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In Vivo Metabolism of 1,5-Anhydro-d-fructose to 1,5-Anhydro-d-glucitol

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Abstract. *Background/Aim: 1,5-Anhydro-D-fructose (1,5-AF, saccharide) and 1,5-anhydro-D-glucitol (1,5-AG) converted from 1,5-AF via the glycemic pathway have health benefits. However, this metabolism has not been sufficiently elucidated. To clarify the in vivo metabolism of 1,5-AF to 1,5-AG, porcine (blood kinetics) and human (urinary excretion) studies were conducted. Materials and Methods: Microminipigs were administrated 1,5-AF orally or intravenously. Blood samples were obtained to analyse the kinetics of 1,5-AF and 1,5-AG. Urine samples were collected from human subjects who had orally ingested 1,5-AF, and the amounts of 1,5-AF and 1,5-AG excreted in the urine were analysed. Results: In blood kinetics analysis, the time to the maximum concentration of 1,5-AF after intravenous administration was 0.5 h, whereas 1,5-AF was not observed after oral administration. The times to the maximum concentration of 1,5-AG after intravenous and oral administration were 1.5 h and 2 h, respectively. In urinary excretion, the concentration of 1,5-AG in urine rapidly increased after the administration of 1,5-AF, peaked at 2 h, whereas 1,5-AF was not detected. Conclusion: 1,5-AF was rapidly metabolized to 1.5-AG in vivo in swine and human.*

1,5-Anhydro-D-fructose (1,5-AF) is a saccharide that has been isolated from several fungi (1) and an edible seaweed (2) as an intermediate of the microthecin biosynthesis pathway. This saccharide is considered to be widely distributed among organisms, including animals (3). It is believed that 1,5-AF is biosynthesized from α-1,4-glucans such as starch and glycogen by the action of α-1,4-glucan lyase (4). Furthermore, 1,5-AF is produced as a food material in Japan *via* the enzymatic degradation of starch. 1,5-AF has antioxidant (5) and antibacterial properties (6) and health benefits (7), thus it has utility as a food preservative and healthy food material.

1,5-Anhydro-D-glucitol (1,5-AG) is present in the blood of humans and rats (8). The blood 1,5-AG concentration is clinically measured because it is correlated with blood glucose levels and is used in blood glucose control. Blood 1,5-AG originates from two pathways, namely the consumption of food containing 1,5-AG and the biosynthesis of glycogen with 1,5-AF as an intermediate. It was estimated that 0.438 mg of 1,5-AG is synthesized per day from 1,5-AF *via* the glycemic pathway (9).

In Japan, the fermentative production method of 1,5-AG from 1,5-AF has been reported. Also, 1,5-AG has beneficial properties for human health such as anti-inflammatory (10) but can also act as a blood glucose regulator in diabetes (11), and an inhibitor of COVID-19 infection (12).

Following the oral administration of ^{14}C-labelled 1,5-AF in mice, it has been reported that 62.9, 4.5, and 10.7% of the radioactivity is recovered from urine, faeces, and CO_2,

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Key Words: 1,5-anhydro-D-fructose, 1,5-anhydro-D-glucitol, high-performance lipid chromatography, *in vivo* metabolism, pharmacokinetics.

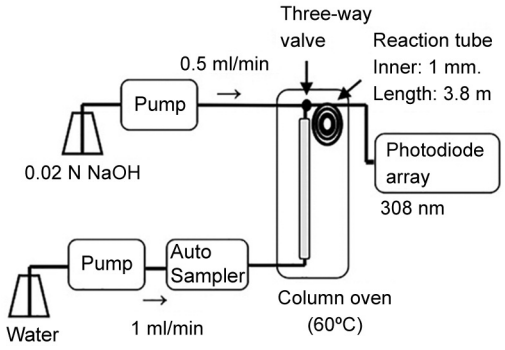

Figure 1. *High-performance liquid chromatography system for the specific determination of 1,5-anhydro-D-fructose.*

