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Research Article

MOST RELIABLE ANIMALS MODELS OF PARKINSON'S DISEASE FOR SCREENING NEW CHEMICAL ENTITIES

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A B S T R A C T

Parkinson's disease is an auto degenerative disorder of the motor system in the central nervous system. It is caused due to the death of the dopamine generating cells of the mid brain. Parkinson's disease is considered a synucleiopathy due to an abnormal accumulation of alpha -syncline protein in the brain in the form of Lewy bodies, as opposed to other diseases such as Alzheimer's disease where the brain accumulates tau protein in the form of neuro fibrillary tangles. Many authors have given details regarding the chemicals that causes parkinson's disease in various animal models which serve as the test subjects. This present review mainly focuses on briefly describing the present advanced chemicals which are used to induce Parkinson's disease to animals.

INTRODUCTION

Parkinson's disease is the second most common neurodegenerative disorder, after Alzheimer´s disease which is mostly due to the progressive loss of about 50–70% of the dopaminergic neurons in the substantia nigra pars compacta (SNc) region, a profound loss of dopamine (DA) in the striatum, and the presence of intracytoplasmic inclusions called Lewy bodies (LB), which are composed mainly of α-synuclein and ubiquitin. Therefore, this α-synuclein protein may play an important role in the pathogenesis of this disease. Loss of DA neurons or decrease in dopamine levels may cause mitochondrial dysfunction, oxidative stress, neuroinflammation, and insufficient autophagic or proteosomal protein degeneration. In addition, PD (Parkinson's disease) also affects the dorsal motor nucleus of the vagus nerve, the nucleus basalis of Meynert, the locus coeruleus, olfactory structures, and the hypothalamus. While these structures may account for the movement and mobility disorders associated with PD Lewy bodies have also been observed in the myenteric plexus[1].

Nonmotor symptoms of PD include depression, incontinence, disturbances of sleep patterns, anosmia, cognitive impairment, constipation and disturbances of some autonomic function.

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There is no known cause of PD however, environmental factors or genetic mutations have been theorized to be implicated. As age increases, the risk of developing the disease also increases[2].

In the last decade, many researchers have focused on the genetic mutations that caused due to the transfer of gene into the animal model through the rDNA technology, with the development of various mammalian (mice and more recently rats) and non-mammalian transgenic models that replicate most of the disease - causing mutations identified for monogenic forms of familial PD. Both toxic and transgenic classes of animal PD models have their own specificities and limitations, which must be carefully taken into consideration when choosing the model to be used. If a substantial and reproducible nigrostriatal lesion is required, a classic toxic model such as one based on the administration of 1-methyl-4-phenyl1,2,3,6-tetrahydropyridine or 6-hydroxydopamine will adequately serve the purpose. Since the clinical symptoms are due to loss of dopamine neurons and dopamine, drugs have been developed to treat the disease and animal models developed to study the progression and possible cause of the disease[3]. This review focused mainly on animal models of Parkinson's disease which are very much standardized and are used successfully to screen new molecules.

Chemical Induced Animal Models

The various Chemicals that can induce the parkinson's disease are

- 6-OHDA (6-Hydroxy Dopamine)

- MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)
- Rotenone
- Paraquat (N,N-dimethyl-4-4-4-bypiridinium)
- Annonacin
- l-trans-pyrrolidine-2,4-dicarboxylate(EAATs inhibitor)
- LPS
- α-Synuclein preformed fibrils

6-OHDA (6-Hydroxy Dopamine)

6-hydroxy dopamine (6-OHDA) is a hydroxylated analog of dopamine isolated by Senoh and Witkop in 1959. It is capable of inducing degeneration of both dopaminergic and noradrenergic neurons. Currently, 6-OHDA is used to lesion the dopaminergic system as a model for PD. But the disadvantage of a 6-OHDA animal model is that 6-OHDA does not cross the blood brain barrier, therefore direct injections are needed into the SNpc, medial forebrain bundle or striatum. There is also no olfactory loss, the brain stem is not affected. This model has been extensively studied in monkeys, rats, and mice and has the disadvantages that surgical preparations (stereotaxic injections) are needed, the possibility of infections from the surgery exists, and it is time consuming[5].

