Dysphagia in 6-Hydroxydopamine-Treated Rodents: Behavioral and Manometric Studies

Shin-ichi Iwata, MD

Abstract

Dysphagia is a serious problem in patients with Parkinson's disease (PD) because it causes malnutrition and aspiration pneumonia. In this study, dysphagia was examined in a PD rodent model induced with 6-hydroxydopamine (6-OHDA). Video-recording observation showed that the animals could gnaw food pellets but could not swallow them. However, they could swallow gelled food. L-DOPA administration did not improve the dysphagia. The result suggested that the upper esophageal sphincter (UES) may be impaired. Therefore, pressure of the UES was measured using a catheter with a small balloon in awake animals because UES pressure decreases under anesthesia. The pressure was unaltered in the PD model. Administration of neither haloperidol, which induces muscle rigidity, nor atropine, which relaxes esophageal smooth muscle, altered UES pressure. These results suggest that the pharyngeal phase in deglutition may be involved in dysphagia in the PD model.

Key words: Dopamine, Dysphagia, 6-Hydroxydopamine, Parkinson's disease

Dysphagia is a serious problem in patients with Parkinson's disease (PD) because it induces malnutrition and aspiration pneumonia (Kalf et al. 2012). Although several reports have elucidated mechanisms of dysphagia in PD (Ertekin 2014; Johnston et al, 2001), an effective remedy against dysphagia has not been developed. In general, animal models are effective tools for clarifying the mechanisms of diseases. Although 6-hydroxydopamine (6-OHDA)-treated rodents show dysphagia (Ungerstedt 1971), the precise mechanism has not been examined. Therefore, dysphagia in a 6-OHDA rodent model of PD was examined in the present study.

METHODS

6-OHDA rodent models

Male 10-to 12-week-old DDY mice (Kyudo Company, 883-1 Tateishimachi, Tosu, Saga 841-0075), weighing 43-50 g, were used in a behavioral study. All surgeries were performed under 50 mg/kg pentobarbital anesthesia (Nembutal, Abbott Laboratories, North Chicago, IL, USA). To induce degeneration of striatal dopaminergic terminals, a 40-μg dose of 6-OHDA (Sigma, St. Louis, MO) was dissolved in 4 μl 0.9% saline containing 0.01% ascorbic acid and was manually infused bilaterally into the striatum (20 μg per side, 40 μg total per animal) using a 1-μl Hamilton microsyringe at a rate of 1 μl /min. 6-OHDA infusions were performed using a stereotaxic apparatus (Model 900, David Kopf Instruments, Pembroke Pines, FL) with the tip of the needle inserted vertically −3.5 mm ventral to the bregma through a small hole in the skull 0 mm rostral and 2.2 mm lateral to the bregma. The needle was left in place for 2 min following the infusion to minimize the spread of the drug through the injection track. Desipramine hydrochloride (Sigma) at 20 mg/kg was dissolved in 40% propyleneglycol containing 10.5% ethanol and 0.34 mg/ml NaOH and intraperitoneally administered 20 min before the 6-OHDA treatment to preserve noradrenergic nerve terminals in the striatum. Control animals received vehicle solution instead of 6-OHDA.

Male 13-week-old Wistar rats (Kyudo Company) weighing 430-480 g were used for the manometric study. To induce degeneration of bilateral striatal

Department of Nutrition, Kagoshima Junshin University
E-mail: shinichi@jundai.k-junshin.ac.jp

dopaminergic terminals, a 120-μg dose of 6-OHDA dissolved in 12 μl 0.9% saline containing 0.01% ascorbic acid (i.e., the concentration was 10 μg/μl) was manually infused bilaterally into the striatum using a 10-μl Hamilton microsyringe at a rate of 1 μl/min. 6-OHDA infusions were performed using the stereotaxic apparatus with the tip of the needle inserted vertically −6.0 mm from the surface of the skull. The first infusion site was at 2.0 mm rostral and 2.5 mm lateral to the bregma, the second was at 0 mm rostral and 3.0 mm lateral to the bregma, and the third was at −1.0 mm rostral and 4.0 mm lateral to the bregma. Therefore, six lesions were made in each rat, and the dose was 20 μg/site. The needle remained in place for 2 min following the infusion. Desipramine hydrochloride was administered as described above. Control animals received the vehicle solution instead of 6-OHDA.