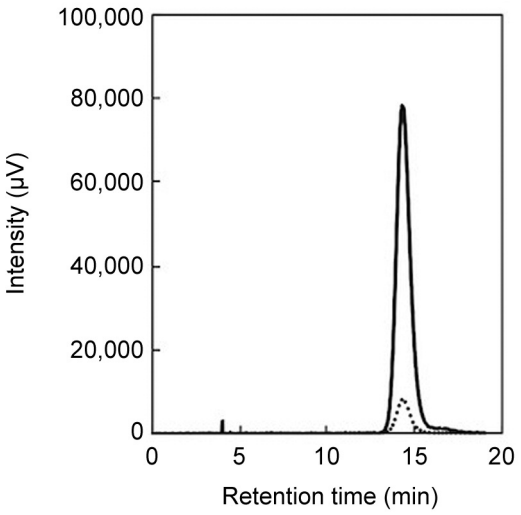

Figure 2. *Chromatograms of 1,5-anhydro-D-fructose standard. Solid line: 100 μg/ml; dot line: 10 μg/ml.*

respectively (13). In addition, it has also been observed that the urine 1,5-AG levels were dramatically increased in mice administrated non-labeled 1,5-AF orally (13). The oral consumption of 1,5-AF has been proven safe in mice, rats (14), and humans (15). However, limited research has examined the pharmacokinetics and *in vivo* metabolism of 1,5-AF to 1,5-AG. An enzymatic determination method for 1,5-AG in blood has been established. In addition, 1,5-AF quantification methods based on gas chromatography–mass spectrometry after derivatization have been reported (2) although these methods are complex, and a simpler protocol is desired.

In 2011, the Microminipig (MMPig) was developed as the smallest miniature pig in the world (16), and this strain has similar general biochemistry and haematology profiles (17, 18) as domestic swine and humans. The utility of MMPigs as experimental animals has been demonstrated in life science research. Similarly, as other minipigs, the MMPig represents a potentially appropriate experimental model because its lipoprotein metabolism (19-21), anatomy, physiology, and feeding and sleep habits (22-24) are similar to those of humans.

Therefore, this study examined the pharmacokinetics of 1,5-AF and 1,5-AG after the intravenous and oral administration of 1,5-AF in MMPigs to clarify the *in vivo* metabolism 1,5-AF to 1,5-AG. Furthermore, we conducted an oral administration test of 1,5-AF in humans and quantified 1,5-AF and 1,5-AG excreted in the urine for evaluation of 1,5-AF supplement as 1,5-AG precursor. Additionally, a simpler method for blood 1,5-AF determination was investigated.

Materials and Methods

Test article. 1,5-AF was prepared by SUNUS Co., Ltd. (Kagoshima, Japan) as previously described (5). The samples for intravenous injection were applied after filtration with a molecular weight cut-off of 10 KDa.

Experiment 1 (Porcine study: blood kinetics).
Animals and diet. Two adult MMPigs (male and female; body weight of 22.6 and 26.9 kg, respectively) were obtained from a breeder (Fuji Micra Inc., Shizuoka, Japan) and maintained in a dedicated room with filtered air laminar flow at Kagoshima University. The room was maintained at a temperature of 24±3˚C and a relative humidity of 50±20% with a 12-h/12-h light/dark cycle. Tap water was available *ad libitum*, and the animals were provided a commercial porcine diet (Horeborekobuta; Marubeni Nisshin Feed Inc., Tokyo, Japan). All protocols were approved by the Ethics Committees of Animal Care and Experimentation, Kagoshima University (MD19094). Finally, the research was performed according to the Institutional Guidelines for Animal Experiments and in compliance with the Japanese Act on Welfare and Management of Animals (Act No. 105 and Notification No. 6).

Blood kinetics study design. Unilateral sinus venarum cavarum catheterization was performed in all animals under deep anaesthesia with isoflurane inhalation as previously reported (25). All animals were first intravenously administered 1,5-AF (20 mg/kg body weight). After a 1-week follow-up period, all animals were orally administered the same dose of 1,5-AF. Five millilitres of blood were obtained from each animal before and 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h after 1,5-AF administration for pharmacokinetic analysis. The maximum concentration (Cmax) and time to maximum concentration (Tmax) were measured for 1,5-AF and 1,5-AG, and the half-lives of 1,5-AF and 1,5-AG were calculated.

