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≪Research Note≫

Half-life of Glycated Tryptophan in the Plasma of Chickens

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Tryptophan, an essential amino acid, is enzymatically metabolized to two compounds, kynurenine and serotonin, and 95% of tryptophan is metabolized to kynurenine. As chickens have hyperglycemia and high temperature, tryptophan glycation occurs more easily in chickens than in mammals. Part of tryptophan is non-enzymatically converted to two types of glycated tryptophan, tryptophan-Amadori product and (1R, 3S) -1-(D-gluco-1, 2, 3, 4, 5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (PHP-THβC). Although these compounds are detected in the plasma of chickens, information on the half-life of PHP-THβC in the blood circulation is limited. Therefore, the present study aimed to measure the half-life of plasma PHP-THβC in chickens. PHP-THβC (114 nmol/0.2 mL/70 g body weight) was intravenously administered to chickens via the wing vein, and blood samples were collected at 0, 15, 30, 60, 180, 360, 720, and 1440 min after administration. Plasma concentrations of PHP-THβC were measured by liquid chromatography-mass spectrometry. Plasma PHP-THβC reached to a peak concentration of 16.1 μM at 30 min after administration, and then decreased rapidly to return to the physiological level (0 min) at 360 min after administration. The half-life of plasma PHP-THβC was calculated by non-linear regression analysis, and it was found to be 107 min. This study was the first to measure plasma half-life of glycated tryptophan.

Key words: chicken, glycation, PHP-THβC, plasma half-life, tryptophan

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Introduction

Biological molecules having amino groups, such as proteins and amino acids, bind non-enzymatically to reducing sugars, such as glucose and fructose, via the so-called Maillard reaction or glycation. Although it is known that tryptophan is metabolized to two compounds, kynurenine and serotonin, by two enzymatic pathways named the kynurenine pathway and the serotonin pathway, respectively, part of tryptophan can be non-enzymatically converted to two types of glycated tryptophan, tryptophan-Amadori product and (1R,3S)-1-(D-gluco-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (PHP-THβC) (Nishimagi and Kita, 2012). Amadori products undergo further complex reactions to form advanced glycation end products (AGEs). The acceleration of glycation during hyperglycemia increases AGE production and accumulation, which is implicated in the gradual development of diabetic complications in diabetes mellitus (Brownlee 2001; Singh *et al*., 2001). As avians have hyperglycemia and high body temperature, they more easily develop AGEs from Amadori products than mammals. Pentosidine, one of the classical AGEs, is a fluorescent cross-link molecule generated from glycated extracellular proteins having lysyl and arginyl residues (Sell and Monnier, 1989; 1990). In broiler chickens, pentosidine was initially detected in collagen, which is the main structural protein of connective tissues of the skin and tendons (Iqbal *et al*., 1997; 1999; 2000). Our recent study revealed that approximately 10% of plasma tryptophan was non-enzymatically glycated and that glycated tryptophan was linearly related to the plasma tryptophan concentration (Makino *et al*., 2015).

In the concept of dynamic equilibrium (Schoenheimer, 1942), the mass of body constituents is apparently maintained at a steady state, in which the renewal or replacement of a biological substance is leveled between different compartments (Waterlow, 2006). In this concept, the half-life of a body substance has been used as an index of its renewal or replacement. Although plasma concentrations of glycated tryptophan, tryptophan-Amadori product, and PHP-THβC have been determined previously (Makino *et al*., 2015), information on the half-lives of these compounds remains limited. Therefore, in the present study, the half-life of plasma PHP-THβC was examined in chickens intravenously administrated PHP-THβC.

