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ARTICLE

Comparison of Absorption of 1-Deoxynojirimycin from Mulberry Water Extract in Rats

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In this study, we compared the absorption and excretion of DNJ in mulberry leaf extract against that of the purified compound (DNJ) using GC-TOF-MS, a newly developed analytical method, when administered orally to rats. Moreover, we also compared absorption levels in small intestinal cells using the Caco-2 cell line. In the cell study, DNJ absorption from the mulberry extract seemed to be inhibited when compared to that of the purified DNJ compound. The concentration of DNJ in rat plasma was also significantly ($p < 0.05$) lower when the mulberry extract was administered versus the purified DNJ compound. The metabolic pattern of DNJ from the mulberry leaf extract indicated that most was excreted in the feces, whereas a lower amount was detected in the urine, which was similar to the purified DNJ compound. These findings indicate that the bioavailability of DNJ in mulberry leaf extract might be lower than that of highly purified DNJ.

KEYWORDS: 1-Deoxynojirimycin; mulberry leaves; bioavailability; Caco-2; absorption

INTRODUCTION

Imino sugars have been considered the most promising active compounds for α-glucosidase inhibition over the past three decades. In particular, 1-deoxynojirimycin (DNJ), which is abundant in mulberry leaf (*Morus alba L.*), is believed to be a typical naturally occurring imino sugar with potent biological activity (*1*, *2*). DNJ is a D-glucose analogue with an NH group substituting the oxygen atom of the pyranose ring (**Figure 1**). Despite its excellent ability to inhibit α-glucosidase, DNJ has been deemed an improbable drug due to its moderate postprandial hypoglycemic activity (*3*, *4*). Instead of DNJ, derivatives with altered inhibitory specificities, such as miglitol and emiglitate, have been developed (*5*). However, because the chronic use of purified α-glucosidase inhibitors can have undesirable side effects such as flatulence, diarrhea, and abdominal cramping, their use may be limited. Therefore, attention has focused on alternative hypoglycemic agents from natural products with effective and safe α-glucosidase inhibition activities and fewer side effects (*6*). However, because DNJ is isolated from natural products, it has been suggested that the intake of these natural dietary products, including mulberries or other sericulture products, might be beneficial in suppressing blood glucose levels (*4*). Recently, several animal and human studies have reported that mulberry or sericulture products containing DNJ suppressed postprandial increases of glucose (*7*, *8*).

At the present time, 130 cultivars of mulberry are cultivated in Korea where they are grown for sericulture and used for medicinal purposes. Mulberries contain various functional ingredients besides 1-deoxynojirimycin, such as soluble dietary fibers, resveratrol, quercetin, rutin, astragalin, and γ-aminobutyric acid (*9*, *10*). Thus, mulberry leaf may be a promising functional ingredient for hyperglycemic agents.

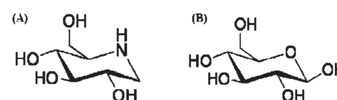

Figure 1. Chemical structures of DNJ (**A**) and glucose (**B**).

In spite of its numerous beneficial effects, there are few reports on the absorption and metabolism of DNJ. Faber et al. (*11*) investigated the pharmacokinetics of 1-deoxymannojirimycin (DMJ), which is structurally related to DNJ, after intravenous administration in rats. They reported that 120 min after i.v. administration, 52% of the DMJ dose was detected in the unchanged form in urine. Nakagawa et al. (*12*) also studied plasma levels of DNJ after oral consumption. They found that orally administered mulberry DNJ was absorbed in the intact form from the alimentary tract and then quickly excreted from the body. However, because Nakagawa's study (*12*) used purified DNJ compounds, the bioavailability of DNJ in mulberry leaf extract, which contains a similar DNJ form and is also abundant in fiber and other phytochemicals such as quercetin, was not determined.

Therefore, in this study, we compared the absorption and excretion of DNJ from the mulberry leaf extract against that of the purified compound when administered orally to rats. Moreover, we also compared absorption levels in small intestinal cells using the Caco-2 cell line. Caco-2 cells are well-differentiated human intestinal cells, which have been extensively characterized (*13*, *14*). As an in vitro model of the principal barrier of intestinal absorption, Caco-2 cells were mounted in Ussing type

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chambers. To detect trace levels of DNJ in plasma, urine, and feces, we developed a simple method using gas chromatography coupled with time-of-flight mass spectrometric detection (GC-TOF-MS).

MATERIALS AND METHODS

Reagents. The purified 1-deoxynojirimycin (DNJ, > 98.0%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Adonitol and pyridine were obtained from Sigma Aldrich (St Louis, MO, USA), and *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing trimethylchlorosilane (TMCS) was purchased from Supelco (Bellefonte, PA, USA). High performance liquid chromatography (HPLC) grade methanol, acetonitrile, and acetic acid were purchased from J. T. Baker (Phillipsburg, NJ, USA). Nine-fluorenylmethyl chloroformate (FMOC-Cl) was purchased from Fluka (Buchs, Switzerland). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (DPBS), MEM nonessential amino acids (MEM NEAA), penicillin/streptomycin, and 0.25% Trypsin-EDTA were purchased from GIBCO (Grand Island, NY, U.S.A.).