New measurement methods of blood 1,5-AF and 1,5-AG concentrations. The concentration of 1,5-AF was analysed *via* high-performance liquid chromatography (HPLC) using a MCIGEL CK08S column (8.0×500 mm, Mitsubishi Chemical Co.). The flow rate of eluent water was 1.0 ml/min. The injection volume was 100 μl. A three-way valve was provided at the outlet of the separation column, and 0.02 N NaOH was mixed with the eluent at a flow rate of 0.5 ml/min. The mixed eluent was introduced into a photodiode

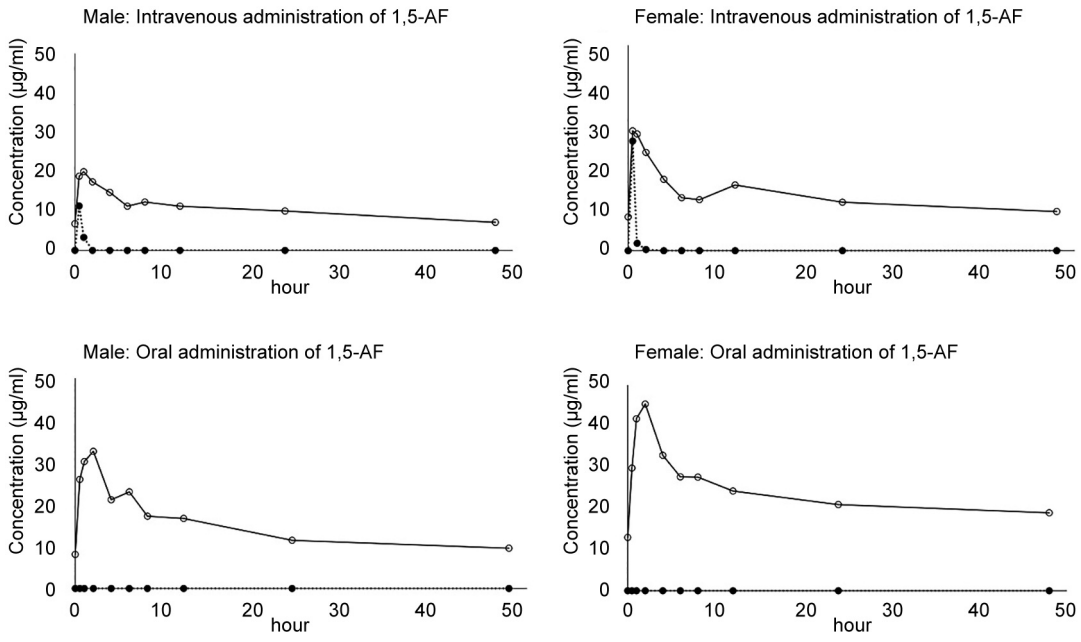

Figure 3. *Pharmacokinetics of blood 1,5-anhydro-D-fructose (1,5-AF) and 1,5-anhydro-D-glucitol (1,5-AG) concentrations after the intravenous and oral administration of 1,5-AF in Microminipigs. Closed circle: blood 1,5-AF concentration; open circle: blood 1,5-AG concentration.*

array detector after reaction in a tube with an inner diameter of 1 mm and a length of 3.8 m. The separation column and reaction tube were placed in a column oven set at 60˚C (Figure 1). 1,5-AG content was measured using Lana AG® (Nihonkayaku Co., Ltd., Tokyo, Japan) following the manufacturer's instructions. To develop the quantitative method for 1,5-AF, the absorption spectrum of 1,5-AF in alkaline solution was analysed. One thousand microlitres of 1 μg/ml 1,5-AF were mixed with 500 μl of 0.02 N NaOH at 60˚C, and the absorption spectrum from 270 to 330 nm with a 1-cm cell was analysed.

