Nutrition 27 (2011) 471–478

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Time course of vitamin C distribution and absorption after oral administration in SMP30/GNL knockout mice

Mizuki Iwama M.S. [a,b], Kentaro Shimokado M.D., Ph.D. [a], Naoki Maruyama M.D., Ph.D. [b], Akihito Ishigami Ph.D. [b,c,*]

[a] Department of Geriatrics and Vascular Medicine, Tokyo Medical and Dental University, Tokyo, Japan
[b] Aging Regulation, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[c] Department of Biochemistry, Faculty of Pharmaceutical Sciences, Toho University, Chiba, Japan

ARTICLE INFO

Article history:
Received 12 December 2009
Accepted 30 April 2010

Keywords:
Gluconolactonase
Knockout
Plasma
Senescence marker protein-30
Urine

ABSTRACT

Objective: Because vitamin C (VC) has multiple metabolic and antioxidant functions, we investigated the movement of VC throughout the tissues of senescence marker protein-30 (SMP30)/gluconolactonase (GNL) knockout (KO) mice.
Methods: SMP30/GNL KO mice, which cannot synthesize VC in vivo, were divided into two groups: VC sufficient and VC deficient. Starting at 2 mo of age, both groups had free access to water containing 1.5 and 0.0375 g/L of VC for 1 mo.
Results: The average rate of VC retention in 20 tissues of VC-deficient SMP30/GNL KO mice was only 13.7% of that in VC-sufficient mice. Tissues that retained over 20% of VC were the cerebellum, white fat, testes, eyeballs, and pancreas, and those with less than 5% VC were the kidneys and heart. These results clearly indicate the different VC retention capacities among tissues. Next, we examined the time course of VC distribution and absorption in VC-deficient SMP30/GNL KO mice. After oral VC administration, VC content in the liver and kidney peaked at 3 h and then decreased. VC content in the lungs, adrenal glands, skin, white fat, and pancreas peaked at 6 h and in the cerebellum, cerebrum, skeletal muscles, eyeballs, thyroid gland, and testes at 12 h.
Conclusion: In this study, we found that exogenous VC administered orally in VC-deficient SMP30/GNL KO mice was distributed at distinctly different rates within individual tissues. The SMP30/GNL KO mice used in this study are a useful animal model that provides unique opportunities for investigating VC movement and metabolism in the entire body.

Introduction

Many animals can synthesize vitamin C (VC) in vivo; however, others such as humans and guinea pigs have lost the ability to make VC because of mutations in the L-gulono-γ-lactone oxidase gene, which is essential for VC synthesis in vivo [1]. Therefore, animals without enzyme activity of L-gulono-γ-lactone oxidase must obtain VC from dietary sources.

Vitamin C has numerous metabolic functions that are largely dependent on its potent reducing properties [2]. VC acts as a cofactor in reactions catalyzed by several metal-dependent oxygenases, e.g., Cu^{+}-dependent mono-oxygenases including peptidylglycine α-amidating mono-oxygenase involved in peptide hormone synthesis [3,4], dopamine β-hydroxylase involved in norepinephrine synthesis [5,6], and Fe^{2+}/α-ketoglutarate-dependent dioxygenases including prolyl and lysyl hydroxylases involved in collagen synthesis [7], 6-N-trimethyllysine dioxygenase and γ-butyrobetaine dioxygenase involved in carnitine synthesis [8], and asparaginyl hydroxylase, which modifies hypoxia-inducible factor-1 [9]. Moreover, VC has non-enzymatic reductive activity in chemical reactions. That is, VC has a strong antioxidant function evident in its ability to scavenge superoxide radicals in intracellular and extracellular reactions [10]. VC decreases oxidative DNA and protein damage, low-density lipoprotein oxidation, lipid peroxidation, oxidants and nitrosamines in gastric juice, and extracellular oxidants from neutrophils [10]. VC enhancement is evident in its ability to increase endothelium-dependent vasodilation [11].

