Effect of Chronic Glutathione Administration on Striatal Dopaminergic Terminals in Intrastriatal 6-Hydroxydopamine-Treated Rats

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Abstract

Many reports have shown that abnormalities in glutathione are related to the onset of Parkinson's disease. Therefore, the ameliorating effect of glutathione in an animal model of Parkinson's disease was studied. The model was made by injections with 6-hydroxydopamine (6-OHDA) into both sides of the striatum. In this model, dopaminergic terminals in the striatum gradually regenerate after an initial degeneration. The amount of tyrosine hydroxylase, which is a marker of dopaminergic terminals, in the striatum after glutathione (100 mg/kg, intraperitoneally) administration twice a week for 8 weeks was compared with vehicle-treated 6-OHDA-treated rats. No significant differences were observed between the two groups. Thus, glutathione does not affect dopaminergic terminals.

Key words: dopaminergic neuron, glutathione, 6-hydroxydopamine, Parkinson's disease, striatum

A small group of physicians have been promoting glutathione therapy for Parkinsonian patients and charging a fee for infusion of glutathione in their office practices in Japan and North America (Okun and Lang, 2010). This treatment is based on a report by Sechi et al. (1996) in which intravenous infusion of glutathione into nine patients with early untreated Parkinson's disease produced a 42% decline in disability during the infusions. However, in a study that examined 21 patients, intravenous administration of glutathione produced no significant improvement (Hauser et al, 2009).

Many reports have shown that glutathione modulates degeneration of dopaminergic neurons in Parkinson's disease (Smeyne and Smeyne, 2013). Therefore, in the present study, an animal model of Parkinson's disease was assessed to investigate the beneficial effects of glutathione on dopaminergic neurons.

METHODS

Male 12-week-old Wistar rats (Kyudo Company, 883-1 Tateishimachi, Tosu, Saga 841-0075, Japan) weighing 350-410 g were used. The animals were housed with free access to standard food in an air-conditioned room. All efforts were made to minimize animal suffering and to reduce the number of animals used. The present experiments were carried out after obtaining permission from the Committee of Animal Experimentation of Kagoshima Junshin University.

Pentobarbital (50 mg/kg, intraperitoneally (i.p.) was used for anesthesia. To induce degeneration of dopaminergic terminals in the striatum, a 12-µg dose of 6-hydroxydopamine (6-OHDA) (Sigma, St. Louis, MO) dissolved in 3-µl 0.9% saline containing 0.1% ascorbic acid, was injected into both sides of the striatum using a 30-gauge needle at a rate of 1 µl/min. Desipramine hydrochloride (Sigma, 20 mg/kg) dissolved in 40% propyleneglycol containing 10.5% ethanol and 0.34 mg/ml NaOH was administered i.p. 20 min before the 6-OHDA treatment to preserve noradrenergic terminals in the striatum. Using a stereotaxic apparatus (Model 900, David Kopf Instruments, Pembroke Pines, FL), the tip of the needle was inserted vertically through a small hole in the skull 0.5 mm rostral and 3.0 mm lateral to the bregma and −5 mm ventral to the brain surface.

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The needle remained in place for 2 min following the infusion to minimize the spread of the drug through the injection track.

Glutathione (Tathion®, Astellas Pharma Inc., Tokyo, Japan, 100 mg/kg) was dissolved in distilled water and administered i.p. twice a week (every Tuesday and Friday) for 8 weeks. Control rats were given saline.

Rats were decapitated 56 days following lesioning, and both sides of the striatum were removed onto an ice-cold glass plate using a brain-cutting block and then sonicated in 500 μl 1% sodium dodecylsulfate. The animals were deeply anesthetized with ether before they were decapitated. A portion of the solution was used for a BCA protein assay (Pierce, Rockford, IL).

Total protein (5 μg/lane) was separated on a 10% polyacrylamide gel. The proteins were transferred onto a PVDF membrane (immobilon-P, Millipore Co., Billerica, MA) at 30 V for 16 h. The blots were blocked in Tris-buffered saline (10 mM Tris, 150 mM NaCl, pH 7.4) containing 0.1% Tween 20 and 1% bovine serum albumin (blocking buffer) and incubated with anti-tyrosine hydroxylase (TH) monoclonal antibody (MAB 318, 1:4000, Chemicon International, Inc., Temecula, CA) for 1 h. The blot was then incubated with anti-mouse IgG horseradish peroxidase (A5278, 1:40,000, Sigma) for 30 min. Immunoreactivity on the blot was visualized with enhanced chemiluminescence (ECL) techniques (ECL Plus, Amersham Co., Arlington Heights, IL). Image analysis was performed by Image-J (Abramoff et al, 2004).

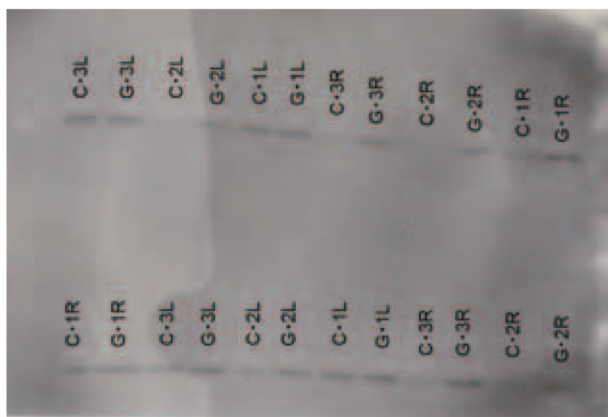

Fig. An immunoblot of TH in the striatum 56 days after 6-OHDA.

