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Characterization of Acetoxyscirpendiol of *Paecilomyces tenuipes* as Inhibitor of Sodium Glucose Co-transporters Expressed in *Xenopus laevis* Oocytes

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Abstract – Cordyceps possesses numerous health-promoting ingredients including hypoglycemic agents. The mechanism for the reduction of circulatory sugar content, however, is still not fully understood. In this study, 4-beta acetoxyscirpendiol (ASD) was purified from the methanolic extracts from fruiting bodies of *Paecilomyces tenuipes*. Na+/Glucose transporter-1 (SGLT-1) was expressed in the *Xenopus* oocytes. The effect of ASD on the oocyte expressed SGLT-1 was analyzed utilizing the voltage clamp and 2-deoxy-D-glucose (2-DOG) uptake studies. ASD was shown to significantly inhibit SGLT-1 activity compared to the non-treated control in a dose-dependent manner. In the presence of its two derivatives (diacetoxyscirpenol or 15-acetoxyscirpendiol), SGLT-1 activity was greatly inhibited similarly as ASD. Between ASD derivatives, 15-acetoxyscirepenol showed inhibition equivalent to that of ASD while diacetoxyscirpenol did less degree of inhibition. In summary, these results strongly indicate that ASD in *P. tenuipes* may serve as a functional substance in lowering blood sugar in the circulatory system. ASD and its derivatives can be utilized as inhibitors of SGLT-1.

Keywords □ SGLT-1, Acetoxyscirpendiol, Cordyceps, Diabetes, Voltage clamp, *Xenopus* oocytes

Cordyceps, an entomopathogenic fungus, contains many ingredients with potentials in promoting health, wellness, and fitness. Cordyceps have been consumed as tonics and antitussives from ancient times in Asia. Especially in China, cordyceps has been highly regarded as one of the most effective medicines with anti-aging and cure-all potentials. Among the numerous effects presented by cordyceps including *Paecilomyces tenuipes*, hypoglycemic effect is considered one of the most prominent (Kikuchi *et al.*, 2004, Talpur *et al.*, 2002; Kiho *et al.*, 1999). Many scientists make an effort to understand the basis for the hypoglycemic effects of cordyceps while others to isolate the functional chemical components responsible for lowering the blood sugar content from cordyceps. Neither potential ingredients nor the mechanism, responsible for the hypoglycemic effect of cordyceps, has been fully understood to date.

Glucose transporters are structurally and functionally diverse. GLUTs (Gene ID; SLC5A) are almost expressed in most tissue and facilitate passive diffusion of glucose across tissue barriers by energy independent stereo-specific mechanisms (Juan *et al.*, 2004). Thirteen different glucose transporters GLUT-1~GLUT-12 and HMIT - are identified in GLUT family and divided into three classes (Wood and Trayhyun, 2003): GLUT-1-4 (Class I); GLUT-5, 7, 9, 11 Class II); GLUT-6, 8, 10, 12, HMIT (Class III). SGLTs (Gene ID; SLC2A) play important roles in the absorption of glucose in the intestine and the reabsorption of glucose in the kidney. It has been reported that there are at least three isoforms; SGLT-1, SGLT-2, SGLT-3. Most tissues express a number of different glucose transporters, and the expression pattern of glucose transporters in each tissue is related to specific metabolic requirement and tissue's function. When GLUT and SGLT are compared, blood-glucose level largely depends on SGLT since SGLT helps glucose to rapidly move against the concentration gradients. Thus, final glucose concentration is governed by SGLTs.

Potential components responsible for reducing blood sugar content, fruiting bodies of *P. tenuipes* were isogated by methanolic extraction in this study. The 4-beta-acetoxyscirpene-3 alpha, 15-diol (ASD) was subsequently purified from methanolic extracts. Analysis on its chemical structure reveals that ASD belongs to a class of trichothecenes. Since inhibition of the small intestinal Na+/glucose co-transporter (SGLT-1) may help reduce blood-glucose concentration, this study investigates

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the effect of ASD on the activity of SGLT-1. Since the initial cloning of SGLT-1 from the rabbit small intestine, Xenopus oocyte expression system has been employed to study the functional and structural characteristics of the glucose transporters. Following the heterologous expression of human SGLT-1 in Xenopus laevis oocytes, the expressed SGLT-1 was subjected to electrophysiological measurements and D-glucose influx studies in the presence of ASD or its analogs. Pair of ASD's structural analogues were also tested in terms of inhibitory strength on SGLT-1.