Procedure

Thirty minutes prior to operations, weigh each animal and record the weight. Systemically administer desipramine hydrochloride and pargyline hydrochloride at 10 ml/kg by intra – peritoneal injection (i.p) to one mouse using a 27 g needle attached to a 1 ml syringe. Warm up the heating disc and place this under the ear and incisor bar. The heating disc will help to maintain the temperature of the animal during surgery and keep the animal at an ideal height to fit the ear and incisor bars of the stereotaxic frame. Fifteen minutes following administration of the desipramine HCl and pargyline HCl solution, place the mouse in a closed anaesthesia chamber, and anesthetise the animal using isofluraneinhalation. The animal is sufficiently anesthetised when it shows no response to hind leg pinch and no blink reflex. Shave the top of the mouse's head, and apply the topical analgesic lidocaine directly to the skin using cotton wool. Five minutes following lidocaine application, sterilize the head of the animal with betadine solution. Place the animal in a stereotaxic frame adapted for mice. Firstly place the animal into the incisor bars with the anaesthesia mask placed over the face of the animal. Adjust the oxygen flow to 1 L/min, and the isoflurane to 1 . 5 – 2 %. The incisor bar should be at a level relative to the ear cups such that the top of the skull is level. Insert the ear cups. The ear cups have been inserted correctly when the head is completely flat, and cannot be moved in either direction. Cut along the midline of the skin on top of the head of the mouse using a scalpel blade, and retract the skin. Dry the surface of the skull using gauze. Push down plunger of the 1.0 µl syringe and place the 33 gauge injection needle into the 1 ml tube covered in tin foil containing 6 – OHDA solution (15 mg/ml). Record the coordinates at which 6-OHDA is administered in small amounts in bregma and lambda regions. Retract the needle, and burr a hole into the skull using a 25 gauge needle. Return the injection needle to the bregma and lambda region co-ordinates, and insert the needle to DV : - 5 mm . Infuse 0.2 µl of 6 – OHDA or vehicle unilaterally into the median forebrain bundle at a rate of 0.1 µl/min. Upon completion of administration of 6 – OHDA or vehicle, leave the needle in place for a further five minutes to allow diffusion away from the injection site. Slowly retract the needle. Close the incision to the scalp using three sutures, and deliver 1 ml of Lactated Ringer's solution subcutaneously. Remove the animal from stereotaxic frame, and place in the recovery cage until consciousness is regained [6].

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

1 - methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was first discovered in intravenous drug users in the early 1980's by Davis and Langston. It is a neurotoxin used to induce parkinsonism symptoms in experimental models of Parkinson's disease (PD). MPTP can produce irreversible neurological damage indistinguishable from PD in humans and animals. MPTP is lipophilic, easily crosses the blood brain barrier and is metabolized by astrocytes where it is converted to its toxic active cation MPP+.The most commonly used regimens in monkeys are the multiple intraperitoneal or intramuscular injections and the intracarotid infusion of MPTP. Conversely, rats injected with much higher doses of MPTP do exhibit significant dopaminergic neurodegeneration although, at these high doses, rats have to be pretreated with guanethidine to prevent dramatic peripheral catecholamine release and extensive mortality [7].

Procedure

On the evening before the experiment, all animals are weighed and coded. About a half - hour before starting the injection schedule, sterile MPTP solution should be prepared to the desired working concentration. During animal injection, care must be taken to avoid self - inoculation, special attention to animal restraint will significantly reduce this risk. For injection, place the mouse cage in the fume hood and when injecting, hold the animal so that any urine spray will fall into the cage and not on the surrounding areas, since mice, when held, tend to expel urine which can contain significant amounts of MPTP. Make sure the mouse is not held so tightly as to cause back flow of the injected MPTP from the peritoneum. Larger animals such as squirrel monkeys must be placed in restrainers for injection. It is not practical to inject large animals in a fume hood. Inspect injection site for leakage or spilled solution and wipe with a small pad dampened with 1 % bleach solution. When discarding syringes , do not clip , recap or remove needles from syringes, fill the syringe with 1 % bleach solution and then place the syringe with attached needle in a sharps container to be disposed of as biohazardous waste. At the end of the injection schedule, the remaining MPTP solution must be destroyed with an equivalent volume of 1 % bleach solution [8].

Rotenone

Rotenone is both an insecticide and herbicide, is widely used, occurs naturally in tropical plants, lipophilic and crosses the blood brain barrier. Rotenone has a half - life of less than 5 days and chronic exposure of low doses of rotenone results in inhibition of the mitochondrial transport system in the rat brain. Administered orally, rotenone

causes very little neurotoxicity, however in the Lewis rat strain chronic systemic administration via osmotic pumps has demonstrated neurotoxicity, while intravenous injection causes damage to the nigrostriatal neurons with Lewy body - like formations, oxidative stress and Intestinal problems mimicking symptoms of PD [9].