Preparation of Hot Water Extract of Mulberry Leaves. Hot water mulberry leaf extract (MLE) was obtained from Jeonnam Biotechnology Center. The mulberry leaves (*Morus alba* L.) used in this study were cultivated in Naju by Donguinara. Co., Ltd. (Naju, Jeonnam, Korea). Briefly, dry mulberry leaves (81.09 g) were extracted with water (4 L) by sonication and heating at 60 °C for 1 h and then centrifuged at 12,000*g* for 15 min to remove insoluble substances. The supernatant was freeze-dried for 37 h. The residue was freeze-dried after repeating the extraction step. The extracts were concentrated to give the MLE (28.23 g). DNJ was analyzed using a reversed-phase high-performance liquid chromatography system (RP-HPLC, Shiseido Co. Ltd., Tokyo, Japan) equipped with a fluorescence detector after derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) according to the procedure of Kim et al. (*15*). Briefly, 10 μL of standard or sample solution were mixed with 10 μL of 0.4 M potassium borate buffer (pH 8.5) in a 1.5 mL microtube. Twenty microliters of 5 mM FMOC-Cl in CH_3CN was added with immediate mixing and allowed to react at 20 °C for 20 min. Ten microliters of 0.1 M glycine was added to terminate the reaction by quenching the remaining FMOC-Cl. The mixture was diluted with 0.1% aqueous acetic acid to stabilize the DNJ-FMOC and then filtered. The filtrate was injected into the RP-HPLC using a Unison US-C18 column (250 × 4.60 mm I.D., 5 μm, Imtakt, Kyoto, Japan) with a flow rate of 1.0 mL/min and detection by a fluorescence detector (excitation 254 nm, emission 322 nm). The analyte was eluted with a mobile phase of acetonitrile−0.1% aqueous acetic acid (1:1, v/v).

In Vitro Absorption of DNJ by Human Intestinal Cell Monolayers. The human colon adenocarcinoma cell line Caco-2 was obtained from Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in a humidified incubator in an atmosphere of 5% CO_2−95% air at 37 °C. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% NEM nonessential amino acids, and 1% penicillin−streptomycin mixture. For the in vitro absorption studies on semipermeable membranes (Polyester Transwell inserts, Costar 3460, pore size 0.4 μm, Corning Incorporate Life Sciences, Lowell, MA, USA), the cells were seeded at a density of 1×10^4 cells/cm^2 onto membranes. The inserts were placed into six-well plates, and the cells were allowed to grow and differentiate to confluent monolayers for 11−14 days. The culture medium was changed every 3 days at the luminal side and every 7 days at the serosal side. To ensure that the monolayers exhibited properties of a tight biological barrier, transepithelial electrical resistance (TEER) was monitored using a Millicell-ERS voltometer (Millipore, Billerica, MA, United States). Monolayers with TEER above 400 Ω were exclusively utilized in these experiments. For the experiments, the medium was decanted, and the cells were carefully washed with prewarmed (37 °C) PBS. To the upper compartments, test media containing DNJ or MLE were added, and a blank medium was added to the lower compartment. After the cells were incubated for 2 h in an atmosphere of 5% CO_2−95% air at 37 °C, each membrane was washed with ice cold PBS three times, and then the medium in the lower compartment was used to analyze DNJ content. Aliquots of 10 μL were used to determine protein concentrations by bichinchonic acid (BCA protein assy, Bio-Rad, Hercules, CA, USA). The concentration of DNJ was determined by RP-HPLC in the same manner as that described above unless stated otherwise (*15*).

Animals. Ten male Sprague−Dawley (SD) rats at 9 weeks of age (Orient Bio Inc., Kyeonggi-do, Korea) were used to determine DNJ excretion in the urine and feces as well as levels in plasma. Another 50 SD rats at 6 weeks of age were used to examine time course changes of DNJ in plasma. The rats were housed in a controlled room maintained at 23 ± 1 °C with a 12 h light−dark cycle. The rats were randomly divided into experimental groups according to blood glucose (n = 5). The rats were fed a commercial pellet diet (Orient Bio Inc., Kyeonggi-do, Korea) and allowed free access to water.

Time Course Changes of DNJ Concentrations in Plasma. To evaluate time course changes of DNJ concentrations in plasma, the animals were fasted for 12 h on day 9, and then the DNJ compound (3 or 6 mg/kg body weight) or mulberry leaf extract (MLE, 1.7 g/kg body weight, 6 mg as DNJ) was orally administered. Before and at 15, 30, 45, 60, 120, 240, 360 min after MLE administration and before and at 0.5 min after DNJ administration, blood samples were collected directly from the heart using syringes under ethyl ether anesthetization. Each blood sample was collected in a tube containing EDTA as an anticoagulant (1.5 mg/mL blood). The plasma was immediately prepared by centrifugation at 1500*g* for 15 min at 4 °C. The obtained plasma was transferred to microtubes and stored at −80 °C until use.

Administration of MLE and DNJ to Rats for the Determination of DNJ in Urine, Feces, and Plasma. For the determination of DNJ in urine and feces, the animals were fasted for 12 h at day 9, and then purified DNJ compound (30 mg/kg body weight) or MLE (0.85 g/kg body weight, 3 mg as DNJ) was orally administered. Using a metabolic cage, urine was collected between 0 to 24 h, and feces samples were collected between 0 to 48 h to evaluate DNJ concentrations. To determine plasma DNJ, the animals were fasted for 12 h at day 12 and then purified DNJ compound (30 mg/kg body weight) or MLE (0.85 g/kg body weight, 3 mg as DNJ) was orally administered. Thirty minutes after the administration, the animals were sacrificed with ethyl ether anesthetization, and blood samples were collected directly from the heart using a syringe. Each blood sample was collected in a tube containing EDTA as an anticoagulant (1.5 mg/mL blood). The plasma was immediately centrifuged at 1500*g* for 15 min at 4 °C. The plasma was transferred to microtubes and stored at −80 °C until use.