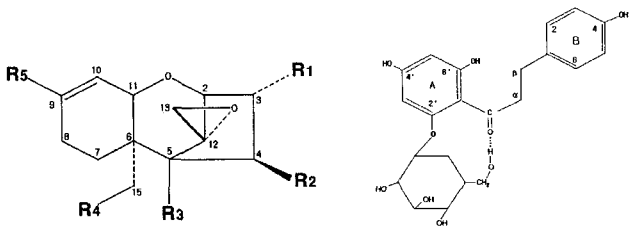

Fig. 1. Structures of ASD, 2-AS 15-AS, and phloridzin. 4-beta ASD has five functional groups: R_1 (OH), R_2 (OAc), R_3 (H), R_4 (H) and R_5 (H). 2-AS has an additional -OAc at R_5 instead of (H). In 15-AS, R_2 and R_5 positions switched compared to 4-beta acetoxyscirpendiol; thus, R_2 and R_5 positions have -H and -OAc, respectively.

MATERIALS AND METHODS

Microinjection of SGLT-1 cRNA into oocytes

A piece of ovary was manually excised from an adult *Xenopus* and treated with type III collagenase for defolliculation. Defolliculated oocytes were injected with hSGLT-1 cRNA as previously described (Lee et al. 1995, 1998). Before microinjection, the oocytes were washed in Barth Solution [5-mM KOH, 100-mM NaOH, 0.5-mM $CaCl_2$, 2-mM $MgCl_2$, 100-mM methanesulfonic acid, and 10-mM HEPES (pH 7.4)] and stage 4 or 5 oocytes were injected with 50 nl of the injection mixture containing 50 ng cRNA. These cRNAs of human SGLT-1 were synthesized from pSP6-hSGLT-1 including cloned cDNA by Sp6 polymerase according to the manufacturers protocol (Promega, WI, USA). Following injection, oocytes were incubated in Barth Solution at 14°C for 24 h before uptake and electrophysiologial assays. Natural ASD was isolated according to Nam *et al.* (2001). Briefly, artificially cultivated conidophores of *P. tenuipes* were first extracted in methanol and the extracts were concentrated up to approximately 10 times at 45°C under reduced pressure. The concentrated samples were suspended in distilled water and the aqueous layer was extracted with a same volume of ethyl acetate to obtain ASD. Its analogs and phloridzin, a known SGLT-1 blocker, were purchased from Sigma Chemicals (Figure 1): 15-Acetoxyscirpendiol (Natural Mycotoxin Registry Number: 2623-22-5) was named 15-AS while diacetoxyscirpenol (Natural Mycotoxin Registry Number: 2270-40-8) was referred to as 2-AS. Phloridzin, a glucoside of the flavonoid-like polyphenol phloretin, has long been known to be a specific nontransportable inhibitor of SGLT1.

Analysis of hSGLT-1 expression

Following microinjection and incubation, SGLT-1 was extracted in phosphate-buffered saline (PBS) with 0.2% mercaptoethanol using a Dounce homogenizer. The transmembranal feature of the SGLT-1 was assayed using surface biotinylation according to Lee et al (1998). Injected oocytes were subjected to biotinylation with 1.0 mg/ml EZ-link-sulfo-NHS-LC-biotin (Pierce, Rockford, IL) and, subsequently, to precipitation by Neutravidin-conjugated beads. The precipitated proteins were electrophoresed and detected by Western blotting using antisera against hSGLT-1 (Acris Antibodies, Hiddenhausen, Germany). In addition, hSGLT-1 expression was functionally analyzed by the entry of α-^{3}H-2-deoxy-D-glucose (2-DOG) into the oocytes according to the procedure as described in the following segment.

