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Neuroprotective Effect of D-Psicose on 6-Hydroxydopamine-Induced Apoptosis in Rat Pheochromocytoma (PC12) Cells

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We evaluated the neuroprotective effects of D-psicose, one of the rare sugars, on 6-hydroxydopamine (6-OHDA)-induced apoptosis in catecholaminergic PC12 cells, the *in vitro* model of Parkinson's disease (PD). Apoptotic characteristics of PC12 cells were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling (TUNEL) assay. The results showed that D-psicose at a concentration of 50 mM, exerted significant protective effects against the 6-OHDA (200 μM)-induced PC12 cell apoptosis, while other sugars had little or no protective effects. We have observed a significant increase in the level of intracellular glutathione after 24 h in 6-OHDA (200 μM) treated cells, while a decrease in the level was observed at 3 h and 6 h. Also, a synergistic exposure to D-psicose and 6-OHDA for 24 h showed a significant increase in intracellular glutathione level. Therefore, these results suggest that D-psicose may play a potential role as a neuroprotective agent in the treatment of neurodegenerative diseases by inducing an up-regulation of intracellular glutathione.

[**Key words:** rare sugars, D-psicose, 6-hydroxydopamine (6-OHDA), Parkinson's disease (PD), apoptosis, antioxidant, PC12 cells]

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the preferential degeneration of dopaminergic neurons in the substantia nigra pars compacta and a depletion of the neurotransmitter dopamine in the striatum (1). Several biochemical evidences suggest that oxidative stress mainly caused by an overproduction of reactive oxygen species (ROS) is involved in the pathogenesis of Parkinson's disease (PD). Previously, it was indicated that ROS could injure cells and induce cell death via apoptosis (2–4). Also, several studies showed that natural antioxidants can reverse neuronal cell death *in vitro*, and may have therapeutic properties for neurodegenerative diseases (5, 6).

Rare sugars are defined by the International Society of Rare Sugars (ISRS) as monosaccharides and their derivatives that are rare in nature (The 1st International Symposium of ISRS, Takamatsu, Japan, 2002). These sugars have received increasing significant attention in recent years for a variety of usages, such as low-calory carbohydrate sweeteners and bulking agents (7). However, the biological functions and physiological implications of rare sugars have been rarely reported.

Recently, the establishment of rare sugar production method by Takeshita *et al*. enabled the sufficient upscale production of rare sugars, D-psicose from D-fructose using an immobilized D-tagatose 3-epimerase bio-reactor (8), and D-allose from D-psicose using an immobilized L-rhamnose isomerase one (9). The inter-conversion between D-glucose, D-fructose, D-psicose and D-allose catalyzed by D-xylose isomerase, D-tagatose 3-epimerase and L-rhamnose isomerase, respectively is schematically shown in Fig. 1. Recently, we have started an extensive study to elucidate the physiological functions of rare sugars. We observed that D-psicose and D-allose possess a stronger scavenging activity toward ROS than D-glucose and D-fructose, and that D-allose has a potent inhibitory effect on the production of ROS from stimulated neutrophils (10). The intriguing inhibitory effect on ROS production led us to examine the potential role of these rare sugars in various pathological systems where ROS is involved, such as in ischemia-reperfusion injuries in various organs and tissues. A neuroprotective effect of D-allose and D-psicose has also been observed in the delayed neuronal death of gerbil hippocampus induced by a 5 min ischemia following reperfusion (manuscript in preparation).

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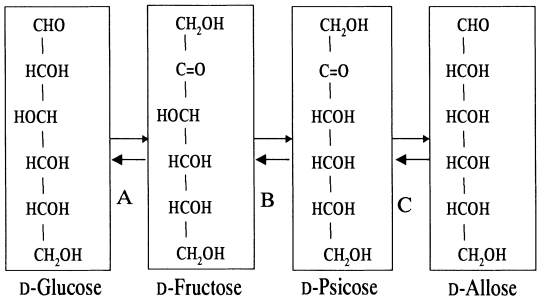

FIG. 1. Interconversion between D-glucose, D-fructose, D-psicose, and D-allose. A, D-Xylose isomerase; B, D-tagatose 3-epimerase; C, L-rhamnose isomerase.