DNJ Analysis in Feces, Urine, and Plasma Using GC- TOF-MS. A 200 μL amount of sample was sonicated, and then 800 μL of methanol and 100 μL of adonitol [1 ppm (v/v) in methanol] as an internal standard compound were added and mixed thoroughly with vortexing for 30 s. Next, the samples were incubated on ice, sonicated, and centrifuged at 3000 rpm and 4 °C for 10 min. The supernatant was collected, dried, and then mixed with 120 μL of pyridine, 90 μL of acetonitrile, and 90 μL of *N*, *O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) before sonication at 30 °C for 1 h. A gas chromatograph (Agilent 6890N, Agilent Technologies, Palo Alto, CA, USA) connected to a time-of-flight (TOF) Pegasus III mass spectrometer (Leco, St. Joseph, MI, USA) and equipped with a DB-5MS column (30 m length ×0.25 mm i.d. × 0.25 μm film thickness, J & W Scientific) was employed. The GC oven temperature was increased from 80 to 230 °C at a rate of 5 °C/min and then kept at 230 °C for 5 min. Both the injector and detector transfer line temperatures were 250 °C. A 1 μL amount of derivatized extract was injected in the splitless mode with helium as the carrier gas (1.0 mL/min). The data acquisition rate of scanning was 20 scans s^{-1}, and the recorded mass range was m/z 45 to 550. The signal-to-noise (S/N) values of the peaks lower than 10 were rejected. The baseline offset was set to 1, the data points for averaging was set to auto, and the peak width was set to 1.333. Other MS conditions used were as follows. Em voltage, −70 eV; transfer line temperature, 220 °C; ion souce temperature, 180 °C; and vacuum state 2.3×10^{-7} torr. Quantification was performed by comparing the unique ion peak area of the derivatized DNJ to that of the derivatized internal standard compound following the same method as that described above. The unique ion value of both the internal standard compound and DNJ for quantification was 73.

Statistics. All statistical analyses were performed using the SAS program package version 9.1. Differences between the control and treatment groups were analyzed by Student's *t*-test. As a way to compare

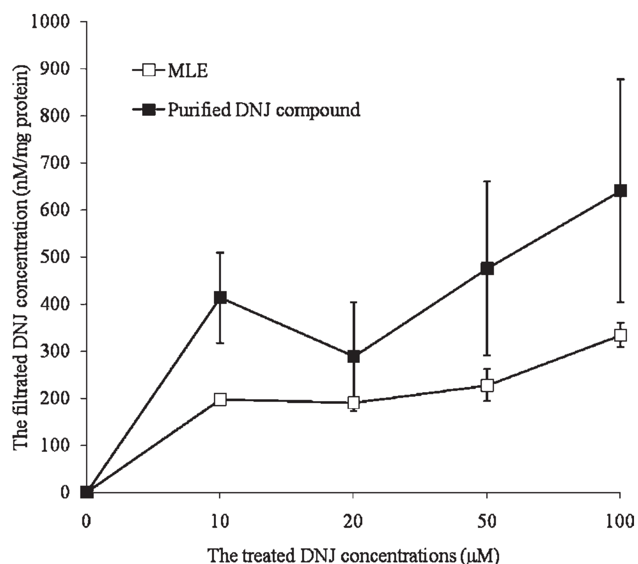

Figure 2. DNJ concentrations at the basolateral side of cell monolayers when incubated for 2 h. Purified DNJ compound or MLE at DNJ concentrations of 0, 10, 20, 50, 100 μM were treated to Caco-2 cells in transport chambers. After 2 h of incubation, the concentrations of DNJ in the serosal side (filtrate) were determined by HPLC. Data are expressed as mean values with standard errors shown by vertical bars.

several groups, one-way analysis of variance (ANOVA) with Duncan's multiple range tests was used at $p < 0.05$. All results are expressed as the mean $\pm$ standard error (SE).

RESULTS

In Vitro Absorption of DNJ from Mulberry Extracts by Caco-2 Human Intestinal Cell Monolayers. Figure 2 shows the absorbed concentrations of DNJ from mulberry extract or purified DNJ compound in basolateral solutions after incubation of the Caco-2 monolayers with DNJ concentrations of 0, 10, 20, 50, and 100 μM. After 2 h of incubation, the concentrations of DNJ in the basolateral side (filtrate) were determined by HPLC. Concentrations of absorbed DNJ increased in a dose−response manner in both treatments. However, concentrations of absorbed DNJ from the MLE were lower than those by treatment with the purified DNJ compound.