α-^{3}H-2-deoxy-D-glucose (2-DOG) uptake inhibition by ASD

The extent of SGLT-1's transport was measured using ^{3}H -labeled α-2-deoxy -D-glucose (2-DOG), a non-metabolized model substrate. ASD inhibition of 2-deoxy-D-glucose (2-DOG) uptake was assayed by incubating 5 oocytes in 2-mM ^{3}H-2-DOG with ASD concentrations ranging from 0 to 5 mM in 1 mL of Barth solution. After a 10-minute incubation, the oocytes were thoroughly washed with the cold Barth solution. Glucose entry was analyzed using ^{3}H-2-DOG (2 mCi/0.5 mL) under a 30-min influx period. The influx of 2-DOG was initiated by incubating five oocytes in 1 mL of Barth's solution containing 2.5 mCi of ^{3}H 2-DOG and cold 2-DOG at concentrations of 1 to 50 mM and at a constant osmolarity of 179.1 mOsm/L achieved by adding a 1-M sucrose solution. Oocytes were removed to a scintillation vial containing 0.5 ml of Barth's solution. After 2 minute incubation, oocytes were transferred to another scintillation vial. Five hundred microliters of 0.1% SDS was added to both vials containing NEN scintillation cocktail

(DuPont NEN, Boston, MA, 5 ml) and mixed by gentle tapping before counting.

Electrophysiological experiments

Electrophysiological experiments were performed at 22-25 °C using the two-microelectrode voltage-clamp method with a OC-725 voltage clamp amplifier (Warner Instrument, Hamden, CT, USA). The oocytes were perfused in a solution containing (in mM) 88 NaCl, 2 KCl, 1.8 $CaCl_2$, and 10 HEPES-NaOH, pH 7.4. In the Na^+-free solution, the Na^+ was replaced with choline, and the pH was adjusted with KOH in the equilibrating buffer. The electrodes were filled with 3 M KCl and the membrane potential was normally maintained at a holding potential of -50 mV. ASD inhibition of the oocyte-expressed SGLT-1 was assayed following incubation of oocytes at ASD concentrations ranging from 0 to 1 mM. Membrane voltage control and data collection were achieved using pCLAMP 6 software (Axon Instruments).

Kinetic analysis and inhibition by ASD and its derivatives

Michaelis-Menten kinetics was obtainined after oocytes were incubated with five different 2-DOG concentrations (5, 15, 30, 60, and 120 mM) for 5 h. Zero trans influx was analyzed using 2-DOG (2 mCi/0.5 ml) under a 30-min influx period. The influx of 2-DOG was initiated by incubating five oocytes in 1 mL of Barths solution containing 2.5 mCi of ^{3}H-2-DOG and cold 2-DOG at concentrations of 1 to 50 mM, at a constant osmolarity of 179.1 mOsm/L achieved using a 1 M sucrose solution. The oocytes were transferred to a scintillation vial containing 0.5 mL of Barths solution. For the control, oocytes were injected with water under specified conditions. The transport rates were standardized by subtracting the control reading from the transport rates of oocyte-expressing SGLT-1. Following overnight incubation at 18°C in 1 mL of Barth's solution containing unlabeled 2-DOG at concentrations of 1 to 50 mM, the oocytes were subjected to the 3-OMG equilibrium-exchange influx experiments at the osmotic condition of 179.1 mOsm/L. Values of *Km* and *Vmax* were calculated using a software package GraphPad Prism™ (GraphPad Software, San Diego, CA).

RESULTS

We have previously shown that ASD inhibits GLUT activity in a dose dependent manner and the inhibition of GLUT may constitute a basis for the anti-tumoric effect by cordyceps (Lee and Kim, 2004). We further investigated whether ASD of *P. tenuipes* and its derivatives serve as the functional ingredient in lowering blood-sugar content by inhibiting SGLT-1. The inhibitory effect of ASD on SGLT-1, including its derivatives, was characterized in the *X. laevis* oocyte system expressing the glucose transporter.

Following injection of cRNA for human SGLT-1, SGLT-1 were significantly expressed in *Xenopus* oocyte membranes. When expressed proteins were initially precipitated by avidin-conjugated agarose and detected by immunoblotting using anti-hSGLT-1, hSGLT-1 was detected in the pool of biotinylated proteins (Bottom panel in Figure 2). The detected molecular size was confirmed according to the molecular size markers. No hSGLT-1 was detected in the oocytes injected with water and saponin The graph part of Figure 2 shows that the oocyte-expressing SGLT-1 showed a significant increase in the uptake of 2-DOG. In terms of the amount of mRNA injected, there was an evident difference in the degree of uptake. The oocytes