The neurotoxin 6-OHDA (a hydroxylated analogue of dopamine) induces apoptosis in catecholaminergic rat pheochromocytoma PC12 cells and is most commonly used in animal models of PD both in *in vivo* and *in vitro* studies (11, 12). The 6-OHDA, a hydroxylated analogue of the neurotransmitter dopamine, generates ROS via two major mechanisms, being a substrate for monoamine oxidase (MAO) and its auto-oxidation activity, inhibits mitochondrial respiratory chain, leading to the degeneration of dopaminergic neurons (1, 13).

In the present study, we observed the neuroprotective effect of D-psicose in 6-OHDA-treated PC12 cells where we observed an increased intracellular glutathione level.

MATERIALS AND METHODS

Materials Rare sugars were supplied by the Rare Sugar Research Center of Kagawa University (Kagawa). PC12 cells were provided by RIKEN GENE Bank (Ibaraki). Dulbecco's modified Eagle's medium (DMEM), 6-OHDA and the reduced form of glutathione (GSH) were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum was purchased from JRH biosciences (Lenexa, KS, USA). Horse serum was obtained from Thermo Trace (Noble Park, Australia). The total glutathione quantification kit and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye were provided by Dojindo Molecular Technologies (Kumamoto). DeadEND Fluorometric TUNEL System kit was supplied by Promega (Madison, WI, USA).

Culture of PC12 cells PC12 cells were cultured in DMEM containing 10% v/v horse serum, 5% v/v fetal calf serum, 50 units/ml penicillin and 50 μg/ml streptomycin at at 37°C in a 5% CO_2/95% air humidified environment. The cells were plated at an appropriate density according to each experimental scale. After 24 h, the cells were treated with 6-OHDA alone or pretreated with different concentrations of the sugars and/or GSH for 3 h. The PC 12 cells were pretreated with sugars and/or GSH for 3 h prior to 6-OHDA treatments in order to study the effect of sugars and GSH versus 6-OHDA treated cells in the medium. After 3 h pretreatment, the medium was replaced with fresh medium, and then 6-OHDA at 200 μM was added.

Measurement of cell viability by MTT assay PC12 cells were seeded at a density of 1.0×10^4 cells/100 μl/well in 96-well collagen coated plates and the cell viability was determined by the MTT reduction assay (14). After the treatment, at the indicated time, 10 μl of 5 mg/ml MTT stock solution was added into the medium and incubated for 4 h at 37°C in a 5% CO_2/95% air humidified environment. The insoluble dark blue formazan crystal formed in living cells were solubilized with the lysis buffer (20% sodium dodecylsulfate in 50% *N*,*N*-dimethyl formamide) and the absorbance at 570 nm was determined with a microplate reader (Thermo Labsystems, Vantaa, Finland).

Terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling (TUNEL) assay DNA fragmentation was detected by using a commercially available DeadEnd Fluorometric TUNEL System kit (15). PC12 cells were treated with D-psicose or D-fructose onto 8-chamber collagen-coated glass slides. After a 6-OHDA exposure for 24 h, the cells were fixed in 4% methanol free formaldehyde solution in PBS (pH 7.4). The cells were then treated with 0.2% Triton X-100 for permeabilization for 5 min at room temperature and degraded DNA strands were labeled with TUNEL reaction mixture for 1 h at 37°C. After stopping the reaction with 2×SSC, the cells were stained with propidium iodide. Apoptotic cells were detected as localized bright green cells (positive cells) in a red background by fluorescence microscopy. At least four fields were observed and more than 500 cells were counted to determine a statistically significant percentage of apoptotic cells.

Measurement of intracellular glutathione concentration Intracellular total glutathione concentration was measured by using a total glutathione quantification kit (16, 17). Cells were gently washed with PBS and resuspended with 10 mM HCl. After freezing and thawing twice, a solution of 5% sulfosalicylic acid was added at a final concentration of 1% and samples were centrifuged at 15,000 rpm for 10 min. Supernatants were incubated with a reaction mixture containing 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), NADPH, and glutathione reductase in a 96-well plate. The absorbance was measured at 405 nm using a microtiter plate reader (Thermo Labsystems).

