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≪Research Note≫

Half-life of Fructosyl-valine in the Plasma of Chicks

Natsuki Takahashi[1], Ryosuke Makino[2] and Kazumi Kita[1]

[1] Department of Animal Sciences, Faculty of Agriculture, Iwate University, Morioka, Iwate 020−8550, Japan
[2] Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matsuyama 790−8566, Japan

Eighty14-d-old single-comb White Leghorn male chicks were divided into 16 groups with five birds each. Fructosyl-valine, which is a valine-glucose-Amadori product, was intravenously (2,250 nmol/kg body weight) or orally (300 μmol/kg body weight) administered to chicks. Blood samples were collected 15, 30, 60, 120, 180, 360, 720 and 1440 min after administration. Plasma concentrations of fructosyl-valine were measured by using a liquid chromatography / mass spectrometry (LC/MS). The time course change in plasma fructosyl-valine concentration showed an exponential curve, as $y=a+be^{-\lambda t}$. The half-life of plasma fructosyl-valine was calculated by the following equation: $(\log_e 2)/\lambda$. When fructosyl-valine was injected intravenously, the highest value for plasma fructosyl-valine concentration was observed 15 min after administration. When injected intravenously, the half-life of plasma fructosyl-valine was calculated to be 231 min. When fructosyl-valine was administered orally to chicks, the highest value for plasma fructosyl-valine concentration was observed 180 min after administration. When administered orally, the half-life of plasma fructosyl-valine was calculated to be 277 min. We conclude that the half-life of fructosyl-valine in plasma was approximately 4 h, which is longer than that of glycated tryptophan.

Key words: Amadori product, fructosyl-valine, gastrointestinal absorption, plasma half-life

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Introduction

Glycation, which involves the Maillard reaction (Maillard, 1912), is a non-enzymatic reaction between carbonyl groups of reducing sugars and amino groups of amino compounds like amino acids and proteins. This reaction results in the formation of the Schiff base, and the subsequent formation of Amadori product through Amadori rearrangement (Hodge, 1955). Amadori product undergoes further complex reaction such as cross-linking, oxidation, dehydration, and condensation to form advanced glycation end products (AGEs), which are likely to associated with the development of diabetic complications (Brownlee, 2001).

Compared to mammals, avian species have unique characteristics, such as high blood glucose concentrations and a high body temperature (Hazelwood and Lorenz, 1959), which can easily lead to the generation of many AGEs. It was reported that pentosidine, one of the typical and classical AGEs, was detected in the skin of chickens (Iqbal *et al*., 1997; 1999; 2000). Recently, glycated hemoglobin was measured in the blood of chickens (Aggarwal *et al*., 2009). We also measured plasma concentrations of two types of glycated tryptophan, fructosyl-tryptophan (glucose-derived Amadori product of tryptophan) and (1R, 3S)-1-(D-gluco-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-beta-carboline-3- carboxylic acid (PHP-THβC) (Kita *et al*., 2013; Makino *et al*., 2015) and fructosyl-valine (N-α-(1-deoxyfructosyl) valine) (Honma *et al*., 2017).

The mass of body constituents is maintained at the steady state, which is known as dynamic equilibrium. The term 'turnover' described in a single word by Schoenheimer (1942), covers the renewal or replacement of a biological substance as well as the exchange of material between different pools of biological substances. Based on this concept, biological substances are taken in and out by both inflow (intake and degradation) and outflow (synthesis and excretion). It was also recognized that the rate of degradation can be expressed by half-life of biological substances (Waterlow, 2006). Recently, we examined the time-course change in the plasma concentrations of PHP-THβC of chickens to calculate the half-life of PHP-THβC (Makino and Kita, 2018). However, the dynamics of fructosyl-valine in the plasma of chickens has not been previously examined. Therefore, in the present study, chicks were administered fructosyl-valine intravenously or orally, and the time-course

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Correspondence: Dr. Kazumi Kita, Laboratory of Animal Nutrition, Faculty of Agriculture, Iwate University, Morioka, Iwate 020−8550, Japan. (E-mail kitak@iwate-u.ac.jp)

change in plasma concentrations of fructosyl-valine was determined to estimate the plasma half-life of fructosyl-valine in chicks.