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Materials and Methods

Animals and Experimental Procedures

One-hundred newly hatched single-comb White Leghorn male chicks were obtained from a local hatchery (Koiwai Farm Co., Ltd., Iwate, Japan). The chicks were fed a commercial chick mash diet (crude protein 207 g/kg, metabolizable energy 12.1 MJ/kg; Toyohashi Feed Mills Co., Ltd, Aichi, Japan) from hatching until 7 days of age in an electrically heated brooder, to provide sufficient nutrients as recommended by NRC (1994). At this age, 60 birds were divided evenly over 10 experimental groups of 6 birds each. The chicks were kept in individual cages. PHP-THβC dissolved in Dulbecco's phosphate-buffered saline (DPBS) was intravenously administered at 114 nmol/0.2 mL/70 g body weight to chicks in 7 groups via the wing vein. DPBS was administrated to chicks in 2 groups in the same manner as a control. After anesthesia with diethyl ether, blood samples were taken from the heart at 15, 30, 60, 180, 360, 720, and 1440 min (15 and 1440 min in the control groups) after administration. The remaining 1 group was used for blood sampling as a baseline control at time 0. Blood samples were centrifuged for 20 min at 5,000 $\times g$ to obtain plasma. Plasma samples were stored at -20℃ until analyzed. Animal care was provided in compliance with applicable guidelines from the Iwate University Animal Care and Use Committee.

Deproteinization and Delipidation of Plasma Samples

The protocol used to remove protein and lipid from plasma samples was based on a previous report by Kita *et al*. (2013), with small modification. Briefly, before deproteinization and delipidation, 62.5 μL of 0.33 mM N-α-acetyl-L-tryptophan (Watanabe Chemical Industries, Ltd., Hiroshima, Japan) was added into 500 μL of plasma as internal standard. Then, plasma samples were mixed with 2 mL of acetonitrile for deproteinization. After deproteinization, lipid was removed by mixing with chloroform and methanol (2:1, v/v). For purifying PHP-THβC, cation-exchange resin (Dowex 50W $\times$8, Wako Pure Chemical Industries Ltd., Osaka, Japan) was used.

Measurement of PHP- THβC using Liquid Chromatography-Mass Spectrometry (LC-MS)

PHP-THβC and N-α-acetyl-L-tryptophan were quantified using LC-MS with an atmospheric pressure chemical ionization interface (LCMS-8040, Shimadzu Corporation, Kyoto, Japan). The selected ion monitoring mode was used to detect PHP-THβC at m/z +367 and N-α-acetyl-L-tryptophan at m/z +247. The two compounds were separated on a reverse phase HPLC column (50$\times$2 mm I.D., Gemini 3 μm C18 110 Å; Phenomenex, Torrance, CA, USA) with a gradient elution. Mobile phase A consisted of 0.1% (v/v) formic acid in ultrapure water. Mobile phase B consisted of 100% acetonitrile. The gradient elution was started at 5% of mobile phase B (0−2 min). Mobile phase B was increased up to 10% (2−3 min), and to 35% B (3−5 min), and then decreased to 5% for equilibration (5−7 min). The flow rate was 0.4 mL/min and the injection volume was 3 μL.

Statistical Analysis

The time course change in plasma PHP-THβC concentration from 30 to 720 min after administration was calculated using least squares nonlinear regression analysis (NLIN procedure) in SAS (version 9.4, SAS Institute, NC). The equation representing the time course change in plasma PHP-THβC concentration was as follows:

$$\text{PHP-TH}\beta\text{C } (\mu\text{M}) = a \cdot \exp^{(-\lambda \cdot \text{time})} + b$$

The plasma half-life of PHP-THβC was calculated by the above equation as follows:

$$T_{1/2} = \log 2/\lambda$$

Results and Discussion

The administration of DPBS alone did not affect the plasma concentration of PHP-THβC (Fig. 1). Intravenous injection of PHP-THβC rapidly increased the plasma PHP-THβC concentration. At 30 min after administration, plasma PHP-THβC peaked at 16.1 μM, and then decreased rapidly. Plasma PHP-THβC returned to the physiological level (0 min) at 360 min after administration. A non-linear regression equation was calculated as follows: plasma PHP-THβC concentration (μM) $= 8.77 \cdot \exp^{(-0.0065 \cdot \text{time (min)})} + 8.84$. The plasma half-life of PHP-THβC calculated by solving the above equation was 106.6 min. The plasma half-life of tryptophan in rats reportedly was 93 min (Iizuka *et al*., 2011), which suggests that the plasma half-life of PHP-THβC may be slightly lower than that of tryptophan.