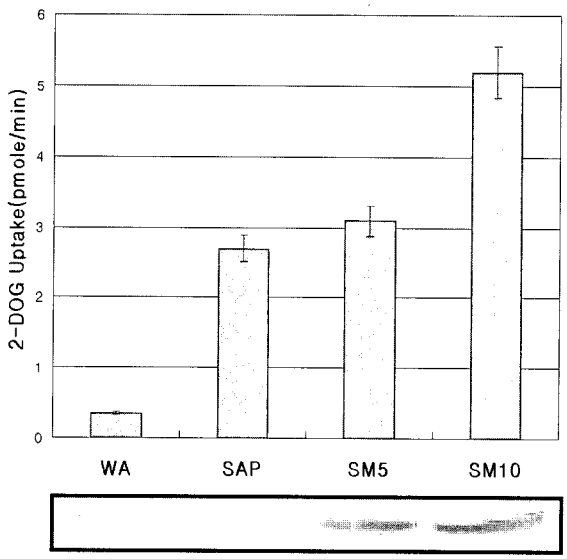

Fig. 2. Comparison of 2-DOG uptake in oocytes expressing hSGLT-1. The entry of 2-DOG was compared among oocytes that were injected with water (WA), saponin (SAP) and SGLT-1 messages (GM). GM5 refers to oocyte injected with 5 ng of SGLT-1 cRNA while GM10 with 10 ng. Following injection, oocytes were incubated with 5 mM 2-DOG for 6 hours. Entry of 2-DOG into oocytes was measured following copious washing. Bottom panel refers to the expression of SGLT-1 in *Xenopus* oocytes. Membrane fractions were prepared from oocytes injected with cRNA for human SGLT-1. Proteins were precipitated and detected by immunoblotting by hSGLT-1. Lane 1 refers to water injected oocyte. Lane 2 refers to the proteins that are precipitated by avidin-conjugated agarose while lane 3 refers to the proteins that are immunoprecipitated by anti-hSGLT1 antisera.

injected with 10 ng of the SGLT-1 mRNA showed higher uptake activity compared to those injected with 5 ng of SGLT-1 mRNA. This uptake pattern is consistent with those of previous; i.e., that the amount of mRNA injected into oocytes correlates with glucose-transporter protein expression and affects transport rates of GLUT-1 (Lee and Kim, 2004) and SGLT-1 (Mandal et al., 2003).

Since SGLT-1 glucose transporter involves an ion trafficking of sodium, electrophysiological experiments were performed to determine whether ASD affect the activity of SGLT-1 expressed in the oocytes. Figures 3 shows that hSGLT-1 expressing Xenopus oocytes results in a substantial current increase with addition of 100 mM D-glucose. When pre-incubated with 5 mM phloridzin, a known SGLT blocker, the oocytes did not respond to the addition of glucose (Fig. 3-A). With addition of 0.5 mM ASD, Na+ inward current significantly attenuated similarly as phloridzin (Fig. 3-B). When ASD was removed by washing the oocyte with the perfusion media, however, the currents were restored back to the level with the ASD-free treatment. This observation indicates that the inhibitory effect by ASD is reversible.

When the effect of ASD on SGLT-1 was measured in the presence of 0.5 mM 2-DOG, SGLT-1 activity was significantly reduced in a dose-dependent manner (Figure 4). The proportional entry rate was calculated as V/V_0, where V refers to inhibited and V_0 to non-inhibited entry rate: The fractional value decreased in a dose-dependent manner. For non-inhibited control, SGLT-1-expressing oocytes were not treated with ASD. The transport of 2-DOG into oocytes expressing SGLT-1 was significantly reduced to levels measured for water injected. This is consistent with phloridzin.

ASD's inhibition of SGLT-1 was further characterized according to the kinetic analysis. Based on the kinetics affected by ASD, the functional characteristics of ASD inhibition on SGLT-1 activity was studied under variable concentration of 2 DOG. In the presence of ASD, reduction in the half-saturation rate constant by ASD was obvious. The half-saturation rate constant and the maximal velocity of zero-trans 2-DOG uptake leveled off under treatment with ASD. The equilibrium-exchange analysis further supported that ASD reduced half-saturation rate when 3-OMG was used as a glucose analog (Figure 5). Unlike 2-DOG, 3-OMG is transported through SGLT-1, but not phosphorylated, following influx into the cell. Accumulation of 3-OMG at 100-mM 3-OMG equilibrium concentration becomes significantly impeded in comparison to the ASD-free counterpart. For this impeded accumulation, the most probable cause would be the significant impairment of SGLT-1 in its activity by ASD. The values of the Michaelis-Menten constants of SGLT-1 (equilibrium exchange and zero-trans influx experiments) correspond with previous reports (Mandel *et al.*, 2003; Oulianova *et al.*, 2001).