GC-TOF-MS Analysis of Standard DNJ and Rat Biological Samples. The GC-TOF-MS unique ion chromatogram of the authentic DNJ compound after derivatization is presented in **Figure 3A**. Trimethylsilyl (TMS) derivatization was performed in order to increase the volatility of the nonvolatile DNJ before GC-TOF-MS analysis. The increased volatility could be obtained by elimination of polar OH and NH groups in nonvolatiles such as sugars and amino sugars (*16*). The detection limit of this method was below 6×10^{-4} μmol/L. In addition, the calibration curve showed a good linearity ($r^2 > 0.99$) in the range of 6.13×10^{-2} μmol/L and 3.07×10 μmol/L. For the recovery of DNJ, it was found to be about 35% when a known amount of DNJ standard was added to the plasma sample. However, the recovery test could provide relatively consistent results. A defined peak ascribed to the derivatized DNJ was detected at a retention time of around 22.40 min. The typical unique ion (m/z 73) chromatogram of a rat plasma extract is presented in **Figure 3B**. Although there were several small peaks ascribed to the background of the mulberry extract, DNJ itself was not detected in the rat plasma extract before administration. However, 30 min after oral DNJ administration, a clear peak ascribed to DNJ (22.40 min) was observed in the plasma extract (**Figure 3C**). The same analytical methods were applied to both the urine and feces samples for the determination of DNJ concentrations.

Occurrence of DNJ in Rat Plasma. Figure 4 shows the time courses of DNJ concentration when the mulberry extract was administered. Plasma samples were obtained by scarification at 0, 15, 30, 45, 60, 120, 240, and 360 min after MLE administration (1.7 g/kg body weight, 6 mg as DNJ). The concentration of DNJ in plasma increased to its highest level (12.01 μmol/L) 30 min after single oral administration and then rapidly decreased (**Figure 4A**). The DNJ concentrations in plasma 4−6 h after administration were below the detection limit. When the purified DNJ compound was administered at different levels (3 or 6 mg/kg body weight, respectively), concentrations of DNJ in plasma were significantly increased in a dose-dependent manner after 30 min ($p < 0.0001$, **Figure 4B**). To determine the absorption of DNJ from the mulberry extract, we compared DNJ concentrations in plasma after the extract was administered versus DNJ in plasma after the purified DNJ compound was administered. A statistically significant ($p < 0.05$, **Figure 4B**) difference in DNJ concentration in plasma was found 30 min after administration in which the level was 25.66 $\pm$ 1.96 μmol/L when the purified DNJ compound was administered and 12.01 $\pm$ 4.95 μmol/L when the mulberry extract was administered.

Metabolic Fate of DNJ. In order to evaluate the metabolic fate of DNJ, MLE (0.85 g/kg body weight, 3 mg as DNJ) and purified DNJ compound at a high dose (ten times that contained in the mulberry extract) were administered, and concentrations of DNJ in urine and feces were analyzed. When DNJ was administered at about 9.6 mg per rat, the amount of DNJ detected in the urine (4.08 $\pm$ 0.83 mg) in the intact form was less than that of DNJ excreted in the feces (7.22 $\pm$ 2.26 mg) over 48 h (**Figure 5A**). However, when MLE was administered at 0.98 mg of DNJ per rat, the majority of the DNJ was excreted in the feces (1.27 $\pm$ 0.60 mg per rat) and little amount of DNJ was detected in the urine (0.07 $\pm$ 0.07 mg per rat) (**Figure 5B**). Although the pattern of metabolic fate was similar to that of the purified DNJ compound, when DNJ was administered as an extract, the excretion rate seemed to be high compared to that with the purified form used.

DISCUSSION

Although various beneficial effects of DNJ on human health have been suggested (*1*−*5*), the absorption and metabolism of DNJ from mulberry leaves or leaf extracts still remain unresolved. A recent study (*12*) using rats showed that DNJ was absorbed into plasma in the intact form. However, there is no reported data on the absorption of DNJ when administered as an extract. This prompted us to investigate the absorption of DNJ using mulberry extract in vitro and in vivo. It has been reported that Caco-2 cells can undergo spontaneous differentiation under culture conditions and exhibit characteristics of mature enterocytes (*17*). The cell surface facing the top medium develops a brush border that resembles the luminal membrane of the intestinal epithelium. The cell surface attached to the permeable membrane and facing the bottom medium develops into a basolateral membrane. These cells have been used to evaluate cell transport and/or accumulation of pure phytochemicals such as quercetin and flavones, as well as flavonoids in the food form (*18*−*20*). In the current study, we evaluated the bioaccessibility of DNJ in the mulberry leaf extract against that of purified DNJ using a Caco-2 cell model. We found that DNJ was transported through the Caco-2 cell monolayers in the intact form but that its absorption rate was low when the mulberry extract was applied.

In order to confirm the absorption of DNJ in an animal model, we evaluated the level absorbed through the intestinal barrier