Each sample was loaded in two different gels (an upper lane and a lower lane). G: glutathione-administered rats. C: vehicle-administered rats. R: right striatum. L: left striatum.

RESULTS

The densities of the TH bands (average ± standard error (number of striata)) in glutathione-administered rats and in control rats were 228 ± 10 (6) and 215 ± 10 (5), respectively (Fig.). The difference was not statistically significant (Student's t-test, p = 0.41).

DISCUSSION

The present result shows that chronic administration of glutathione had no effect on the amount of TH in rats treated with 6-OHDA.

We used a striatal 6-OHDA injection rat model in the experiments. This model is suitable for investigating degeneration and regeneration of dopaminergic neurons, because dopaminergic terminals gradually degenerate, allowing researchers to assess the effect of drugs (Blandini et al, 2008). It was previously shown that dopaminergic terminals are gradually restored with this model (Iwata et al, 2001).

Two experimental protocols can be used in 6-OHDA animal models to identify a substance with a beneficial effect on nigrostriatal dopaminergic neurons. In one protocol, the substance is pretreated before 6-OHDA injection. In the other, the substance is administered after 6-OHDA. Pretreatment with a substance implies protection of dopaminergic neurons from the toxic effect of 6-OHDA. Many substances are protective against this toxicity (Santos, 2012). The pretreatment regimen is not suitable for identifying a substance that preserves dopaminergic neurons in Parkinson's disease for two reasons. The first is that a longitudinal prospective cohort positron emission tomography study showed that dopaminergic neurons die faster in the early phase of Parkinson's disease, i.e., 5-6 years before disease onset (Hilker et al, 2005), when parkinsonian symptoms are not apparent. Therefore, a substance considered to be protective should be taken at least 5-6 years before disease onset. As we are currently unable to predict who will develop Parkinson's disease, such protective measures are inappropriate. The second rationale is that, in the 6-OHDA striatal model, dopaminergic terminals in the striatum initially degenerate due to the direct effect of 6-OHDA. Then, the dopaminergic neuronal soma with severally damaged terminals due to the direct effect of 6-OHDA gradually degenerate

(retrograde degeneration). Subsequently, the terminals that survived the direct effect of 6-OHDA begin to degenerate due to the degeneration of the dopaminergic soma (anterograde degeneration; Wallerian degeneration). This implies that successive degeneration of dopaminergic neurons cannot be inhibited by a substance that blocks the direct toxic effect of 6-OHDA. Therefore, the post-treatment regimen in 6-OHDA animal studies is more suitable than pre-treatment for identifying disease-modifying substances.

Several substances show a protective effect on dopaminergic neurons after administration of 6-OHDA, including calcitriol (Smith et al, 2006), melatonin (Ozsoy et al, 2015), hesperidin (Antunes et al, 2014), fibroblast growth factor (Sleeman et al, 2012), and hydrogen water (Ito et al, 2012).

In the present study, glutathione failed to regenerate dopaminergic terminals in the striatum, as shown by the absence of an increase in TH. Glutathione should not be used in Parkinsonian patients as both an animal and a human study showed no effect on Parkinson's disease.

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パーキンソン病モデルラットにおけるグルタチオンの
ドパミン神経終末再生効果の研究

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要　旨

パーキンソン病治療薬としては，L-DOPA などのドパミン補充薬などが広く使用されているが，疾患自体を治す薬物，いわゆる disease-modifying drug は存在しない。このような難病の場合，患者心理に付け入る健康食品の類は後を絶たない。グルタチオンは薬物中毒などに処方される医薬品であるが，その還元作用，スーパオキサイド除去作用から，老化防止作用を期待して健康食品としても売られている。一部の医師はパーキンソン病の進行を抑制すると信じて，自費診療しているがその裏付けとなる論文は存在しない。そこで，パーキンソン病モデル動物を使用してグルタチオンのドパミン神経への作用を検討した。

パーキンソン病モデルとしては，ドパミン神経が徐々に逆行変性し，その後，sprouting によりドパミン神経終末が徐々に回復する 6- ハイドロキシドパミン線条体投与ラットモデルを採用した。6- ハイドロキシドパミンは 12 μg を線条体に直接投与した。グルタチオンは 6- ハイドロキシドパミン投与の 3 日後から週 2 回，100 mg/kg を 8 週間，腹腔内投与した。コントロール群は生理食塩水を投与した。8 週後に線条体を取り出して，SDS-PAGE でチロシン水酸化酵素を分離し，免疫ブロットを行い，チロシン水酸化酵素量を両群で比較した。チロシン水酸化酵素はドパミン神経終末のマーカーとして利用した。結果は両群で全く差がなかった。

グルタチオンはスカベンジャーなので，酸化による組織障害を抑制できる可能性はある。しかし，パーキンソン病におけるドパミン神経変性の機序として，酸化説はあるがはっきりとはわかっていない。また，そもそもグルタチオンが脳内に入るかどうかもはっきりとはしていない。健康食品，民間療法の疾患改善効果をすべて否定するわけではないが，今回の実験プロトコルではグルタチオンの効果は否定された。パーキンソン病の患者は，症状に対する精神状態の影響が強く，そのためプラセボ効果が強く，治験の難しさが指摘されている。グルタチオンの場合も患者へ効果を証明するたまにはダブルブラインドなどのエビデンスの高い臨床治験が必要と思われる。また，条件を変えた動物実験も行うべきかもしれない。