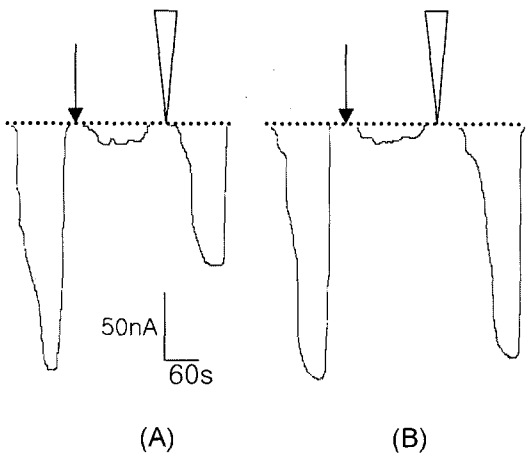

Fig. 3. Inhibition of glucose-induced current by ASD in a single oocyte expressing hSGLT-1. An oocyte expressing hSGLT-1 was subjected to electrophysiological measurement with membrane potential of -50 mV. (A) With addition of 100 mM glucose, oocytes showed -150 nA as the Na+-inward current. Treatment with 0.5 mM phloridzin for 1 minute, however, inhibits oocytes from exerting any significant current even with addition of 100 mM glucose. (B) When pre-incubated with 0.5 mM ASD, the oocytes did not show any significant Na+-induced current similarly as phloridzin. When the oocyte was washed out with perfusion media (downward arrow), the electric current was induced to the previous level (~120 nA).

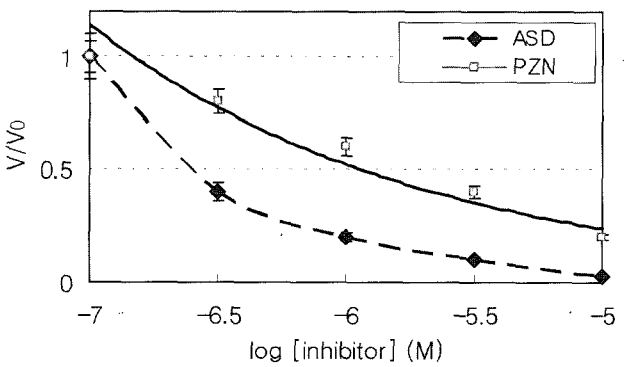

Fig. 4. Effect of ASD on SGLT-1 on 2-DOG uptake. The effects of ASDs on SGLT-1 were measured in the presence of 0.5 mM 2-DOG. *Xenopus* oocyte expressing SGLT-1 were incubated with ASD or phloridzin (PZN). The measurements (V/V_0) were calculated as fractional value of inhibited per non-inhibited uptake rates.