Procedure

For ST infusion, the female Sprague – Dawley rats weighing 220 – 260 g, were anesthetized with chloral hydrate (400 mg/kg in 0.9 % NaCl , I.P) and fastened on a cotton bed over a stereotaxic frame. Rotenone dissolved in DMSO (Dimethyl sulfoxide) was infused into the right VTA (AP : 5.0 mm ; L : 1.0 mm; DV : 7.8 mm) at a flow rate of 0.2 ml/min. The needle was left in place for additional five minutes for complete diffusion of the drug. Rotenone was infused into the right SNc (AP: 5.0 mm; L : 2.0 mm ; DV : 8.0 mm) at a flow rate of 0.2 ml/min, with a five – minute needle retention. After needle withdrawal, proper postoperative care was given until the animals recovered completely. The animals were given ibuprofen and penicillin in their drinking water for 24 hours to alleviate potential postsurgical discomfort and to prevent infection. For the SYS infusion, rotenone dissolved in sunflower oil was injected subcutaneously (2 mg/kg/d) into the back of these rats daily for four weeks.

Paraquat

Paraquat is a herbicide widely used in agriculture that exhibits a structural resemblance to MPP+, and because of this structural similarity, it was reasoned that PQ should behave like MPP+. PQ exerts its deleterious effects through oxidative stress mediated by redox cycling, which generates ROS. This model have been extensively studied in rats and monkeys [10].

Procedure

All the experiments were performed on Male Wistar rats (body weight , 210 ± 15 g). Animals were housed in groups of 4 in polypropylene cages with a controlled light and dark cycle of 12 hours each at 24 - 26 °C. Food and water were available at libitum. PQ dissolved in normal saline, was injected at 10 mg/kg i.p. daily for 3 consecutive weeks to induce neurotoxicity. Equal number of rats served as control and received normal saline in similar way. At the end of three weeks of treatment and after 30 min of last treatment with PQ, the individual groups of rats were subjected to different tests such as Western immunoblot analysis to detect the levels of α - synuclein protein in the brain [11].

Annonacin

Annonacin is a chemical compound with toxic effects, especially in the nervous system, found in some fruits such as the paw paw (custard apple), soursop, and others from the family Annonaceae. Annonacin is a potentially lethal neurotoxin. Like other acetogenins, it is a mitochondrial complex I (NADH – dehydrogenase) inhibitor. The LD50 of annonacin is 0.018 μM to dopaminergic neurons, and it is the damage done to these neurons that results in the neurodegenerative effects of the toxin. Annonacin is 100 times more toxic than 1-methyl-4-phenylpyridinium (MPP+), another potent mitochondrial complex I inhibitor. This model have been extensively studied in rats and monkeys[12].

Procedure

Male Lewis rats were implanted with Alzet osmotic mini pumps containing annonacin in dimethylsulfoxide and polyethyleneglycol 400 (v/v) as described previously to deliver the substance for 28 days through a catheter in the left femoral vein.

Tissue preparation

To prepare brains for histological analysis, rats were anaesthetized with 30 mg/kg sodium pentobarbital i.p. and killed by transcardial perfusion. Brains were then postfixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline for 24h, cryoprotected in 10% sucrose in 0.1 M phosphate - buffered saline for 24h, frozen in isopentane at 30^{o}C for 2 min and stored at 80^{0}C. To prepare brain tissue for ATP measurements and annonacin detection, rats were perfused rapidly with ice - cooled phosphate – buffered saline to wash out blood from the vessels. Brains were frozen immediately after removal in isopentane at 30^{0}C and stored at 80^{0}C. Correct intravenous placement of the catheter and complete emptying of the pumps were verified after perfusion.

1–trans–pyrrolidine–2,4–dicarboxylate (EAATs inhibitor)

The role of excitatory amino acid transporters (EAATs) in glutamatergic transmission has been extensively studied using EAAT blockers or deficiency but their function in feeding glutamate - dependent metabolic pathways just begins to be explored. Levels of GSH, a tripeptide with major antioxidant functions in the brain that is synthesized from glutamate, cysteine and glycine are highly dependent on EAAT activity in both neurons and astrocytes. Besides glutamate, glial EAATs also mediate the transport of cystine, a cysteine precursor, whereas the neuronal excitatory amino acid carrier 1 (EAAC1) / EAAT3 directly transports cysteine. EAAT dysfunction can directly trigger GSH depletion and astrocyte oxidative death. Deficiency of EAAC1 has been associated with reduced neuronal GSH, increased oxidant levels, and neurodegeneration in the hippocampus at advanced age. This model have been extensively studied in rats [13].