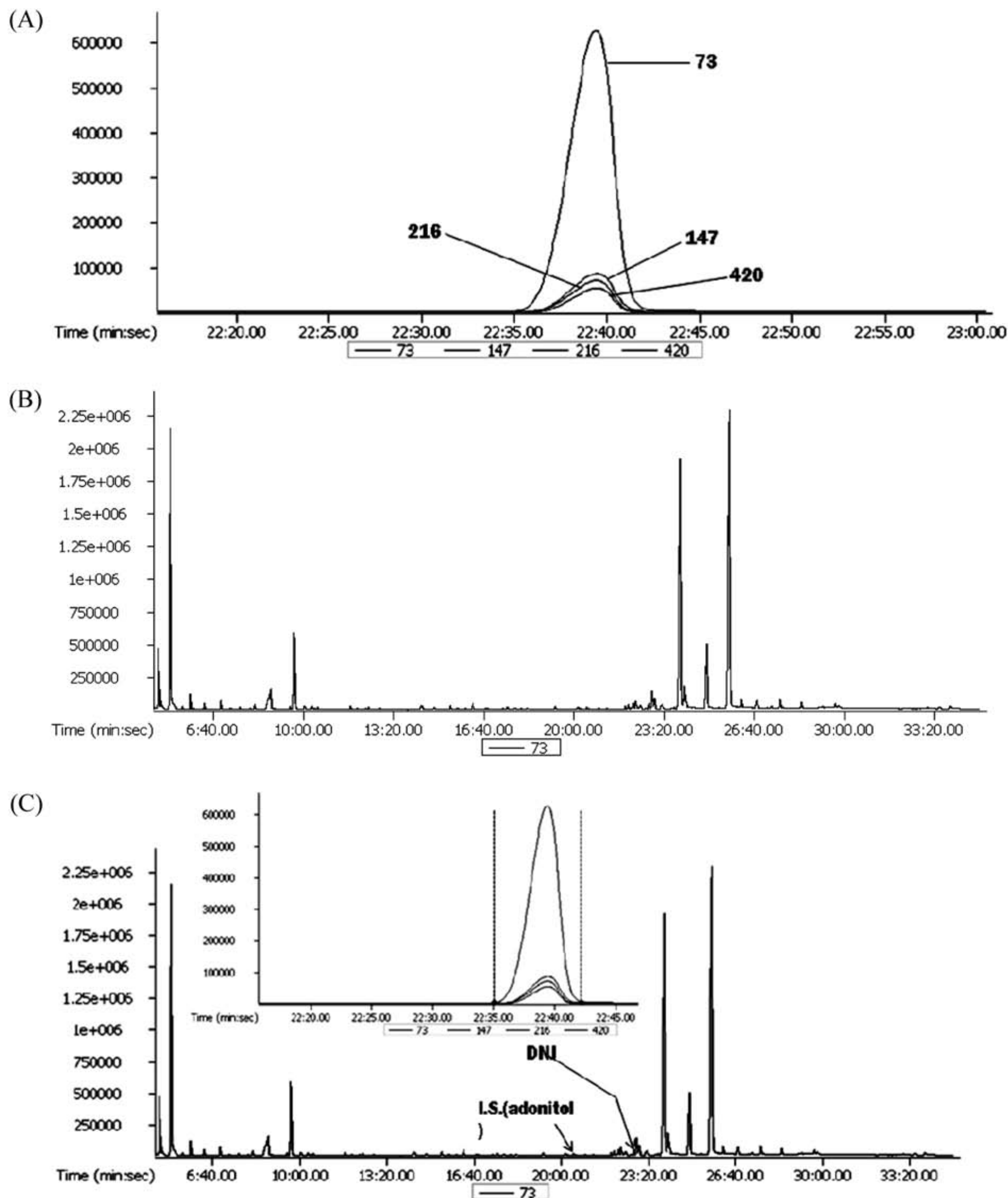

Figure 3. GC-TOF-MS analysis of rat plasma extract before (**B**) and after (**C**) oral administration of DNJ. Rat plasma extract before and 30 min after oral administration of DNJ (6 mg/kg body weight) was analyzed using GC-TOF-MS. (**A**) MS spectrum of standard derivatized DNJ compound. (**B**) Extract of rat plasma before administration. (**C**) Extract of rat plasma at 30 min after administration of DNJ.

using SD rats after oral administration of mulberry extract or purified DNJ compound. To analyze DNJ in biological samples such as plasma, urine, and feces, it was important to develop a highly reproducible and sensitive analytical method. We therefore developed a simplified method that employed GC-TOF-MS after derivatization of DNJ by BSTFA. The derivatization procedure allowed for the relatively easy detection of DNJ in complex biological samples using GC-TOF-MS. It is less expensive compared to other analytical approaches such as LC-MS and has high reproducibility, high resolution, and few matrix effects (*21*). Although there was no study on the derivatization of DNJ for the analysis using GC-MS, we confirmed that DNJ, which has a structure very similar to that of sugars and amino sugars, could be readily derivatized to be analyzed by GC-MS following the same procedure as that used for sugars and amino sugars. Also, this method could provide increased selectivity and sensitivity as well as a lower detection limit. Compared to the detection limit using HILIC-TOF-MS, which was lower than 6 μmol/L (*12*), the detection limit of this study was below 6×10^{-4} μmol/L. With this newly developed analytical method, we investigated the bioavailability of DNJ in mulberry leaf extract using SD rats. Recently, Nakagawa et al. (*12*) reported that DNJ was absorbed