Behavioral observation

Natural behavior, food handling, gnawing, mastication, and swallowing in the 6-OHDA-treated mice were observed by video-recording in a transparent plastic cage 4 days after 6-OHDA injections. To investigate the ameliorating effect of L-DOPA on dysphagia, 6-OHDA-treated mice were administered 60 mg/kg L-DOPA + 15 mg/kg benserazide. L-DOPA (Sigma) was dissolved in 0.05 N HCl, and benserazide HCl (Ro 4-4602, Santa Cruz Biotechnology) was dissolved in distilled water. Both drugs were injected simultaneously twice a day from the day after the 6-OHDA injection for 3 days. Then, the state of dysphagia was observed 30 min after the last administration of L-DOPA + benserazide.

Manometric study

The upper esophageal sphincter (UES) pressure was measured using a catheter equipped with a small plastic balloon (Fig. 1) connected to a transducer (PAS-111, Star Medical, Arakawa, Tokyo) and recorded with an X-Y recorder (8U16, San-ei Sokki Co. LTD, Kodaira, Tokyo) 4 days after 6-OHDA injections. Preliminary experiments showed that the pressure was greatly reduced under ether anesthesia (Fig. 3B), and the pressure was therefore measured in awake animals. The rat was anesthetized with ether, and the catheter and a deflated balloon were inserted into the stomach. Insertion of the catheter into the esophagus after intubation with a plastic tube into the trachea was feasible. When the catheter reached the stomach, the balloon was filled with 0.3 ml air. The rat was food-deprived for 24 hours before the measurement, since food in the stomach disrupts the pressure measurement. The rat was restrained manually by two persons, and the mouth was kept open by one pair of incisor bars from the stereotaxic apparatus. The catheter was slowly pulled out, and intra-esophageal pressure was recorded when the rat woke up from the anesthesia. The effect of drugs on esophageal pressure was examined with 2 mg/kg haloperidol and 1 mg/kg atropine. Haloperidol was intraperitoneally administered 45 min before (when the back muscle showed rigidity) the measurement of esophageal pressure. Atropine was intraperitoneally administered 15 min before the measurement of esophageal pressure.

Immunoblotting

The level of tyrosine hydroxylase (TH) protein in the striatum was measured to determine the degree of lesioning in 6-OHDA-treated rodents as previously described (Iwata et al, 2001). Rodents were decapitated just after the behavioral or manometric study ended, and the entire bilateral striata were removed onto an ice-cold plate. The striata were then sonicated in 250 μl 1% SDS. A portion of the solution was used for a BCA protein assay (Pierce, Rockford, IL). TH protein was detected by immunoblotting, and the densities of bands

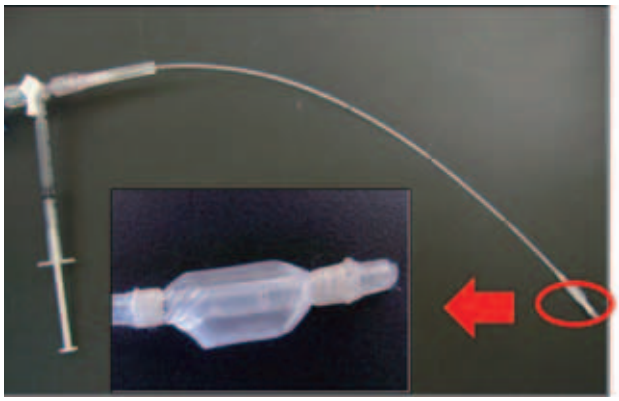

Fig.1 The apparatus used for manometric measurement of intra−esophageal pressure.

The diameter and length of the balloon were 5 and 15 mm, respectively. The balloon was deflated when it was inserted into the stomach, and then it was filled with 0.3 ml air. The balloon was slowly pulled by hand.

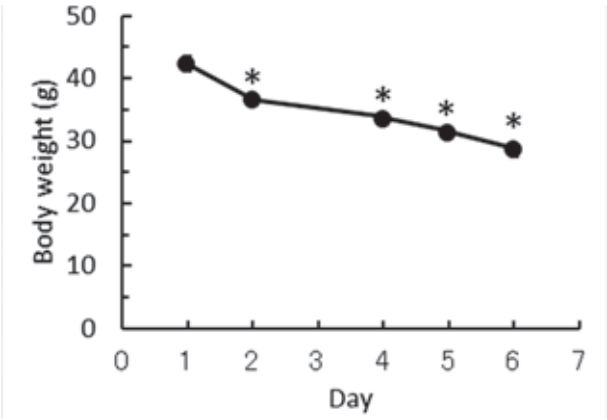

Fig.2 Time course of the effect of 6-OHDA injections into the striatum on the body weight of mice.