Materials and Methods

Synthesis of Fructosyl-valine

Fructosyl-valine was prepared by modifying the method previously reported by Wang *et al*. (2008). Briefly, anhydrous glucose (20 mmol), malonic acid (5 mmol) and L-valine (20 mmol) were refluxed in anhydrous methanol (30 ml) under nitrogen gas for 6 h. After filtration to remove the unreacted glucose and L-valine, the filtrate was concentrated under reduced pressure, and subsequently almost the same volume of anhydrous acetone was added, which resulted in precipitating fructosyl-valine. Precipitated fructosyl-valine was dissolved in ultrapure water and then precipitated with anhydrous acetone in order to eliminate any remaining unreacted valine. The product was vacuum-dried and stored under nitrogen atmosphere until used. The identification and determination of the product's purity were performed using LC/MS.

Chicks and Experimental Procedures

Newly hatched single-comb White Leghorn male chicks were obtained from a local hatchery (Koiwai Farm Co., Ltd, Shizukuishi, Iwate, Japan). Chicks were fed a commercial chick mash diet (crude protein: 207 g/kg, metabolizable energy: 12.1 MJ/kg; Toyohashi Feed Mills Co., Ltd, Toyohashi, Aichi, Japan) from hatching until 14 d of age in an electrically heated brooder. Continuous illumination was provided and ambient temperature was controlled at 29± 2℃. At 14 d of age, 80 chicks were selected and divided into 16 groups with five chicks each. After short-term food-deprivation for 12 h, fructosyl-valine dissolved in distilled water was intravenously (2,250 nmol/kg body weight) or orally (300 μmol/kg body weight) administered to chicks. Chicks were allowed free access to the commercial diet and water after fructosyl-valine administration. Fifteen, 30, 60, 120, 180, 360, 720, and 1,440 min after administration, blood samples were collected by heart puncture after light anesthesia with diethyl ether. Blood samples were centrifuged for 20 min at 5,000×g at 4℃ to separate plasma. Plasma samples were stored at −20℃ until analysis. Animal care was in compliance with applicable guidelines from the Iwate University Animal Care and Use Committee.

Sample Preparation

Protein and lipid were removed from plasma samples according to our previously developed method, with slight modification (Kita *et al*., 2013). Before deproteinization and delipidation, 62.5 μl of 0.33 mM N-methyl-leucine was added into 500 μl of plasma samples as an internal standard. Then, the plasma samples were mixed with 2 ml of acetonitrile for deproteinization. After deproteinization, lipid was removed by mixing samples with a mixture of chloroform and methanol (2:1, v/v). To extract fructosyl-valine, cation-exchange resin was used.

Measurement of Plasma Fructosyl-valine Using LC/MS

Separation and detection of fructosyl-valine and N-methyl-leucine were performed using LC/MS with an electrospray positive ionization (LCMS-2020, Shimadzu Corporation, Kyoto, Japan). The mobile phase was ultrapure water. The flow rate was 400 μl/min. The sample injection volume was 10 μl. Reverse phase HPLC column (100×2 mm I.D., Gemini 3 μm C18 110 Å, Phenomenex, Torrance, CA, USA) was used. Fructosyl-valine and N-methyl-leucine (an internal standard) were measured in positive ion mode using single ion monitoring. The values for m/z of positive ion of fructosyl-valine and N-methyl-leucine were 280.29 and 146.20, respectively. The concentration of fructosyl-valine was corrected with the recovery rate of N-methyl-leucine.

Statistical Analysis

All data are presented as mean±standard error (SE). Statistical analysis of data was performed by one-way ANOVA and Duncan's HSD test for multiple comparisons (with significance set at $P<0.05$) using the general linear model (GLM) procedures of SAS (SAS version 9.4, SAS Institute, 2012).