RESULTS

Distinct effects of D-psicose and D-fructose on PC12 cell viability PC12 cells were treated with various concentrations of 6-OHDA (100–400 μM) for 24 h and cell viability was measured by the MTT reduction assay. The viability of 6-OHDA-treated cells significantly decreased in a dose-dependent manner (Fig. 2A). We chose the dosage of 6-OHDA at 200 μM which induced about 50% reduction in PC12 cell viability.

We examined the two rare sugars (D-psicose and D-allose) and their epimers (D-fructose and D-glucose), for their neuroprotective activity against 200 μM 6-OHDA-induced apoptosis of PC12 cells. The results showed that D-psicose had a significant protective effect against 6-OHDA-induced apoptosis (Fig. 2B). The survival rate in the presence of 50 mM D-psicose was greater than 75%, while that in the absence of any sugar (6-OHDA alone) was about 45%. The effects of D-glucose and D-allose were weakly protective (56% and 55%, respectively) but were not statistically significant. On the contrary, D-fructose markedly enhanced the 6-OHDA toxicity (by approximately 14%).

Attenuation of 6-OHDA-induced apoptotic cell death by D-psicose The DNA of apoptotic cells is typically detected as oligonucleosomal DNA showing a ladder-like appearance of 180–200 bp fragments in many cell types. TUNEL staining is a commonly used method for detecting DNA fragmentation of apoptotic cells *in situ* (15). Nuclear fragmentation in PC12 cells was observed after exposure to 200 μM 6-OHDA for 24 h (Fig. 3B–D). Treatment with 200 μM 6-OHDA alone showed a ratio of TUNEL-positive cells at 39% (Fig. 3E). Treatment with D-psicose reduced

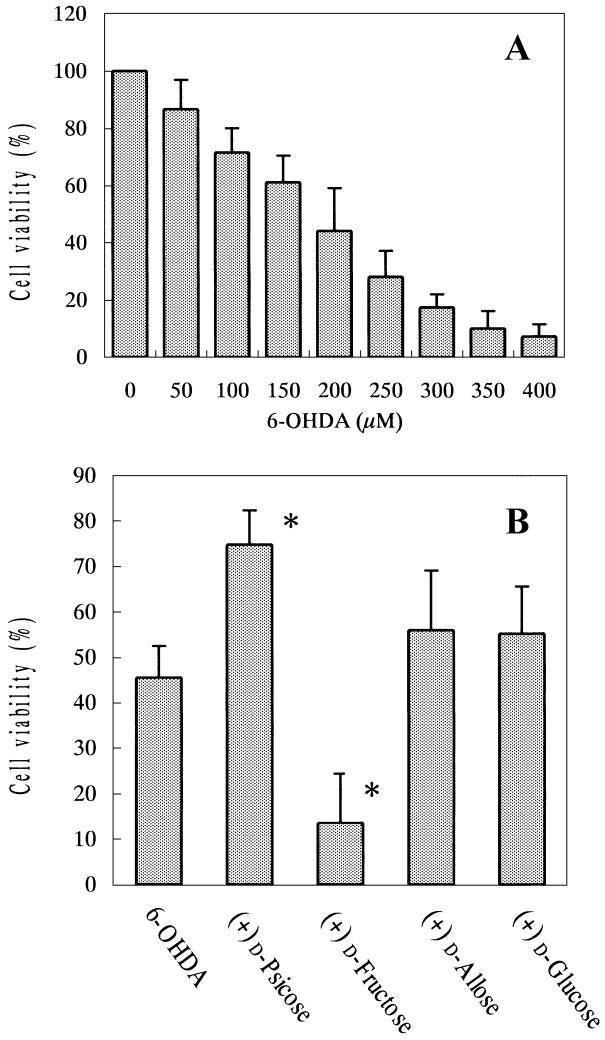

FIG. 2. Effects of sugars on 6-OHDA-induced decrease of PC12 cell viability. Cell viability was measured by MTT reduction assay after treatment with 6-OHDA for 24 h. (A) Dose dependency of 6-OHDA toxicity on PC12 cells. (B) Effect of four sugars (D-psicose, D-fructose, D-allose, and D-glucose) pretreatment for 3 h before 200 μM 6-OHDA treatment. Each value represents mean±SD of quintuplicate determination from a representative experiment. * $p<0.001$ vs 6-OHDA alone using Fisher's PLSD.