When ^{18}F-labeled-N-ε-fructoselysine, which is an Amadori product of glucose with lysine, was intravenously injected into rats, high accumulation (approximately 30% of the injection dose) of ^{18}F-labeled-N-ε-fructoselysine in the kidneys was observed at 5 min after injection, and this high level remained until 60 min after injection. The remaining portion of tracer corresponding to approximately 45% of the injection dose was delivered in the urine (Hultsch *et al*., 2006). Similarly, when mice were intravenously administrated ^{18}F-labeled carboxymethyl-lysine (CML), an AGE developed from N-ε-fructoselysine (Ahmed *et al*., 1986), the uptake of ^{18}F-CML into the kidneys and the excretion of ^{18}F-CML into urine at 60 min after injection were 36% and 45% of the injection dose, respectively. These results suggested that both Amadori products and AGEs may accumulate rapidly in the kidneys, followed by immediate excretion into the urine. A previous study using chickens also indicated that amino acid-derived AGEs intravenously injected into chickens were rapidly incorporated into the kidneys at 30 min after injection (Kita, 2014), which suggested that the rapid excretion of plasma PHP-THβC into the urine can be attributed to the immediate disappearance of PHP-THβC in the plasma of chickens.

In conclusion, PHP-THβC, one of the glycated tryptophans, rapidly disappears from the blood circulation in chickens, with a plasma half-life of 107 min.

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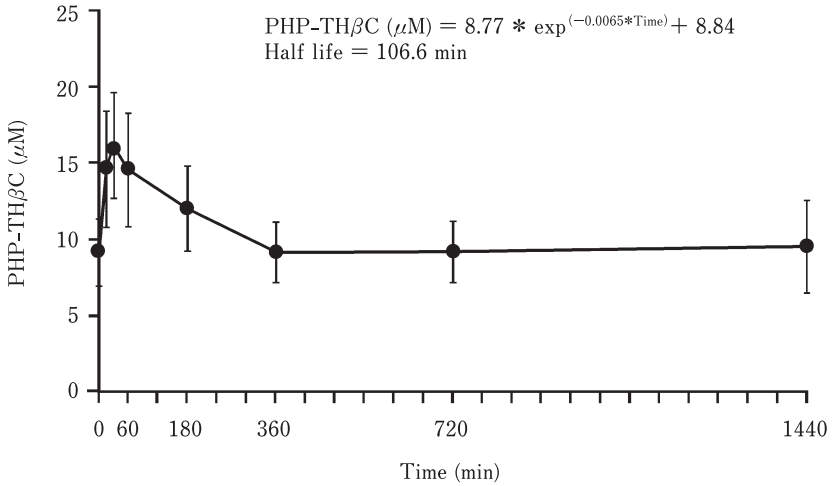

Fig. 1. **Time course changes in plasma PHP-THβC concentration in chickens administrated with PHP-THβC intravenously.** PHP-THβC dissolved in DPBS at 114 nmol/0.2 mL/70 g body weight was intravenously administrated to chickens via the wing vein. Control groups were administrated DPBS alone. At 15, 30, 60, 180, 360, 720, and 1440 min after administration, blood samples were taken by heart puncture after anesthesia with diethyl ether. Blood samples were also taken from chickens at time 0 as a baseline control. PHP-THβC concentration in the plasma was measured using LC-MS. PHP-THβC concentration (μM)=$8.77 \cdot \exp^{(-0.0065 \cdot \text{time (min)})}$ +8.84. Values are means±SEs. $n=6$.

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