Calculation of Plasma Half-life of Fructosyl-valine

The time course change in the plasma fructosyl-valine concentration after administration was described by an exponential equation as $y=a+be^{-\lambda t}$, where y is the plasma concentration of fructosyl-valine in chicks at time t after administration, a is the basal level of plasma concentration of fructosyl-valine, b is the decrement of plasma fructosyl-valine concentration after administration, and λ is the elimination rate constant. All coefficients of exponential equations were estimated by using the non-linear procedure (NLIN) of SAS (2012). For estimating non-linear regression equation, values from 15 to 1,440 min in the intravenous administration group and those from 180 to 1,440 min in the oral administration group were used, respectively. The half-life of plasma fructosyl-valine was calculated using the following equation, $(\log_e 2) / \lambda$.

Results

When fructosyl-valine was injected intravenously, the highest value for plasma fructosyl-valine concentration was observed 15 min after administration (Fig. 1). The non-linear regression equation representing the decline of plasma fructosyl-valine concentration was as follows:

Plasma fructosyl-valine concentration (μM)=

$$5.46\ e^{(-0.0030\times \text{Time (min)})}+6.69$$

From this equation, the plasma half-life of fructosyl-valine was calculated to be 231 min.

When fructosyl-valine was administered orally to chicks, the highest value for plasma fructosyl-valine concentration was observed 180 min after administration (Fig. 2). The non-linear regression equation representing the decline of plasma fructosyl-valine concentration from 180 to 1440 min after administration was as follows:

Plasma fructosyl-valine concentration (μM)=

$$15.25\ e^{(-0.0025\times \text{Time (min)})}+8.29$$

From this equation, the plasma half-life of fructosyl-valine was calculated to be 277 min.

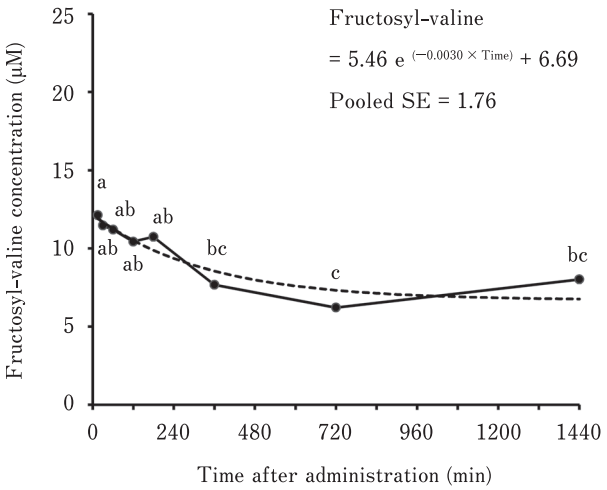

Fig. 1. **Time course change in plasma fructosyl-valine concentration in chicks from 0 to 1,440 min after intravenous administration with fructosyl-valine.** Fructosyl-valine dissolved in distilled water (2,250 nmol/kg body weight) was intravenously administrated to the wing vein of young chicks. Fifteen, 30, 60, 120, 180, 360, 720, and 1,440 min after administration, blood samples were taken from heart puncture, and plasma fructosyl-valine concentration was measured using LC/MS. [a, b, c] Means with different superscript letters are significantly different ($P<0.05$). Values are shown as means±SE, and $n=5$. The dotted line represents the non-linear regression equation estimating the decline of plasma fructosyl-valine concentration from 15 to 1440 min after administration.