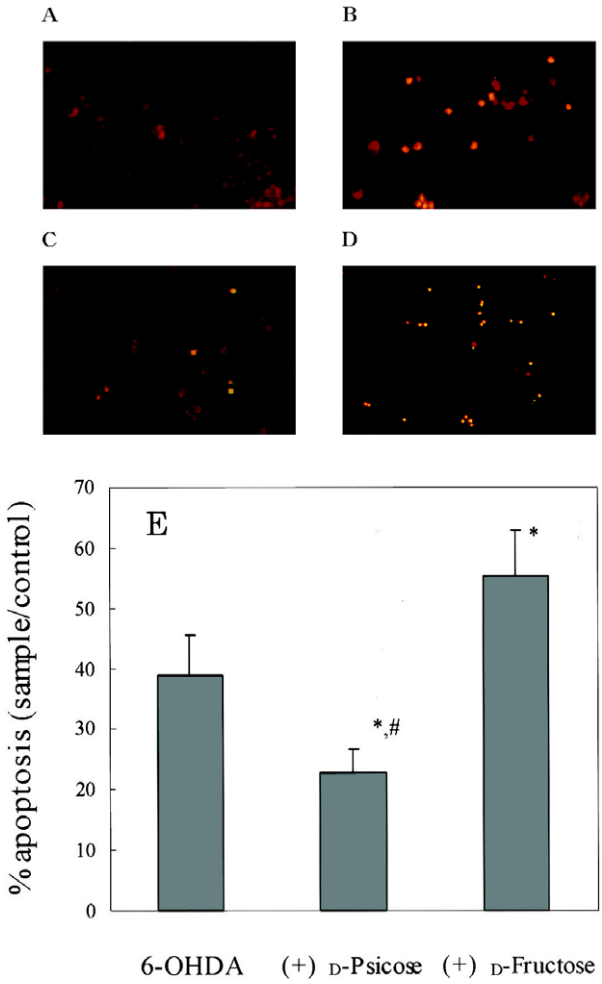

FIG. 3. Effects of D-psicose and D-fructose on 6-OHDA-induced apoptosis in PC12 cells. PC12 cells were treated for 24 h with 200 μM 6-OHDA and apoptosis was detected by TUNEL staining *in situ* as described in Materials and Methods. (A) Control: no 6-OHDA treatment; (B) PC12 cells exposed to 200 μM 6-OHDA for 24 h, PC12 cells pretreated with 50 mM D-psicose (C) and D-fructose (D) for 3 h followed by 200 μM 6-OHDA exposure for an additional 24 h. (E) The percentage of apoptotic cells in each experiment. Results are expressed as mean±SD of triplicate separate experiments. * $p<0.05$ vs 6-OHDA alone, # $p<0.0005$ vs D-fructose using by Fisher's PLSD.

apoptotic cell death rate down to 23%. On the other hand, D-fructose significantly enhanced apoptotic cell death (55%) compared with that by 6-OHDA alone. The degree of cellular protective effect of the two sugars was categorized as D-psicose > none (6-OHDA alone) > D-fructose. These results are inconsistent with the finding of the cell viability shown in Fig. 2.

Effect of pretreatment with D-psicose, D-fructose, and GSH on 6-OHDA-treated PC12 cells After pretreatment with 50 mM D-psicose, D-fructose and 1 mM GSH for 3 h, the medium was replaced with fresh medium and PC12 cells were treated with 200 μM 6-OHDA for 24 h. Cell viability was measured by MTT reduction assay (Fig. 4). Pretreatment with D-psicose for 3 h slightly improved the survival rate of 6-OHDA-treated cells while D-fructose did not (Fig. 4). Pretreatment with 1 mM GSH for 3 h was effective and simultaneous treatment with 1 mM GSH and 50 mM D-psicose induced the best survival rate among the pretreatment materials tested.