This study is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan (to A. I.), and a Grant-in-Aid from the Smoking Research Foundation, Japan (to A. I.).
* Corresponding author. Tel./fax: +81-47-472-1536.
E-mail address: ishigami@phar.toho-u.ac.jp (A. Ishigami).

0899-9007/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2010.04.010

Recommendations for humans' dietary VC intake derive from several sources: steady-state concentrations in plasma and excretion in urine relative to dose after VC intake, saturation of the body with VC and estimates of the urinary response, and amount of VC intake that prevents scorbutic symptoms [10]. In 1979, the metabolism, half-life, turnover rates, elimination rates, and size of body pool of VC for humans were calculated by using radiolabeled VC [12]. Others reported the time course of VC levels in human plasma and urine after oral administration of non-radiolabeled VC [13–18]. Padayatty et al. [15] then documented peaks of VC levels in plasma approximately 3 h after a single administration of VC and a return to the steady-state level at 24 h. Levine et al. [13] considered the rate of actual VC usage for metabolism in the body as most effective after administration of a single 200-mg dose of VC and noted that no VC was excreted in urine after an intake of up to 100 mg of VC. Moreover, administering a single dose of VC higher than 500 mg of VC resulted in lowering the rate of VC absorption, and the large amount of VC absorbed was excreted immediately in urine [13]. Thus, the most recent recommended amount and interval of VC intake for humans have been determined mainly from analyzing plasma and urine, because investigating VC uptake and distribution directly in human tissues is an ethically difficult problem.

Recently, we established senescence marker protein-30 (SMP30)/gluconolactonase (GNL) knockout (KO) mice [19,20], which are incapable of synthesizing VC in vivo, because they lack SMP30/GNL, a necessary component of the VC biosynthetic pathway [21]. By providing VC in the food and drinking water of SMP30/GNL KO mice, we can control their bodily content of VC. Thus, SMP30/GNL KO mice are a useful animal model for investigating the retention capacities of VC in various tissues and its internal movements after oral administration in vivo. In the present study, we investigated VC distribution, absorption, and retention in a tissue-by-tissue study of VC-deficient SMP30/GNL KO mice.

Materials and methods

Animals

The SMP30/GNL KO mice were previously generated by the gene targeting technique [19]. Female KO mice (*SMP30/GNL*$^{-/-}$) were mated with male KO mice (*SMP30/GNL*$^{Y/-}$) to produce the KO mice used in this study, and only males were included. After weaning at 30 d of age, SMP30/GNL KO mice were fed a VC-deprived diet (CL-2, CLEA Japan, Tokyo, Japan) and had free access to water containing sufficient VC (1.5 g/L) and 10 μM ethylenediaminetetra-acetic acid (EDTA) until 2 mo of age. Then, SMP30/GNL KO mice were divided into VC-sufficient and VC-deficient groups. Both groups had free access to water containing 1.5 and 0.0375 g/L of VC containing 10 μM EDTA until 3 mo of age, respectively. Water bottles were changed every 3 or 4 d until the experiment ended. Male wild-type (WT; *SMP30/GNL*$^{Y/+}$) mice at 4 wk old were purchased from Japan SLC (Shizuoka, Japan). WT mice were fed a VC-deprived diet and had free access to water without VC. Throughout the experiments, animals were maintained on a 12-h light/dark cycle in a controlled environment. All experimental procedures using laboratory animals were approved by the animal care and use committee of Toho University (Chiba, Japan) and the Tokyo Metropolitan Institute of Gerontology (Tokyo, Japan).

Preparation of plasma, urine, and tissues

Urine was collected in microtubes by pushing the hypogastrium from both groups of SMP30/GNL KO mice at 3 mo of age. Then, these mice were sacrificed, and their blood was collected from the inferior vena cava. Blood was gently mixed with EDTA and centrifuged at 880 × *g* for 15 min at 4 °C. The resulting supernatants were used as plasma for further analysis. Afterward, mice were systemically perfused with ice-cold phosphate buffered saline through the left ventricle to wash out remaining blood cells, and tissues of interest were collected and stored at −80 °C until use.