6-OHDA was injected on day 1. The body weights at all time points were significantly decreased compared with body weight at day 1 (one-way ANOVA, followed by Fisher's Protected Least Signicicant Difference(PLSD), $p < 0.001$) of the 6-OHDA injection.

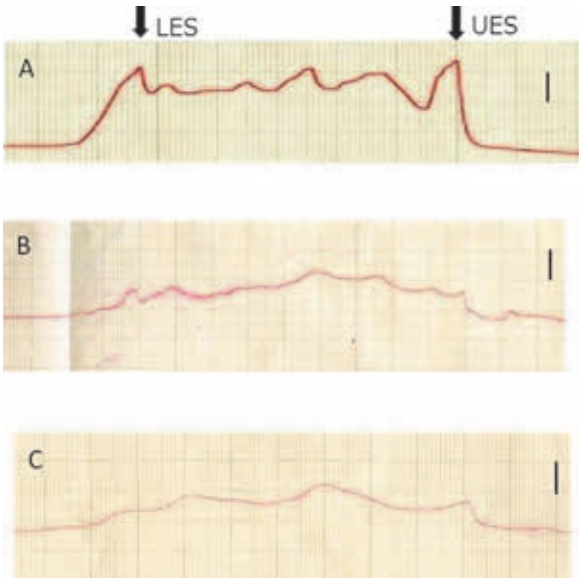

Fig.3 Examples of manometric measurement of intra-esophageal pressure in an awake rat that has emerged from ether anesthesia (A), in a rat under deep anesthesia (B), and in a dead rat (C).

LES: lower esophageal sphincter. UES: upper esophageal sphincter. Bars mean 10 arbitrary units of pressure.

Table UES pressure after various treatments

	pressure (arbitrary unit)
	mean ±SE (n)
Control	32 ± 8 (6)
6-OHDA	34 ± 7 (6)
Haloperidol	34 ± 5 (6)
Atropine	31 ± 4 (6)
Ether anesthesia	14 ± 2 (6) *

* Significant decrease compared to the control (one-way ANOVA followed by Fisher's PLSD, $p < 0.05$).

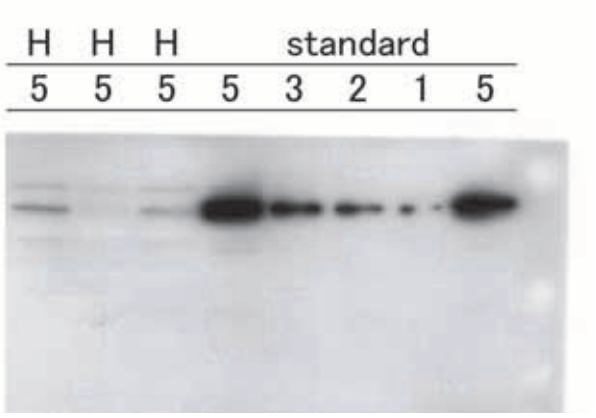

Fig.4 An immunoblot of TH in the mouse striatum treated with 6-OHDA.

The standard was made by loading different amounts of protein (1-5 μg protein). H: 6-OHDA

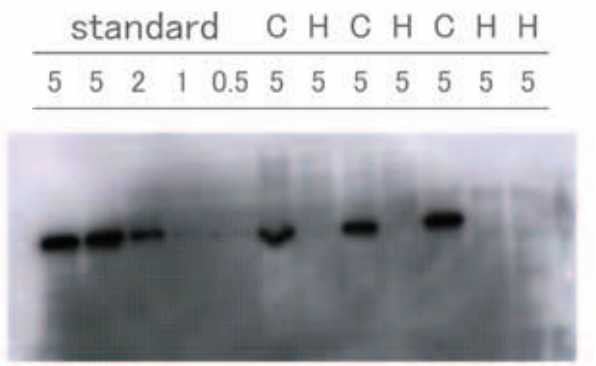

Fig.5 An immunoblot of TH in the rat striatum treated with 6-OHDA.

The standard was made by loading different amounts of protein (0.5-5μg protein). C: vehicle-treated control, H: 6-OHDA were quantified using NIH Image (Abramoff et al, 2004).

The present experiment was carried out after obtaining permission from the Committee of Animal Experimentation, Kagoshima Junshin University.