Experiment 2 (Human study: urinary excretion). This human study was conducted in compliance with the Declaration of Helsinki and the ethical guidelines for human life science and medical research. The bioethics committee reviewed and approved (approval number: USL202203, User Life Science Co.) the study. Four subjects orally ingested 5 g of 20% (w/w) aqueous 1,5-AF solution. All urines were collected from each subject and the volumes were measured before and 2, 4, 6, and 8 h after 1,5-AF administration. The 1,5-AF and 1,5-AG in the urine were quantified by HPLC using a MCIGEL CK08S column with differential refractometer.

Results

Experiment 1 (porcine study).

Measurement of blood 1,5-AF and 1,5-AG concentrations. UV absorption was not observed when water was mixed with 1,5-AF. However, when 0.02 N NaOH was added, the absorption peaked at approximately 308 after 1-3 min. After 20 min, the absorption had almost disappeared. Therefore, the HPLC system was constructed using a reaction time of 2 min and a temperature of 60˚C.

1,5-AF was analysed using this HPLC system over a concentration range of 1-100 μg/ml. The concentrations and peak areas were plotted, and a linear curve passing through the origin was obtained. The coefficient of determination was 0.9999, and quantification was possible within this range. Chromatograms of 10 and 100 μg/ml 1,5-AF are presented in Figure 2. Symmetrical peaks were obtained. The peaks of the absorption spectrum were approximately 308 nm, matching the result of spectrophotometry. Because peaks were not detected for 1 mg/ml glucose and fructose in this HPLC system, 1,5-AF was selectively quantified. Furthermore, 1,5-AF could be effectively quantified because no peaks were obtained near the retention time of 1,5-AF even when swine plasma or serum was analysed using this system.

Blood kinetics (Figure 3, Table I). The Cmax value of 1,5-AF and 1,5-AG after intravenous administration of 1,5-AF were 19.0 and 24.6 μg/ml, respectively. Conversely, The Cmax value of 1,5-AF was not achieved after the oral administration of 1,5-AF, whereas the Cmax value of 1,5-AG was 39.1 μg/ml. After the intravenous administration of 1,5-AF, the average Tmax values for 1,5-AF and 1,5-AG were 0.5 h and 1.5 h, respectively. The average half-lives of 1,5-AF and 1,5-AG were 0.24 h and 3.23 h, respectively. The decay of the blood 1,5-AF concentration was observed at 2 h after the intravenous administration of 1,5-AF. After the oral administration of 1,5-AF, the average Tmax of 1,5-AG was 2 h, whereas no peak blood 1,5-AF concentration was

Table I. *Maximum concentration (Cmax), time to maximum concentration (Tmax), and half-life of 1,5-anhydro-D-fructose (1,5-AF) and 1,5-anhydro-D-glucitol (1,5-AG) in the porcine study.*

Item	Intravenous administration of 1,5-AF	Oral administration of 1,5-AF
Average Cmax of 1,5-AF (male/female)	19.0 μg/ml (11.3 μg/ml/26.7 μg/ml)	–
Average Cmax of 1,5-AG (male/female)	24.6 μg/ml (20.0 μg/ml/29.2 μg/ml)	39.1 μg/ml (32.7 μg/ml/45.4 μg/ml)
Average Tmax of 1,5-AF (male/female)	0.5 h (0.5 h/0.5 h)	–
Average Tmax of 1,5-AG (male/female)	1.5 h (1 h/2 h)	2 h (2 h/2 h)
Average half-life of 1,5-AF (male/female)	0.24 h (0.22 h/0.25 h)	–
Average half-life of 1,5-AG (male/female)	3.23 h (3.25 h/3.21 h)	5.46 h (5.37 h/5.54 h)

recognized. The average half-life of 1,5-AG was 5.46 h. Decay of the blood 1,5-AG concentration was recorded at 48 h after the intravenous and oral administration of 1,5-AF.

Experiment 2 (Human study, Figure 4). The concentration of 1,5-AG in urine rapidly increased after administration of 1,5-AF to humans, peaked at 2 h, and then decreased, whereas the concentration of 1,5-AF was not detected. The excretion amount of 1,5-AG in urine up to 8 h after administration was 122.8±8.6 mg.