Change in intracellular GSH concentration by treatment with sugars GSH, which maintains intracellular redox activity, forms a tripeptide and commonly accounts for more than 99% of the total glutathione (GSH+GSSG, the oxidative form of glutathione) in cells (16, 17). To determine the effect of sugars on endogenous glutathione levels in apoptotic cells, we measured the amount of total glutathione using a total glutathione quantification kit. The decrease in the concentration of endogenous glutathione was evident at 3 h and 6 h in 200 μM 6-OHDA-treated cells in comparison of the control cells (Fig. 5). However, at 24 h, the total GSH concentration was up-regulated in those cells. The treatment with D-psicose significantly elevated the intracellular glutathione concentration at 24 h compared with

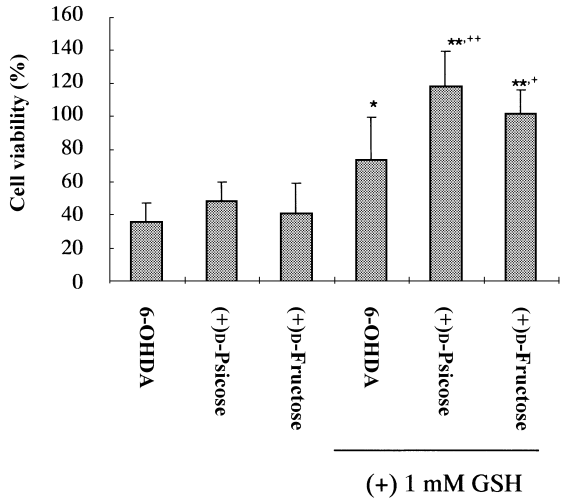

FIG. 4. Effects of pretreatment with D-psicose or D-fructose with or without GSH on 6-OHDA-induced apoptosis in PC12 cells. Cell viability was measured by MTT reduction assay after treatment with 6-OHDA for 24 h. D-Psicose, D-fructose and GSH were added 3 h before 200 μM 6-OHDA treatment. Each value represents mean±SD of quintuplicate determination from a representative experiment. * $p<0.005$ and ** $p<0.0001$ vs 6-OHDA alone, $^{+}p<0.05$ and $^{++}p<0.001$ vs 6-OHDA+1 mM GSH using Fisher's PLSD.

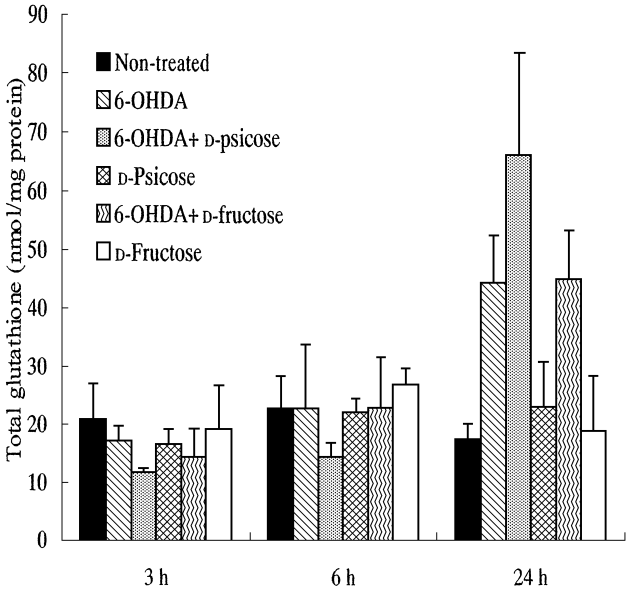

FIG. 5. Total GSH concentration at 3 h, 6 h and 24 h after treatment with 200 μM 6-OHDA. PC12 cells were preincubated with D-psicose or D-fructose and treated with 200 μM 6-OHDA for 24 h. Total GSH concentration was measured using total glutathione quantification kit as described in Materials and Methods.

that of the other 6-OHDA treatments. Hence, the total glutathione concentration of 6-OHDA exposed cells treated with sugars at 24 h was categorized as D-psicose > none (6-OHDA alone) = D-fructose. The result is similar to those of the cell viability assay, therefore, this indicates the effectiveness of D-psicose for 6-OHDA-induced apoptosis of PC12 cells.