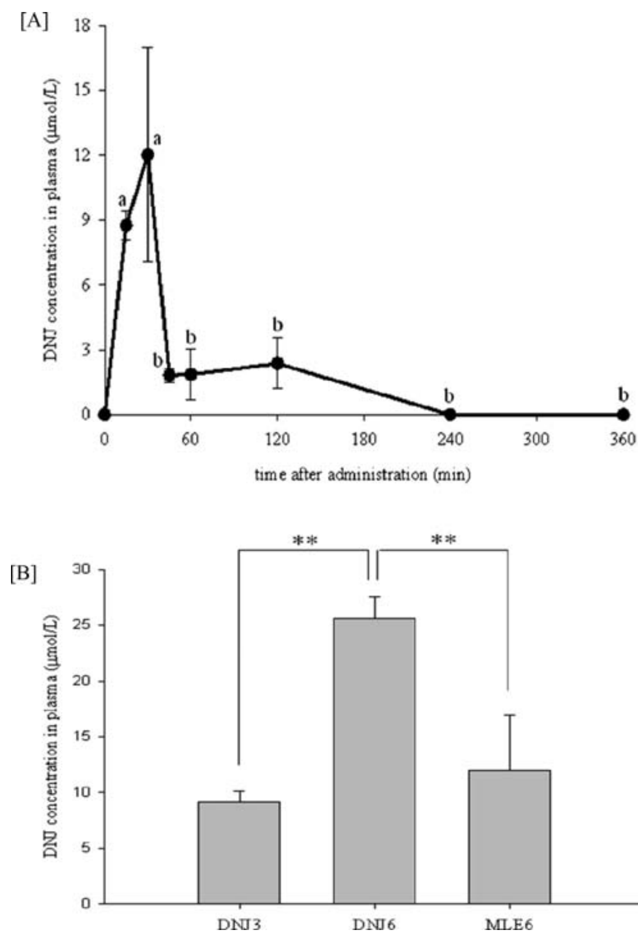

Figure 4. Concentrations of DNJ in rat plasma after single oral administration of purified DNJ compound and mulberry extract. (A) Concentration−time curves for DNJ after oral administration of MLE. MLE was orally administered at 1.7 g/kg body weight (6 mg as DNJ), and then concentrations of DNJ were measured at 0, 15, 30, 45, 60, 120, 240, and 360 min in plasma. Data are expressed as mean values with standard errors shown by vertical bars. Mean values were significantly different at 15 and 30 min using one-way ANOVA at $p < 0.05$. (**B**) Concentrations of DNJ in rat plasma at 30 min after single oral administration of purified DNJ compound and mulberry extract. The purified DNJ compound (3 or 6 mg/kg body weight) and MLE (1.7 g/kg body weight, 6 mg as DNJ) were administered to rats, and then levels of DNJ in plasma were measured. Data are expressed as mean values with standard errors shown by vertical bars. Comparisons were performed between each of the two groups (DNJ3 vs DNJ6 and DNJ6 vs MLE6: ** $P < 0.01$).

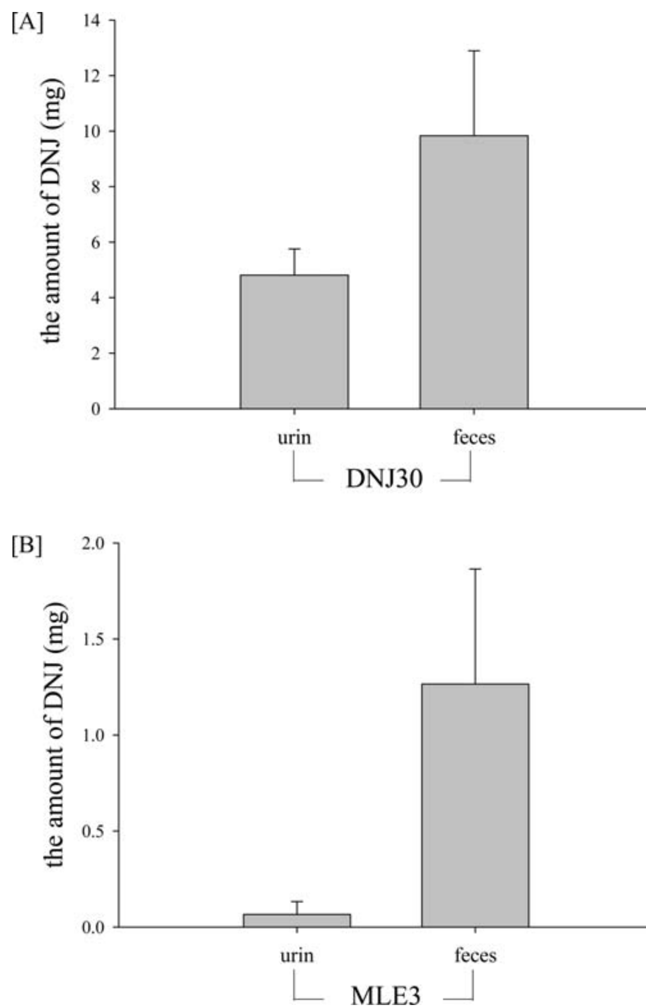

Figure 5. Concentrations of DNJ in urine and feces after single oral administration of purified DNJ compound or mulberry extract. The animals were fasted for 12 h, and then purified DNJ compound (30 mg/kg body weight) or MLE (0.85 g/kg body weight, 3 mg as DNJ) was orally administered at day 9 for the determination of DNJ in urine and feces. Using a metabolic cage, we collected urine between 0 to 24 h, and feces samples were collected between 0 to 48 h to evaluate DNJ concentrations. Data are expressed as mean values with standard errors shown by vertical bars. (**A**) Purified DNJ compound. (**B**) MLE.