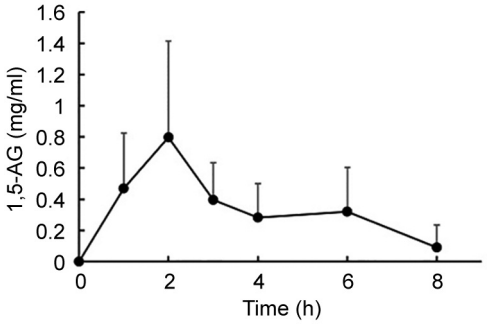

Figure 4. *Urine 1,5-anhydro-D-glucitol (1,5-AG) concentrations after the oral administration of 1,5-AF in human.*

Discussion

In the new measurement methods of blood 1,5-AF and 1,5-AG concentrations, the UV absorption of 1,5-AF increased under alkaline conditions and then disappeared. It has been reported that 1,5-AF is converted to an organic acid *via* ascopylone P (APP) and ascopylone M (APM) under weak alkaline conditions (26). The absorption spectra of APP and APM are reported to be peak at 292 and 262 nm, respectively (27). Therefore, the absorption observed at 308 nm is considered attributable to a substance of unknown structure, and detection of the substance is a future task. Using this method, the blood 1,5-AF concentration could be selectively and highly sensitively measured *via* a simple system. Even when a known amount of 1,5-AF was added to pig serum, it could be quantified accurately. Therefore, this HPLC system is suitable for 1,5-AF detection in blood.

In Experiment 1, 1,5-AF was considered to be metabolized to 1.5-AG *in vivo* because peaks of the 1,5-AG concentration were observed after both the intravenous and oral administration of 1,5-AF. However, following the oral administration of 1,5-AF, an increase in blood 1,5-AG was observed but blood 1,5-AF was not detected. It was thought that 1,5-AF was absorbed in the small intestine and rapidly metabolized to 1.5-AG in the liver, which has a reductase that converts 1,5-AF to 1,5-AG in swine (28). 1,5-AF was also considered to be metabolized to 1.5-AG in other body tissues because a peak of the blood 1,5-AG concentration was noted after intravenous 1,5-AF administration. The production of 1,5-AG from 1,5-AF was reported in erythroleukemia cells (29), but its production in tissues other than the liver remains to be established.

The blood 1,5-AG levels increased after 1,5-AF administration but returned to baseline within 48 h. Previous findings indicated that most of the orally administered dose of 1,5-AG is excreted in urine in mice and humans (30). In the human study, it was thought that 1,5-AF was rapidly metabolized to 1.5-AG, which was excreted in urine *in vivo*. In the porcine study, it is also speculated that 1,5-AG converted from 1,5-AF was excreted in urine *in vivo*.

Advanced glycation end-products (AGEs) are produced by non-enzymatic reactions between the aldehyde groups of reducing sugars and amino groups of amino acids or proteins. AGEs are recognized risk factors for diseases such as diabetes and Alzheimer's disease. An immunological trial using an antibody indicated the existence of novel

AGEs from 1,5-AF (AF-AGEs) in human and animal serum samples (31). 1,5-AF was not detected in blood following oral administration in this *in vivo* trial. Therefore, the *in vivo* risk of AF-AGEs must be lower than that of AGEs produced *via* glucose, which is the most abundant reducing sugar in blood.

In conclusion, 1,5-AF was considered to be metabolized to 1.5-AG *in vivo* in human and swine and the 1.5-AG excreted in urine *in vivo* in human. The *in vivo* metabolism of 1,5-AF and 1,5-AG was also rapid.

Conflicts of Interest

The Authors report no conflicts of interest in relation to this study.

Authors' Contributions

MI, KY, MK, and HK collected sample material. HK and IM planned the study; KY and NM performed the experiments and analysed data; MI, KY, HK, and IM drafted the manuscript; YF and TM revised the draft. All Authors read and approved the final manuscript.

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