DISCUSSION

PD is a neurodegenerative disease mainly caused by oxidative stress and is characterized by a preferential loss of neurotransmitter dopamine due to the degeneration of dopaminergic neurons typically in the substantia nigra (1). Previously, an oxidant 6-OHDA-induced apoptosis of PC12 cells was established using the *in vitro* model for PD (11, 12). The mechanisms of catecholaminergic cell death induced by 6-OHDA neurotoxicity are explained in three main ways: ROS generated by intra- or extracellular autooxidation, hydrogen peroxide formation induced by MAO and direct inhibition of the mitochondrial respiratory chain. Several reports suggested that the excessive ROS generated by 6-OHDA leads to oxidative stress which injures the cells and induces cell death via apoptosis (1). Hence, the removal of excessively generated ROS and the inhibition of its production may have a protective effect on cell death caused by oxidative stresses. Furthermore, studies on antioxidants with polyphenols and vitamin C particularly related for nutritional agents showed that antioxidants attenuate intracellular accumulation of ROS that eventually inhibits the neuronal cell death (4, 18, 19). The chemical and physiological properties of rare sugars are scarcely known, however, recently we have reported the scavenging ability of rare sugars (D-allose and D-psicose) toward ROS by electron spin resonance (ESR) (10). The results showed that 50 mM D-psicose and D-allose have a higher scavenging activity than those of D-glucose and D-fructose, and that D-allose alone induced the inhibition of ROS production (10).

The protective ability of the four sugars (D-psicose, D-allose, D-fructose and D-glucose) by scavenging excess ROS generated by 6-OHDA in the cells was examined. We observed that D-psicose possessed the highest neuroprotective effect in comparison with the other sugars.

In the present study, we demonstrated that 6-OHDA-induced apoptosis via oxidative stresses in PC12 cells was reduced by D-psicose, 50 mM being the most effective. This concentration seems to increase the osmolarity in the medium and may cause damage to cells. However, in other studies no adverse effect on the cell viability was detected on PC12 cells treated even at a higher concentration (13.5 mg/ml) of glucose for 3 d (20). We added 9.0 mg/ml sugar (=50 mM) to the DMEM medium which in itself contains 1.0 mg/ml glucose (=5.5 mM) as energy source. Therefore, the final sugar concentration used in the present study was 10.0 mg/ml (=55.5 mM) and cells cultured in this condition for 1 d showed no adverse effect on cell viability.

Furthermore, apoptotic cells *in situ* were detected by terminal end labeling (TUNEL staining) specific for DNA fragmentation. The observation that D-psicose significantly reduced the ratio of TUNEL-positive cells indicates that D-psicose may possess an inhibitory effect on 6-OHDA-induced apoptosis.

It was unclear whether the effect of D-psicose against ROS generated by 6-OHDA occurs in the medium or in the cells. Therefore, we examined the inhibitory effects of pretreatment with sugars and GSH. When exogenous GSH was added to the medium, GSH diffused into the PC12 cells rapidly and reached a plateau within 1 h which was maintained

for 24 h (data not shown). The pretreatment with GSH for 3 h was therefore enough to elevate the intracellular GSH level, and was effective in improving the survival rate of 6-OHDA-treated PC12 cells (Fig. 4). Even the D-psicose pretreatment (50 mM) showed a slight protective effect, although it was not as effective as the GSH pretreatment. Interestingly, the simultaneous pretreatment with GSH (1 mM) and D-psicose (50 mM) showed a synergic effect (Fig. 4). These results indicate that D-psicose and GSH exert their antioxidative effects not only in the medium but also in the cells. However, a longer pretreatment of D-psicose did not significantly improve the survival rate of the cells (data not shown). We hypothesized that D-psicose may affect the GSH level in the cells and therefore, we conducted an experiment to investigate this as shown in Fig. 5.

To counteract oxidative stresses, there are several intracellular events involved in the alteration of endogenous GSH concentration (21, 22). GSH is the most abundant cellular thiol complex which functions not only as a scavenger of oxygen free radical but also as a key compound for maintaining intracellular redox potential. Recently, it has been observed that GSH plays a significant role in signal transduction as well as in apoptosis (21). Our data demonstrated that the level of total intracellular glutathione was irrespectively reduced in the presence or absence of sugars 3 h after 6-OHDA treatment. After 24 h, we observed that the total glutathione concentration was up-regulated in each 6-OHDA treated sample, and D-psicose-treated cells showed an enhanced concentration of glutathione among the samples tested (Fig. 5).

Previous studies showed the up-regulation of GSH level in the remaining cells which gradually increase endogenous glutathione content to protect themselves from apoptosis (23, 24). Our data of glutathione concentration after 24 h of 6-OHDA treatment similarly suggest that, following the depletion of pre-existing intracellular glutathione by the strong induction of apoptosis, the remaining viable cells increase their endogenous glutathione level to protect themselves from undergoing apoptosis. Additionally, D-psicose may be involved in glutathione generation and have an enhancing effect on glutathione level, although the exact mechanism underlying this effect has not been clarified.