VC distribution and absorption

For the study of VC distribution and absorption after oral administration, VC-deficient groups of 3-mo-old SMP30/GNL KO mice were fasted overnight, after which various amounts (0, 0.5, 1.0, 3.5, and 7.0 mg) of VC dissolved in 250 μL of water were orally administered by using stomach probes (Natsume Seisakusho, Tokyo, Japan). Urine samples were collected immediately or 3, 6, 12, and 24 h later, and then mice were sacrificed. Their urine, plasma, and tissues were stored at −80 °C until use.

Measurement of VC

Vitamin C in urine, plasma and tissues was measured by a high-performance liquid chromatographic electrochemical detection method [22]. In this study we measured only the reduced form of VC because it has numerous metabolic functions and strong antioxidant functions [2,10]. Tissues were homogenized in 14 vol of 5.4% metaphosphate containing 1 mM EDTA and centrifuged at 21 000 × *g* for 10 min at 4 °C. Plasma and urine were mixed with equal volumes of 10% metaphosphate containing 1 mM EDTA and centrifuged at 21 000 × *g* for 10 min at 4 °C. The supernatants obtained were rapidly frozen to prevent oxidation of VC and were kept at −80 °C until use. Samples were analyzed by high-performance liquid chromatography using an Atlantis dC18 5-μm column (4.6 × 150 mm; Nihon Waters, Tokyo, Japan). The mobile phase was 50 mM phosphate buffer (pH 2.8), 0.2 g/L of EDTA, 2% methanol at a flow rate of 1.3 mL/min, and electrical signals were recorded by using an electrochemical detector (2465, Nihon Waters) with a glassy carbon electrode at +0.6 V [23,24]. Creatinine levels in urine were measured with a Wako Creatinine Test kit (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions, and VC levels in urine were normalized by creatinine value. VC retention in tissues was calculated as VC retention (%) = (VC content of tissue from VC-deficient SMP30/GNL KO mice)/(VC content of tissue from WT or VC-sufficient SMP30/GNL KO mice) × 100.

Statistical analysis

Results are expressed as means ± standard errors of the mean. The probability of statistical differences between experimental groups was determined by unpaired *t* test using KaleidaGraph software (Synergy Software, Reading, PA, USA). Statistical differences were considered significant at $P < 0.05$.

Results

VC retention capacity of tissues

The VC content was measured in 20 tissue sites and plasma of WT mice at 3 mo of age and, as Figure 1 shows, amounts of VC per tissue weight varied considerably. Listed in order of the highest to lowest VC content, these were the adrenal gland, cerebellum, cerebrum, spleen, thyroid gland, small intestine, lung, submaxillary gland, testes, large intestine, stomach, kidney, liver, eyeballs, pancreas, brown fat, heart, skin, skeletal muscle, and white fat. The largest amount of VC was in the adrenal glands, which had 8.3 ± 0.8 μmol/g of tissue, and white fat had the lowest VC content at 19.0 ± 3.0 nmol/g of tissue. In addition, the concentration of VC in plasma was 38.0 ± 5.1 μM. We next measured the VC content in 20 tissues and plasma of VC-sufficient SMP30/GNL KO mice at 3 mo of age; after weaning, these mice had free access to water containing 1.5 g/L of VC. Mean water consumption during the experiment was 4.6 ± 0.3 mL/d per mouse (6.9 ± 0.4 mg VC/d per mouse). The amount of VC in tissues and the concentration of VC in plasma from WT mice were almost the same as in VC-sufficient SMP30/GNL KO mice except for the thyroid gland and white fat (Fig. 1). That is, VC contents in thyroid glands from WT and VC-sufficient SMP30/GNL KO mice were 1.9 ± 0.3 and 0.8 ± 0.1 μmol/g of tissue, respectively, and VC contents in white fat from WT and VC-sufficient SMP30/GNL KO mice were 19.0 ± 3.0 and 42.0 ± 9.0 nmol/g of tissue, respectively. In contrast, the amounts of VC in tissues of VC-deficient SMP30/GNL KO mice, which had free access to water containing 0.0375 g/L of VC for 1 mo, were all significantly lower than those of WT and VC-sufficient SMP30/