into plasma in the intact form in a dose-dependent manner. They also found that DNJ has a short half-life and that 1% of ingested DNJ was incorporated into the rat plasma. In our study, which also used isolated DNJ, it was shown that DNJ in mulberry extract was absorbed into plasma in the intact form as well, reaching a maximum at 30 min after oral intake. To determine the metabolic fate of DNJ, we analyzed levels in feces and urine. Most of the DNJ absorbed into the plasma was excreted in the feces and a small portion seemed to be metabolized especially when the mulberry extract was administered (**Figure 5**). Nakagawa et al (*12*) also reported that the absorption rate of DNJ was slightly lower when compared to that of aza-sugars. In our study, we confirmed that the majority of orally administered DNJ is not fully absorbed into the plasma, but rather excreted in the feces. Our results also showed that the metabolic pattern of DNJ existing in the extract was similar to that of the highly purified DNJ compound. However, we found that the bioavailability of DNJ from the mulberry extract was lower by analyzing it in plasma following oral administration of the extract. Mulberry leaves are composed of dietary fiber, quercetin, rutin, and other compounds in addition to DNJ (*9*). Among these compounds, soluble dietary fiber is the major component in mulberry leaf aqueous extract, which is about 60% (*22*). Soluble dietary fiber has the function of inhibiting glucose uptake by binding sugar groups (*23*); therefore, it is assumed that the absorption of DNJ, which is very similar to sugar in its structure, would be inhibited by soluble dietary fiber. Despite the low absorption rate of DNJ from mulberry extracts, it still offers prominent beneficial effects in terms of hypoglycemia control. Miyahara et al. (*8*) reported on the inhibitory effects of an aqueous ethanol extract of mulberry leaves on postprandial hyperglycemia in normal Wistar rats. Kimura et al. (*7*) also showed that food grade mulberry powder suppressed the elevation of postprandial blood glucose in humans. On the basis of these results, although the absorption of DNJ, the active ingredient in mulberry leaves, is lower than that of isolated DNJ, mulberry extract remains a promising candidate

as a hypoglycemic functional ingredient in food or food supplements.

DNJ is generally regarded as a competitive inhibitor of small-intestinal brush-boarder α-glucosidase. Therefore, the therapeutic consideration of DNJ always centers on its postprandial hypoglycemic effects in the gastrointestinal tract. Derivatives of DNJ such as miglitol and emiglitate are systematically absorbed, but they are not metabolized and are rapidly excreted via the kidneys (*24*). However, in the case of DNJ, it appears to be excreted in the feces after administration, and although a small amount of DNJ may be rapidly absorbed into the blood after oral administration, it was removed from the body in our study. Moreover, if DNJ would be administered via mulberry extracts, which is a complex form, its bioavailability would be lower while maintaining its hypoglycemic effect. However, studies revealing the hypoglycemic effects and lysosomal storage of glycogen as a sequel of α-glucosidase inhibition by the absorption of DNJ from mulberry extract over a longer period are required.

In conclusion, the results of our study clearly indicate that the majority of DNJ administered to rats were excreted in feces and that a small amount was absorbed and then rapidly excreted in urine. In addition, the absorption of DNJ in mulberry leaf extract was found to be lower than that of highly purified DNJ. However, further studies examining the effects of DNJ from mulberry leaf on glucose control over long-term intake as well as its safety concerns are required in order to clarify the role of mulberry leaf as a hypoglycemic agent.

ABBREVIATIONS USED

DNJ, 1-deoxynojirimycin; GC-TOF-MS, gas chromatograph coupled with time-of-flight mass spectrometer; DMJ, 1-deoxymannojirimycin; BSTFA, *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide; TMCS, trimethylchlorosilane; HPLC, high performance liquid chromatography; FMOC-Cl, nine-fluorenylmethyl chloroformate; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DPBS, Dulbecco's phosphate buffered saline; MEM NEAA, modified Eagle's medium nonessential amino acids; MLE, mulberry leaf extract; KCLB, Korean Cell Line Bank; BCA, bichinchonic acid; RP-HPLC, reversed-phase high-performance liquid chromatography; TEER, transepithelial electrical resistance; SD, Sprague−Dawley.