RESULTS

Mice greatly lost body weight after 6-OHDA treatment (Fig. 2) because they could not eat food. Behavioral observations revealed that 6-OHDA-treated mice could gnaw a food pellet, but the pellet spilled from the both sides of the mouth. These mice appeared to have difficulty with deglutition, but they were able to eat commercially available gel food (Tomlyon Nutri-StatTM). The average weight loss ratios 3 days after 6-OHDA treatment in L-DOPA-administered mice (n = 4) and in vehicle-administered control mice (n = 5) were 21% and 20%, respectively. Video-recording observation showed that L-DOPA did not ameliorate 6-OHDA-

induced dysphagia. Immunoblotting showed that TH contents in 6-OHDA-treated mice decreased to less than 20% of those in controls (Fig. 4), indicating that dopaminergic neurons were sufficiently destroyed.

Intra-esophageal pressure was successfully recorded (Fig. 3A) and was significantly decreased under ether anesthesia (Fig. 3B). We observed no alterations in pressure between in 6-OHDA-treated rats and vehicle-treated rats. Neither haloperidol nor atropine affected the pressure (Table). The TH level in these rats was reduced to below 10% of control (Fig. 5), indicating that dopaminergic neurons were sufficiently destroyed.

DISCUSSION

The 6-OHDA-treated mice could not swallow a food pellet, but could eat gelled food, indicating that these animals experienced dysphagia and not aphagia. In general, gel food is easier to swallow than regular meals for dysphagic patients. Therefore, the present rodent model is suitable for investigating dysphagia. L-DOPA did not ameliorate the dysphagia. Dysphagia in PD is not solely related to dopamine deficiency (Hunter et al., 1997), and the Hoehn & Yahr score and the degree of dysphagia are not correlated (Nilsson et al, 1996). Furthermore, dopaminergic medication does not affect swallowing ability (Michou and Hamdy, 2010). In the present study, 6-OHDA was injected directly into the striatum, and desipramine pretreatment was performed before 6-OHDA treatment, indicating that the lesion was confined to the nigrostriatal dopaminergic system. These results suggest that dysphagia in PD is induced by impairment of the nigrostriatal dopaminergic pathway, but that it cannot be reversed by L-DOPA supplemental therapy.

The upper one-third of the esophageal muscle consists of striated muscle (Crary and Groher, 2003). 6-OHDA-treated rats and haloperidol-administered rats showed muscle rigidity in their back muscles. Incomplete UES relaxation and reduced UES opening have been reported in PD (Ali et al, 1996; Higo et al, 2001). Therefore, we considered that UES pressure must have increased. However, the present study showed that the UES pressure was unaltered by dopaminergic denervation or haloperidol administration.

Atropine administration did not alter UES pressure in the present study. This is consistent with a study reporting no effects on UES relaxation or swallowing coordination with atropine (Knauer et al, 1990).

Technically, measuring intra-esophageal pressure in an awake rat was difficult. We had to keep the rat immobile because the pressure suddenly increased when the rat moved. The increased pressure of the UES was caused by an increase in tension from the esophageal wall to its proper pressure. For measurement of lower esophageal sphincter pressure in rats, a catheter of 4 Fr outer diameter is more suitable as no tension is present from the esophageal wall (Iino et al. 1983). In the present study, intra-esophageal pressure in the dead animal (Fig. 3C) was not zero, which means that the balloons used detected tension in the esophagus. However, the tension was low enough to allow detection of esophageal pressure, especially in the region of the sphincter.

In summary, lesioning the bilateral dopaminergic system in the striatum is an appropriate model for dysphagia in PD. The dysphagia was not ameliorated by L-DOPA. UES pressure was unaltered in the model, which suggests that the pharyngeal phase in deglutition may be impaired in the model.

ACKNOWLEDGEMENT

This experiment was performed by undergraduate students, A. Onoue, R. Iwashita, M. Sonoda, T. Kamikukita, S. Tou, K. Nishiyama, K. Fujisaki, and M. Yanaidani under the supervision of S.I.

REFERENCES

Abramoff, MD, Magalhães PJ, Ram, SJ. Biophotonics international 2004, 11:36-42.

Ali, G. N., Wallace, K. L., Schwartz, R., DeCarle, D. J., Zagami, A. S., and Cook, I. J. (1996) Mechanisms of oral-pharyngeal dysphagia in patients with Parkinson's disease. Gastroenterology, 110:383-92.

Ertekin, C. (2014) Electrophysiological evaluation of oropharyngeal Dysphagia in Parkinson's disease. J Mov Disord, 7:31-56.

Crary, M. A. and Groher, M. E. (2003) Introduction to adult swallowing disorders. Butterworth-Heinemann, Oxford.

Higo, R., Tayama, N., Watanabe, T., and Niimi, S. (2001) Abnormal elevation of resting pressure

at the upper esophageal sphincter. Eur Arch Otorhinolaryngol, 258:552-6.