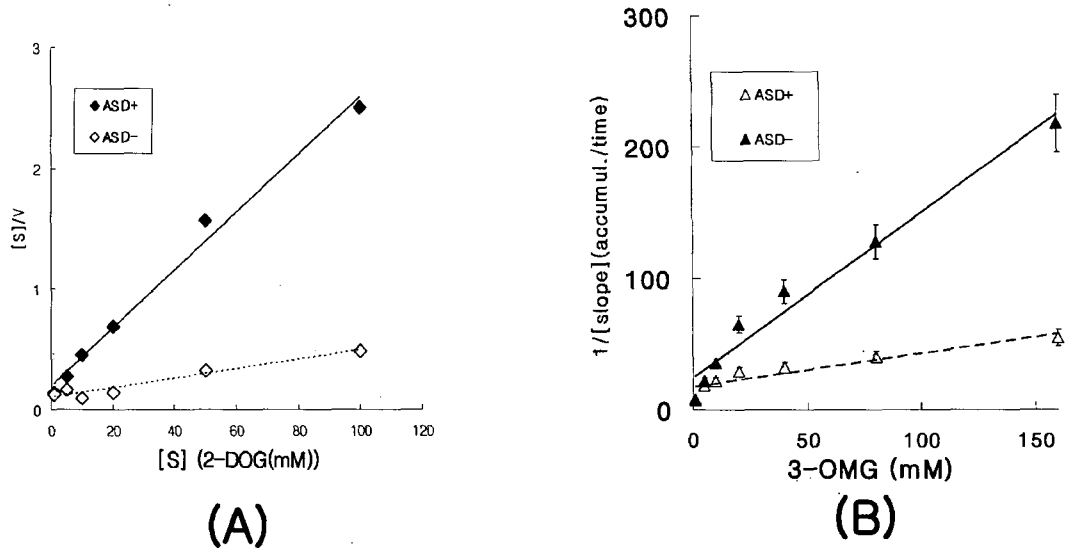

Fig. 5. Kinetic analysis on ASD inhibition of 2-DOG or 3-OMG uptake. (A) Zero-trans influx of 2-DOG at the indicated concentrations was determined in the oocytes expressing GLUT1 in the absence or presence of 0.5 mM ASD (Hans plot). Open diamonds (◆) refer to the ASD-free treatment while closed diamonds (◇) to the ASD treatment. Values were standardized by subtracting those for non-injected oocytes. Each value is derived from the mean of data from 10 oocytes and is corrected for 2-DOG uptake in non-injected *Xenopus* oocytes (ASD treatment: *Km*=8.5 mM *Vmax*=157 pmole/min/oocyte; ASD-free treatment: *Km*=2.9 mM *Vmax*=24 pmole/min/oocyte). (B) Equilibrium exchange influx kinetics was determined at the 3-OMG equilibrium concentrations indicated. Accumulation of 3-OMG was investigated for 1 h and expressed as modified by logarithmic transformation. For each time point, 10 oocytes per group were employed in the assay. The negative reciprocals of the slopes were used to plot against 3-OMG concentrations according to the Hanes plotting application. On the y-axis, 1/[slope] refers to the reciprocal value of each absolute value (ASD-free treatment: *Km*=22.0 mM *Vmax*=198±13; ASD-treatment: *Km*=3.8±0.9 *Vmax*=39±5). Open (△) and closed triangles (▲) refer to the ASD-treated and ASD-free conditions, respectively.

Figure 6 shows electrophysiological measurements on 2-AS and 15-AS. SGLT-1 appears significantly affected in the presence of the two ASD analogs tested. When SGLT-1 activity was assayed in the presence of either ASD analog, the sodium ion influx was significantly reduced in comparison to ASD-free counterparts. The ASD's inhibitory effect on SGLT-1 still holds for its structural analogs. In the two ASD analogs tested in this study, 15-AS shows the higher level of inhibition which even surpass that of ASD. This observation implies that the pool of ASD analogs can be a target for a further study in screening a potent drug to treat diabetes.

DISCUSSION

This study utilized *Xenopus-laevis*-oocyte expression system to assay the transport of the glucose analog 2-deoxy-D-glucose and 3-OMG to characterize electrophysiologically the glucose-transport properties of SGLT-1 in the presence of ASD. The results demonstrate that ASD inhibit significantly SGLT-1's activity; thus, glucose transport is severely impaired by ASD. ASD likely possesses the features affecting the pathway critical

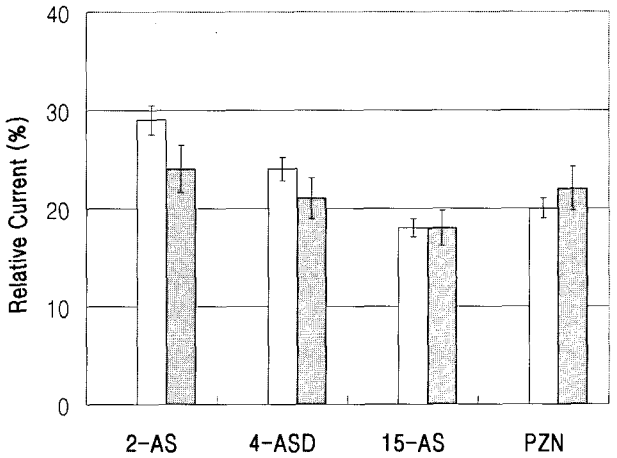

Fig. 6. Inhibition of SGLT-1 by ASD analogs. Inhibitory effects by ASD analogs and phloridzin (PZN) on SGLT-1 was measured and compared using voltage clamp methods. The effects of ASD analogs on SGLT-1 were measured in the presence of 50 mM glucose. The measurements were expressed as relative values considering ASD-free value as 100%. Each column represents the mean ± S.E. (n=5). Light and dark columns refer to application of 0.2 mM and 1 mM of the inhibitor, respectively.