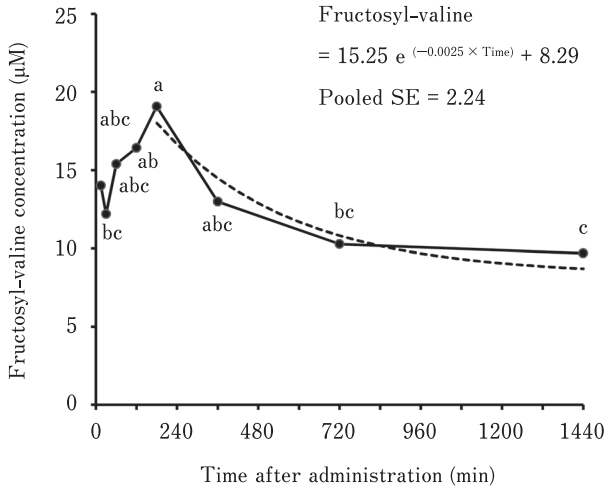

Fig. 2. **Time course change in plasma fructosyl-valine concentration in chicks from 0 to 1,440 min after oral administration with fructosyl-valine.** Fructosyl-valine dissolved in distilled water (300 μmol/kg body weight) was orally administered to the crop of chicks. Fifteen, 30, 60, 120, 180, 360, 720, and 1,440 min after administration, blood samples were taken from heart puncture, and plasma fructosyl-valine concentration was measured using LC/MS. [a, b, c, d] Means with different superscript letters are significantly different ($P<0.05$). Values are shown as means± SE, and $n=5$. The dotted line represents the non-linear regression equation estimating the decline of plasma fructosyl-valine concentration from 180 to 1440 min after administration.

Discussion

When chickens were administered PHP-THβC intravenously, the half-life of PHP-THβC, which is one of the glycated tryptophan, in plasma was estimated to be 99 min from the time-course change in the plasma PHP-THβC concentration (Makino and Kita, 2018). As shown in Fig. 1, the half-life of fructosyl-valine in plasma was calculated to be 231 min. These results suggest that the half-life of fructosyl-valine in plasma is longer than that of glycated tryptophan, PHP-THβC.

When ^{18}F-N-ε-fructosyl-lysine, which is an Amadori product derived from glucose and lysine, was injected intravenously to rats, approximate 30% of the injection dose of fructosyl-lysine accumulated in the kidney 5 min after injection. The remaining portion of fructosyl-lysine, corresponding to approximately 45% of the injection dose, was excreted in the urine (Hultsch *et al*., 2006). Similarly, when mice were intravenously administered ^{18}F-carboxymethyl-lysine (CML), which is the AGE developed from N-ε-fructosyl-lysine (Ahmed *et al*., 1986), the uptake of CML into the kidney and the excretion of CML into urine were 36% and 45% of the injection dose, respectively. It was also reported that CML administered intravenously to rats accumulated in the liver, heart and lung (Li *et al*., 2015). As in mammals, higher rates of accumulation of amino acid-derived AGEs were observed in the kidneys of chickens injected intravenously with amino acid-derived AGE (Kita, 2014). These results suggested that fructosyl-valine administered to chickens may accumulate slowly in kidney followed by immediate excretion into urine. Moreover, the large difference in the half-life of fructosyl-valine compared to that of glycated tryptophan may be explained by the difference in the rate of fructosyl-valine excretion from blood circulation into urine.

As shown in Fig. 2, when fructosyl-valine was administered orally to chicks, the plasma fructosyl-valine concentration increased gradually and reached the highest value 180 min after administration. This is in good agreement with our previous study (Takahashi and Kita, 2016), and these results suggest that fructosyl-valine can be absorbed from gastrointestinal tract and transferred into circulating blood. When administered orally, the plasma half-life of fructosyl-valine in chicks was calculated to be 277 min. This value is longer than that in chicks administered fructosyl-valine intravenously. The reason for the longer half-life of intravenously administered fructosyl-valine compared to orally administered fructosyl-valine may to be attributed to the time required for gastrointestinal absorption of fructosyl-valine.

The time course change in glycated amino acid concentra-

tion after administration was described by an exponential equation, as $y=a+be^{-\lambda t}$. In this equation, the coefficient 'a' refers to the basal level of plasma concentration of fructosyl-valine. As shown in Figures 1 and 2, the value for chicks administered with fructosyl-valine orally was approximately three times higher than that for birds intravenously injected with fructosyl-valine. In addition, the highest concentration of plasma fructosyl-valine in chicks administered fructosyl-valine was apparently higher than that in birds administered fructosyl-valine intravenously. These results suggest that orally administered fructosyl-valine contributed to the elevation in the plasma fructosyl-valine concentration, followed by a rapid decrease in plasma fructosyl-valine.

In conclusion, the half-life of fructosyl-valine in the plasma of chicks administered fructosyl-valine intravenously was 231 min, and this value is higher than that of glycated tryptophan.

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