Interestingly, D-fructose, which is counterchanged from side to side at the third carbon of D-psicose (Fig. 1), exerted an opposite effect to D-psicose. The toxicity of D-fructose for cell viability and the concern of generating advanced glycation end-products (AGE) with enhancement of the neuronal cell death were reported (25, 26). However, the pretreatment with D-fructose for 3 h did not show a toxic effect for cell viability (Fig. 4). We considered that a prolonged treatment with D-fructose might be undesirable for cells, although its underlying mechanism remains unclear (25).

The depletion of endogenous GSH may contribute significantly to PD neuropathology. In recent years, attention has been focused on GSH or related molecules in GSH metabolism as compounds for PD treatment to increase brain GSH level (21).

Our results indicate that D-psicose possesses a neuroprotective effect against 6-OHDA and inhibits apoptosis in PC12 cells. We performed an autographic analysis of organ distribution of intravenously administrated ^{14}C-labelled D-psicose in mice. D-Psicose was distributed at a high concentration in the liver, spleen and kidney within 30 min, and at a low concentration in other organs such as the brain, lungs and intestine. We conclude that D-psicose may play a potential role as a neuroprotective agent for neurodegenerative diseases by elevating the total glutathione level. Moreover, the results suggests that D-psicose-containing products such as healthy food diets, nutrient supplements, and drugs may have a neuroprotective effect and prevent further neuronal degeneration in the patients suffering from these deadly diseases. We have already finished the fundamental safety assessment for D-psicose such as acute toxicity trials, including a primary allergy/irritant test to the eyes and skin, and a biodegradable test, and D-psicose was proved to be a safe sugar (data not shown). Also, several groups in the Faculty of Agriculture, Kagawa University, have already shown that D-psicose is a useful sugar for healthy food diets (27–29). However, the mechanism underlying the elevation of glutathione level in cells with D-psicose treatment needs to be further studied in detail. Further research including ours will provide better a understanding of the action of D-psicose in *in vitro* and *vivo* models and will give insights on the usefulness of D-psicose.

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REFERENCES

1. **Blum, D., Torch, S., Lambeng, N., Nissou, M., Benabid, A. L., Sadoul, R., and Verna, J. M.:** Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson’s disease. Prog. Neurobiol., **65**, 135–172 (2001).
2. **Kosel, S., Hofhaus, G., Massen, A., Vieregge, P., and Graeber, M. B.:** Role of mitochondria in Parkinson disease. Biol. Chem., **380**, 865–870 (1999).
3. **Metodiewa, D. and Koska, C.:** Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders. An overview. Neurotox. Res., **1**, 197–233 (2000).
4. **Di Matteo, V. and Esposito, E.:** Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis. Curr. Drug Targets CNS Neurol. Disord., **2**, 95–107 (2003).
5. **Jang, J. H. and Surh, Y. J.:** Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells. Mutat. Res., **496**, 181–190 (2001).
6. **Nie, G., Jin, C., Cao, Y., Shen, S., and Zhao, B.:** Distinct effects of tea catechins on 6-hydroxydopamine-induced apoptosis in PC12 cells. Arch. Biochem. Biophys., **397**, 84–90 (2002).
7. **Livesey, G. and Brown, J. C.:** D-Tagatose is a bulk sweetener with zero energy determined in rats. J. Nutr., **126**, 1601–1609 (1996).
8. **Takeshita, K., Suga, A., Takada, G., and Izumori, K.:** Mass production of D-psicose from D-fructose by a continuous bioreactor system using immobilized D-tagatose 3-epime-