to transport of glucose governed by SGLT-1.

In the presence of ASD and its derivatives, the entry of glucose significantly decreases in a dose-dependent manner. *Xenopus laevis* oocytes demonstrated that ASD significant decreases glucose transport in *Xenopus* oocytes expressing SGLT-1. This observation virtually hold true for ASD's structural analogs despite difference in inhibitory strength. This result can be considered important since we can further study the potential role and structural importance of ASD and its derivatives as model structures for blockers of glucose transporters. In addition, the pool of ASD analogs can be subjected to a further screening study for a potent drug to treat diabetes.

The kinetic study indicates that ASD is involved even in the equilibrium binding of glucose to the glucose transporter. In terms of the normalized *Vmax* with ASD, SGLT-1 was significantly lower than that of SGLT-1 compared to ASD-free controls. According to the inhibition mode index, ASD can be comparable to that of phloridzin. This lowered transport activity can be explained with the decreased affinity of 2-DOG. This result strongly indicates that inhibition of SGLT-1 by ASD is directly targeted at SGLT-1 rather than at signaling pathways leading to glucose uptake and metabolism. The lowered affinity was also shown in a *Km* change for 3-OMG. ASD inhibition of 3-OMG uptake showed more than a six-fold reduction in the binding affinity. Interference with the translocation of glucose transporters is the basis for the inhibitory effect of ASD. Rather, it is a direct inhibition on glucose transporters. ASD does not lie at the level of glucose metabolism.

Similar inhibitory effect was proven for the GLUT-1 expressed in Xenopus oocytes (Lee and Kim, 2004). In human studies, high levels of GLUT-1 expression in tumors have been associated with poor survival (Rudlowski *et al.*, 2003). Since increased glucose transport in malignant cells has been associated with increased expression of glucose transporters, inhibition of GLUT-1 by ASD can explain the anti-tumor effect of cordyceps. Indeed, GLUT-1 has been well reported that its expression is increased in many cancers and related malignancy.

Recent studies indicate that ASD induces apoptosis and modulates cell cycle genes in cancer cell lines (Nam *et al.*, 2001; Jung *et al.*, 2003). Although the relation between GLUT-1 and apoptosis is least understood, low glucose level in cells is well known as one of apoptosis induction factors. GLUT-1's expression is increased in many cancers and related malignancy. From these reports and our study, the apoptosis of ASD treated cancer cells may be caused low glucose level by inhibited GLUT-1. ASD's dual inhibition of GLUT-1 and SGLT-1 might be a contradictory; however, tissue specific expression of the two transporters may separate the dual inhibitory effect of ASD. The isolation of these compounds indicated that *P. tenuipes* is a promising natural source for producing various biologically active substances.

Considering the inhibitory steps involving glucosides are well understood (Oulianova *et al.*, 2001), the inhibition by ASD, despite their different chemical structure of ASD's, strongly implies that additional inhibitory pathway might exist to reduce overall performance of SGLTs. To date, specific non-transportable inhibitors of the SGLT-1 belong to a glucoside of the flavonoid-like polyphenols such as phloridzin. This present study shows that ASD and its analogs have potentials as an inhibitor for SGLT-1. When ASD structural derivatives were tested for its inhibitory effect on glucose transport, the 15-AS shows the highest degree of inhibition among ASD analogs. These derivatives already exist in the catalogs of chemicals and are readily available in quantity. This result bears a ramification that the chemical library screening can help find useful agents that may exert the inhibitory effect.

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REFERENCES

Doege, H., Bocianski, A., Joost, H.G., and Schurmann, A. (2000). Activity and genomic organization of human glucose transporter 9 (GLUT9), a novel member of the family of sugar-transport facilitators predominantly expressed in brain and leukocytes. *Biochem. J.* **350**, 771-776.