LITERATURE CITED

(1) Asano, N.; Yamashita, T.; Yasuda, K.; Ikeda, K.; Kameda, Y.; Kato, A.; Nash, R. J.; Lee, H. S.; Ryu, K. S. Polyhydroxy alkaloids islated from mulberry trees (*Morus alba L.*) and silkworms (*Bombyx mori L.*). *J. Agric. Food Chem.* **2001**, *49*, 4208–4213.
(2) Kimura, T.; Nakagawa, K.; Saito, Y.; Yamagishi, K.; Suzuki, M.; Yamaki, K.; Shinmoto, H.; Miyazawa, T. Determination of 1-deoxynojirimycin in Mulberry leaves using hydrophilic interaction chromatography with evaporative light scattering detection. *J. Agric. Food Chem.* **2004**, *52*, 1415–1418.
(3) Nojima, H.; Kimura, I.; Chen, F. J.; Sugihara, Y.; Haruno, M. Antihyperglycemic effects of N-containing sugars from *Xanthocercis zambesiaca*, *Morus bombycis*, *Aglaonema treubii*, and *Castranospermum austral* in streptozotocin-diabetic mice. *J. Nat. Prod.* **1998**, *61*, 397–400.
(4) Kong, W. H.; Oh, S. H.; Ahn, Y. R.; Kim, K. W.; Kim, J. H.; Seo, S. W. Antiobesity effects and improvement of insulin sensitivity by 1-deoxynojirimycin in animal models. *J. Agric. Food Chem.* **2008**, *56*, 2613–2619.
(5) Hughes, A. B.; Rudge, A. J. Deoxynojirimycin: synthesis and biological activity. *Nat. Prod. Rep.* **1994**, *11*, 135–162.
(6) Lee, S. K.; Hwang, J. Y.; Song, J. H.; Jo, J. R.; Kim, M. J.; Kim, M. E.; Kim, J. I. Inhibitory activity of *Euonymus alatus* alpha-glucosidase in vitro and in vivo. *Nutr. Res. Pract.* **2007**, *1*, 184–188.
(7) Kimura, T.; Nakagawa, K.; Kubota, H.; Kojima, Y.; Goto, Y.; Yamagishi, K.; Oita, S.; Oikawa, S.; Miyazawa, T. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. *J. Agric. Food Chem.* **2007**, *55*, 5869–5874.
(8) Miyahara, C.; Miyazawa, M.; Satoh, S.; Sakai, A.; Mizusaki, S. Inhibitory effects of mulberry leaf extract on postprandial hyperglycemia in normal rats. *J. Nutr. Sci. Vitaminol.* **2004**, *50*, 161–164.
(9) Song, W.; Wang, H. J.; Bucheli, P.; Zhang, P. F.; Wei, D. Z.; Lu, Y. H. Phytochemical profiles of different mulberry (*Morus* sp.) species from China. *J. Agric. Food Chem.* **2009**, *57*, 9133–9140.
(10) Chae, J. Y.; Lee, J. Y.; Hoang, I. S.; Whangbo, D.; Choi, P. W.; Lee, W. C.; Kim, J. W.; Kim, S. Y.; Choi, S. W.; Rhee, S. J. Analysis of functional components of leaves of different mulberry cultivars. *J. Korean Soc. Food Sci. Nutr.* **2003**, *32*, 15–21.
(11) Faber, E. D.; Oosting, R.; Neefjes, J. J.; Ploegh, H. L.; Meijer, D. K. Distribution and elimination of the glycosidase inhibitors 1-deoxymannojirimycin and N-methyl-1-deoxynojirimycin in the rat in vivo. *Pharm. Res.* **1992**, *9*, 1442–1450.
(12) Nakagawa, K.; Kubota, H.; Kimura, T.; Yamashita, S.; Tsuzuki, T.; Oikawa, S.; Miyazawa, T. Occurrence of orally administered mulberry 1-deoxynojirimycin in rat plasma. *J. Agric. Food Chem.* **2007**, *55*, 8928–8933.
(13) Hidalgo, I. J.; Raub, T. J.; Borchardt, R. T. Characterization of the human colon adenocarcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **1989**, *96*, 736–749.
(14) Artursson, P. Cell cultures as models for drug absorption across the intestinal mucosa. *Crit. Rev. Ther. Drug Carrier Syst.* **1991**, *8*, 305–330.
(15) Kim, J.; Kim, S.; Lee, H. S.; Kim, I.; Ahn, M. Y.; Ahn, M. Y. Determination of 1-deoxynojirimycin in Morus alba L. leaves by derivatization with 9-fluorenylmethyl chloroformate followed by reversed-phase high-performance liquid chromatography. *J. Chromatogr., A* **2003**, *1002*, 93–99.
(16) Knapp, D. R. Carbohydrate. In *Handbook of Analytical Derivatization Reactions*; John Wiley & Sons: Hoboken, NJ, 1979; pp 539−598.
(17) Glahn, R.; Van Campen, D. Iron uptake is enhanced in Caco-2 cell monolayers by cysteine and reduced cysteinyl glycine. *J. Nutr.* **1997**, *127*, 642–647.
(18) Deprez, S.; Mila, I.; Huneau, J.; Tome, D.; Scalbert, A. Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxid. Redox Signaling* **2001**, *3*, 957–967.
(19) Kuo, S. Transepithelial transport and accumulation of flavones in human intestinal Caco-2 cells. *Life Sci.* **1998**, *63*, 2323–2331.
(20) Ferruzzi, M.; Failla, M.; Sarama, R. Estimation of carotenoid bioavailability from fresh stir-fried vegetables using an in vitro digestion/Caco-2 cell culture model. *J. Nutr. Biochem.* **2000**, *11*, 574–580.
(21) Kopka, J. Current challenges and developments in GC-MS based metabolite profiling technology. *J. Biotechnol.* **2006**, *124*, 312–322.
(22) Park, J. M.; Bong, H. Y.; Jeong, H. I.; Kim, Y. K.; Kim, J. Y.; Kwon, O. Postprandial hypoglycemic effect of mulberry leaf in Goto-Kakizaki rats and counterpart control Wistar rats. *Nutr. Res. Pract.* **2009**, *3*, 272–278.
(23) Riccardi, G.; Rivellese, A. A. Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. *Diabetes Care* **1991**, *14*, 1115–1125.
(24) Ahr, H. J.; Boberg, M.; Brendel, E.; Krause, H. P.; Steinke, W. Pharmacokinetics of miglitol, absorption, distribution, metabolism, and excretion following administration to rats, dogs, and man. *Arzneimittelforschung* **1997**, *47*, 734–745.

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