rase. J. Biosci. Bioeng., **90**, 453–455 (2000).
9. **Bhuiyan, S. H., Itami, Y., Rikui, Y., Katayama, T., and Izumori, K.:** D-Allose production from D-psicose using immobilized L-rhamnose isomerase. J. Ferment. Bioeng., **85**, 539–541 (1998).
10. **Murata, A., Sekiya, K., Watanabe, Y., Yamaguchi, F., Hatano, N., Izumori, K., and Tokuda, M.:** A novel inhibitory effect of D-allose on production of reactive oxygen species from neutrophils. J. Biosci. Bioeng., **96**, 89–91 (2003).
11. **Hartley, A., Stone, J. M., Heron, C., Cooper, J. M., and Schapira, A. H. V.:** Complex I inhibitors induce dose-dependent apoptosis in PC12 cells: relevance to Parkinson's disease. J. Neurochem., **63**, 1987–1990 (1994).
12. **Walkinshaw, G. and Waters, C. M.:** Neurotoxin-induced cell death in neuronal PC12 cells is mediated by induction of apoptosis. Neuroscience, **63**, 975–987 (1994).
13. **Glinka, Y., Gassen, M., and Youdim, M. B.:** Mechanism of 6-hydroxydopamine neurotoxicity. J. Neural. Transm., **50(Suppl.)**, 55–66 (1997).
14. **Mosmann, T.:** Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods., **65**, 55–63 (1983).
15. **Gavrieli, Y., Sherman, Y., and Ben-Sasson, S. A.:** Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. J. Cell. Biol., **119**, 493–501 (1992).
16. **Ellman, G. L.:** Tissue sulfhydryl groups. Arch. Biochem. Biophys., **82**, 70–77 (1959).
17. **Griffith, O. W.:** Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem., **106**, 207–212 (1980).
18. **Nie, G., Jin, C., Cao, Y., Shen, S., and Zhao, B.:** Distinct effects of tea catechins on 6-hydroxydopamine-induced apoptosis in PC12 cells. Arch. Biochem. Biophys., **397**, 84–90 (2002).
19. **Jang, J. H. and Surh, Y. J.:** Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells. Mutat. Res., **496**, 181–190 (2001).
20. **Koshimura, K., Tanaka, J., Murakami, Y., and Kato, Y.:** Involvement of nitric oxide in glucose toxicity on differentiated PC12 cells: prevention of glucose toxicity by tetrahydrobiopterin, a cofactor for nitric oxide synthase. Neurosci. Res., **43**, 31–38 (2002).
21. **Bharath, S., Hsu, M., Kaur, D., Rajagopalan, S., and Andersen, J. K.:** Glutathione, iron and Parkinson's disease. Biochem. Pharmacol., **64**, 1037–1048 (2002).
22. **Spencer, J. P., Jenner, P., Daniel, S. E., Lees, A. J., Marsden, D. C., and Halliwell, B.:** Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. J. Neurochem., **71**, 2112–2122 (1998).
23. **Shimizu, E., Hashimoto, K., Komatsu, N., and Iyo, M.:** Roles of endogenous glutathione levels on 6-hydroxydopamine-induced apoptotic neuronal cell death in human neuroblastoma SK-N-SH cells. Neuropharmacology, **43**, 434–443 (2002).
24. **Han, S. K., Mytilineou, C., and Cohen, G.:** L-DOPA up-regulates glutathione and protects mesencephalic cultures against oxidative stress. J. Neurochem., **66**, 501–510 (1996).
25. **Levi, B. and Werman, M. J.:** Fructose and related phosphate derivatives impose DNA damage and apoptosis in L5178Y mouse lymphoma cells. J. Nutr. Biochem., **14**, 49–60 (2003).
26. **Takeuchi, M., Bucala, R., Oukubo, T., Yamazaki, M., Koike, T., Kameda, Y., and Makita, Z.:** Neurotoxicity of advanced glycation end-products for cultured cortical neurons. J. Neuropathol. Exp. Neurol., **59**, 1094–1105 (2000).
27. **Matsuo, T., Baba, Y., Hashiguchi, M., Takeshita, K., Izumori, K., and Suzuki, H.:** Dietary D-psicose, a C-3 epimer of D-fructose, suppresses the activity of hepatic lipogenic enzymes in rats. J. Clin. Biochem. Nutr., **30**, 55–65 (2001).
28. **Matsuo, T. and Izumori, K.:** Effects of supplemental D-psicose on glucose tolerance and serum adipocytokine levels in rats fed a high-fat diet or a low-fat diet. J. Oleo Sci., **53**, 453–460 (2004).
29. **Sun, Y., Hayakawa, S., and Izumori, K.:** Antioxidative activity and gelling rheological properties of dried egg white glycated with a rare ketohexose through the Maillard reaction. J. Food Sci., **69**, C427–C434 (2004).