Hunter, P. C., Crameri, J., Austin, S., Woodward, M. C., and Hughes, A. J. (1997) Response of parkinsonian swallowing dysfunction to dopaminergic stimulation. J Neurol Neurosurg Psychiatry, 63:579-83.

Iino, M., Takahashi, H., Maie, M., Oonuma, N., Nagai, Y., Aoyagi, H., and Iwai, J. (1983) Influence of the catheter diameter on the manometric measurements of the lower esophageal sphincter (LES); with special reference to the application for children. Nihon Heikatsukin Gakkai Zasshi, 19:415-22. '(abstract in English)'

Iwata, S., Nomoto, M., and Fukuda, T. (2001) Regulation of GAP-43 protein and mRNA in nigrostriatal dopaminergic neurons after the partial destruction of dopaminergic terminals with intrastriatal 6-hydroxydopamine. Synapse, 39:16-22.

Johnston, B. T., Colcher, A., Li, Q., Gideon, R. M., Castell, J. A., and Castell, D. O. (2001) Repetitive proximal esophageal contractions: a new manometric finding and a possible further link between Parkinson's disease and achalasia. Dysphagia, 16:186-9.

Knauer, C. M., Castell, J. A., Dalton, C. B., Nowak, L., and Castell, D. O. (1990) Pharyngeal/upper esophageal sphincter pressure dynamics in humans. Effects of pharmacologic agents and thermal stimulation. Dig Dis Sci, 35:774-80.

Michou, E., and Hamdy, S. (2010) Dysphagia in Parkinson's disease: a therapeutic challenge? Expert Rev Neurother, 10:875-8.

Nilsson, H., Ekberg, O., Olsson, R., and Hindfelt, B. (1996) Quantitative assessment of oral and pharyngeal function in Parkinson's disease. Dysphagia, 11:144-50.

Ungerstedt, U. (1971) Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol Scand Suppl, 367:95-122.

6－ハイドロキシドパミンパーキンソン病げっ歯類モデルにおける嚥下障害の研究：行動学的研究と食道内圧の測定

岩田　真一

鹿児島純心女子大学看護栄養学部健康栄養学科

要　旨

嚥下障害はパーキンソン病患者の多くに起こる重大な障害です。患者は食事が摂取できなくなるだけでなく，誤嚥性肺炎をおこし死亡の原因ともなります。パーキンソン患者で嚥下造影検査など多くの研究がされているが，動物モデルによる機序の研究はほとんどされていない。そこで，本研究ではパーキンソン病動物モデルを作成して，嚥下障害の研究を行った。

パーキンソン病モデル作成は6- ハイドロキシドパミンを使用した。6- ハイドロキシドパミンはドパミンの神経終末に取り込まれて，ドパミン神経を破壊する神経毒である。血液脳関門を通過しないので，脳内投与を行った。投与部位は線条体で，マウスの場合は一側の線条体に１ヶ所のみ投与したが，ラットの場合は一側に３ヶ所投与し，線条体を広く十分に傷害するようにした。また，嚥下障害は両側の線条体を破壊しないと出現しないので，両側破壊した。その場合，動物は嚥下障害のために１週間ほどで死亡した。マウスの場合 6- ハイドロキシドパミンは 40 μg 投与し，線条体のドパミン神経終末は 80％以上破壊された。ラットの場合は 120 μg 使用し，線条体のドパミン神経終末は 90％以上破壊された。

パーキンソン病モデル動物は餌のペレットをかじることは出来たが，それを飲み込むことができず，口の両脇からこぼれ落ちた。市販の動物用のゲル状の餌はよく飲み込むことが出来た。ヒトの嚥下障害でもゲル状の形態の食事は嚥下しやすいことが知られており，その現象に類似していると考えられた。L-DOPA の投与は嚥下障害を改善しなかった。

上部食道は組織学的に骨格筋で出来ており，筋強剛が起こり，そのため嚥下障害が起こっている可能性があったので，食道内圧を測定することとした。内圧はプラスチックの風船をカテーテル先端につけたものを使用して計測した。麻酔下では食道平滑筋のトーヌスが低下することが判明したので，無麻酔下で測定した。上部食道括約筋の内圧はパーキンソンモデル動物と正常の動物と同じであった。強い筋強剛を誘発するハロペリドールも平滑筋の収縮を抑制するアトロピンも食道内圧を変化させなかった。

以上の結果から，このモデルでの嚥下障害は咽頭相で生じていると考えられた。