roller, and cylinder tests. In addition to a decrease in the number of DA neurons in the SNpc and injured striatum nerve endings, a significant decrease in cholinergic neurons in the dorsal motor nucleus of the vagus and the intermuscular plexus of the intestine was observed. In addition, Rotenone-ntr1

contralateral motor dysfunction [24]. The PD monkey model established by adeno-associated viral vector-mediated overexpression of α-synuclein closely mimicked human pathological changes. Injection of synuclein into the brain of rodents and non-human

Conclusion

Several different animal models for the pathogenesis of PD have been developed, and each has its advantages and disadvantages. Substantive substantia nigra striatum degeneration is common, and the motor symptoms of PD have been accurately replicated. Neurotoxin models, such as the 6-OHDA, MPTP, and rotenone models, have features consistent with the pathological characteristics of human PD. Genetic studies have elucidated the genetic principles and pathogenesis of PD. The neurotoxin models simulate the late stage of PD, and are not ideal to study potential cures. These are more suitable for the screening of symptomatic treatment drugs. Genetic models use overexpression or gene knockout technology to simulate early stages of PD. There is no progressive loss of DA neurons, which is more helpful in evaluating the role of genes in PD.

In summary, any PD animal model cannot fully simulate the clinical symptoms and pathological processes of PD. The choice of optimal

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