Procedure

Male Wistar rats weighing between 250 and 350 g were housed individually with food and water on a 12h light / dark cycle. After rats were anesthetized with chloral hydrate (400mg/kg, i.p.) , a 21 gauge guide cannula with a stainless steel tip protruding 2 mm below the dura was implanted into each rat as described. A plastic stylet (Plastics One) that also extended 2 mm below the dural surface was screwed into the guide cannula following solidi fication of the dental acrylic. Rats were given a postoperative analgesic ketorolac (Toradol); 3mg/kg, i.p.] and allowed to recover at least 48h before microdialysis probe insertion[14].

LPS

Inflammation has recently emerged as a key player in PD pathogenesis and all neurotoxins currently used in experimental models generate a neuroinflammatory response in the nigrostriatal tract. These considerations have

prompted the introduction of another toxic model, in which bacterial endotoxin lipopolysaccharide (LPS), which causes intense tissue inflammation, is directly infused into the nigrostriatal pathway of rats. Intranigral injection of LPS results in activation of microglia and degeneration of the dopaminergic system. This model have been extensively studied in rats[15].

Procedure

Male Sprague - Dawley (SD) rats (230–250g in weight, 7 weeks of age) were anesthetized by intraperitoneal injection of ketamine (40 - 80 mg/kg) and xylazine (5 - 10 mg/kg). LPS (100 - 500 µg in 250 µl aliquots of sterile PBS) was administered iv through the tail vein. PBS-injected animals were used as controls. Tissue preparation Rats were anesthetized and transcardially perfused with saline solution containing 0.5 % (w/v) sodium nitrate and heparin (10 U/ml), followed by 4 % (v/v) paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, to achieve tissue fixation. Brains were obtained and post-fixed overnight at 4 ^{0} C in 4 % (v/v) paraformaldehyde[16]. Fixed brains were added, at 4 ^{o} C, to 30 % (w/v) sucrose solution until the brain segments sank. Six separate series of coronal brain slices, with each slice being 30 µm in thickness, were obtained using a sliding microtome. For RNA preparation, rats were anesthetized and transcardially perfused with saline solution without paraformaldehyde. Brains were sliced using a Rat Brain Slicer Matrix (1.0 mm slice intervals) and a razor blade. The slice including the needle injection spot was selected, and tissue blocks (2 × 2 × 2 mm3) just below the needle tip were prepared and stored at - 70^{o} C until use[17].

α-Synuclein preformed fibrils

Now a days most of the researchers use a Syn PFFs as a model for PD. These are produced from monomers. α-Synuclein preformed fibrils are produced in three stages include preparing the monomers, generating PFFs from the monomers, and preparing the PFFs for use. For optimal PFF formation, the pH should be between 7-8 (optimal around pH 7.4) and the salt concentration should be approximately 100 mM NaCl[17]. It is recommended to store aSyn monomers at a concentration no higher than 7.5 mg/mL to enable proper PFF formation and sufficient quantity of sample for quality control and experimental use. aSyn PFF samples should not be stored at 4◦C o r - 20◦C. This model have been extensively studied in rodents, rats and mice[18].

Procedure

When using aSyn PFFs for in vivo studies, it is recommended to use freshly - generated aSyn PFFs rather than frozen aliquots of aSyn PFFs. In this case, aSyn PFFs should be generated 1-2 weeks before the surgical session and stored in single - use aliquots at room temperature. For intracerebral injections, it is recommended to use a pulled pipette glass needle attached to a Hamilton syringe to minimize tissue injury and reduce non –target placement and infusion. When performing the injection, it is strongly recommended to slowly infuse the injectate at a rate of at least 0.5 l / minute and to leave the needle in place 2-5 minutes after each injection to allow the solution to permeate the parenchyma and prevent the solution from being suctioned up the needle tract. By far, the striatum is the most common target for aSyn PFF injections [29, 30, 34, 35, 47, 51], with injection into the dorsal striatum leading to midbrain pathology specifically in the SNpc[19].

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