Due, A.D., Qu, Z.C., and Thomas, J.M. (1995). Role of the C-terminal tail of the SGLT-1 glucose transporter in its expression and function in *Xenopus laevis* oocytes. *Biochemistry* **34**, 5462-5471.

Jones, K. (1997). Cordyceps, *Tonic Food of Ancient China*, Sylvan Press, Seattle, Washington, pp.52-61.

Juan, M.P., Dong, W., Beatriz, L., Hong, Y., Xia M., Ru, Y., and Darryl, C., (2004) GLUT1 deficiency and other glucose transporter diseases. *Eur. J. of Endocrin.*, **150**, 627-633.

Kiho, T., Ookubo, K., Usui, S., Ukai, S., Hirano, K. (1999) Structural features and hypoglycemic activity of a polysaccharide (CS-F10) from the cultured mycelium of Cordyceps sinenesis. *Biol. Pharm. Bull.* **22**, 966-970.

Kikuchi, H., Miyagawa, Y., Sahashi, Y., Inatomi, S., Haganuma, A., Nakahata, N., and Oshima, Y. (2004). Novel Spirocyclic Trichothecanes, Spirotenuipesine A and B, Isolated from Entomopathogenic Fungus, Paecilomyces tenuipes. *J. Org. Chem.*

69(2), 352-356.

Kirwan, J. P., and del Aguila, L. F. (2003). Insulin signaling, exercise and cellular integrity. *Biochem. Soc. Trans.* **31**, 1281-1285.

Konno, S., Tortorelis, D.G., Fullerton, S.A., Samadi, A.A., Hettiarachchi, J. and Tazaki, H. (2001) Possible hypoglycemic effect of Maitake mushroom on Type 2 diabetic patients. Diabetic Medicine, **18**, 1010-1015.

Lee, D.H. and Kim H.W. (2004) Inhibition of GLUT-1 expressed in Xenopus laevis Oocytes by acetoxyscirpendiol of p. tenuipes. *J. of Applied Phamocology* **12**(2), 74-78.

Lee, D.H. (1998). Characterization of 27K zein as a transmembrane protein. *J. Biochem. Mol. Biol.* **31**(2), 196-200.

Lee, D.H., Selester, B., and Pedersen, K. (1995). Free movement of 27K zein in the endoplasmic reticulum. *Protein Eng.* **9**, 91-96.

Mandal, A., Verri, T., Mandal, PK, Storelli, C., and Ahearn, GA (2003) Expression of Na(+) /D-glucose cotransport in Xenopus laevis oocytes by injection of poly(A)(+) RNA isolated from lobster (Homarus americanus) hepatopancreas. Comp Biochem Physiol A Mol Integr Physiol. **135**(3), 467-75.

Nam, K.S., Jo, Y.S., Kim, Y.H., Hyun, J.W., and Kim, H.W. (2001). Cytotoxic activities of acetoxyscirpenediol and ergosterol peroxide from Paecilomyces tenuipes. *Life Sci.* **69**(2), 229-237.

Oulianova, N., Falk, S., and Berteloot, A. (2001) Two-step mechanism of phroridzin binding to the SGLT1 protein in the kidney. J. Membrane Biol. **179**, 223-242.

Rudlowski C, Becker AJ, Schroder W, Rath W, Buttner R, and Moser M. (2003). GLUT1 messenger RNA and protein induction relates to the malignant transformation of cervical cancer. *Am J Clin Pathol* **120**(5), 691-698.

Rumsey, S. C., Daruwala, R., Al-Hasani, H., Zarnowski, M. J., Simpson, I. A., and Levine, M. (2003). Dehydroascorbic acid transport by GLUT4 in *Xenopus* oocytes and isolated rat adipocytes. *J. Biol. Chem.* **275**, 28246-28253.

Talpur NA, Echard BW, Fan AY, Jaffari O, Bagchi D., and Preuss HG (2002) Antihypertensive and metabolic effects of whole Mitake mushroom powder and its fractions in two rat strains. Mol Cell Biochem. **237**, 129-136.

Wood, I.S., and Trayhurn P. (2003) Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br. J. Nutr. **89**(1), 3-9.