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Research Paper

L-dehydroascorbic acid can substitute L-ascorbic acid as dietary vitamin C source in guinea pigs

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ABSTRACT

Vitamin C deficiency globally affects several hundred million people and has been associated with increased morbidity and mortality in numerous studies. In this study, bioavailability of the oxidized form of vitamin C (L-dehydroascorbic acid or DHA)—commonly found in vitamin C containing food products prone to oxidation—was studied. Our aim was to compare tissue accumulation of vitamin C in guinea pigs receiving different oral doses of either ascorbate or DHA. In all tissues tested (plasma, liver, spleen, lung, adrenal glands, kidney, muscle, heart, and brain), only sporadic differences in vitamin C accumulation from ascorbate or DHA were observed except for the lowest dose of DHA (0.25 mg/ml in the drinking water), where approximately half of the tissues had slightly yet significantly less vitamin C accumulation than from the ascorbate source. As these results contradicted data from rats, we continued to explore the ability to recycle DHA in blood, liver and intestine in guinea pigs, rats and mice. These investigations revealed that guinea pigs have similar recycling capacity in red blood cells as observed in humans, while rats and mice do not have near the same ability to reduce DHA in erythrocytes. In liver and intestinal homogenates, guinea pigs also showed a significantly higher ability to recycle DHA compared to rats and mice. These data demonstrate that DHA in guinea pigs—as in humans—is almost as effective as ascorbate as vitamin C source when it comes to taking up and storing vitamin C and further suggest that the guinea pig is superior to other rodents in modeling human vitamin C homeostasis.

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1. Introduction

Ascorbate—the reduced form of vitamin C—is a powerful antioxidant and plays an important role as cofactor in many biological reactions [1]. Suboptimal levels of vitamin C have been associated with increased morbidity and mortality from conditions such as cancer and cardiovascular disease in numerous epidemiological studies [2]. Physiologically, ascorbate is under tight endogenous regulation ensuring homeostasis in plasma and tissues by a specialized set of transport proteins and in particular during deficiency, efficient reuptake in the kidneys combined with intracellular recycling by enzymatic systems capable of reducing the oxidized form of vitamin C (L-dehydroascorbic acid or DHA) back to ascorbate ensures a minimal loss of the body stores [3,4]. In recent years, several studies have shown that ascorbate has a variety of physiological functions as co-factor in enzymatic processes such as e.g. neurotransmitter synthesis [5] and vasorelaxation [6]. A widely used approach to investigate the significance of these actions upon health and disease is to use animal models where suboptimal levels of vitamin C can be achieved by adjusting the dietary intake. Guinea pigs have been used for this purpose for decades, but also mice and rats manipulated to be devoid of the intrinsic ability to synthesize ascorbate are available [7,8], moreover supplementation to e.g. rats and mice capable of synthesizing ascorbate has frequently been used. Yet like humans, guinea pigs have adapted to not being able to synthesize ascorbate through millions of years of evolution [9], and such absolute dependency on a dietary supply of this vital micronutrient may have strengthened the preservation mechanisms. It has been reported that DHA administered to scorbutic Osteogenic Disorder Shionogi (ODS) rats had only 10% vitamin C activity [10], however this species—and concurrent strain—has not underwent a comparable degree of evolutionary adaptation. Our aim with the present experiments was therefore to investigate the bioavailability of vitamin C forms in guinea pigs and if the recycling capacity of guinea pigs differs such that DHA—as in humans—will substitute ascorbate more effectively in this species.

Abbreviations: DHA, L-dehydroascorbic acid; RBC, erythrocyte; ODS, Osteogenic Disorder Shionogi

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2. Materials and methods

2.1. Animals

The experiment was approved by the Danish Animal Experiments Inspectorate. In the bioavailability study, 40 female Hartley guinea pigs (Charles River Laboratories, Kisslegg, Germany) weighing 550–600 g upon arrival, were marked with a subcutaneous microchip in the neck, stratified by weight and randomized into eight groups upon one week after arrival to the animal facility. All groups were provided with ad libitum access to a standard guinea pig diet devoid of vitamin C (Ssniff Spezialdiäten GmbH, Soest, Germany).

2.2. Stability of ascorbate and DHA in solution

Ascorbate and DHA are both known to degrade rapidly in dilute aqueous solutions [11]. In order to test the stability prior to the in vivo experiments, all doses of ascorbate (0.1, 0.25, 0.5 and 1 mg/ml) and DHA (0.25, 0.5 and 1 mg/ml) were made freshly in MQ water, transferred to drinking bottles, and left in the animal facility for 24 h with sampling every 12 h. From Fig. 1A, it can be seen that 90% of the originally added amount of DHA was still present after 24 h irrespective of the start concentration. With ascorbate, however, there was a concentration dependent degradation with less than 50% of the initial amount still being present after 24 h for the doses of 0.25 and 0.1 mg/ml. For this reason, it was decided that animals belonging to these two groups should receive fresh solutions twice daily.

2.3. Bioavailability study

Animals were group housed in floor pens in an enriched environment at 20 ± 2 °C with a 12:12 h light–dark cycle, free access to hay, inspected daily by trained personnel, and weighed twice weekly. Ascorbate or DHA was supplied in the drinking water for three weeks as outlined above. Weekly blood sampling took place by puncture of *Vena saphena lateralis* [12] and blood was collected into K_3EDTA BD Microtainer tubes [13]. Three weeks after the initiation of the experiment, the guinea pigs were preanaesthetized with Zoletil mix (Zoletil 50 vet® (125 mg Tiletamin and 125 mg zolazepam; Virbac, Carros, France) dispersed in xylazin (20 mg/ml, Rompun®, Bayer, Leverkusen, Germany) and butorphanol (10 mg/ml, Torbugesic®, ScanVet Animal Health, Fredensborg, Denmark)) and subsequently anaesthetized by isofluran inhalation (Isoba vet, MSD Animal Health, Netherlands). Upon absence of reflexes (interdigital and skin incision) thoracotomy was performed and an intra-cardial blood sample obtained using a syringe with an 18 G, 40 mm needle flushed with 15% K_3-EDTA. Immediately after sampling, the animal was euthanized by decapitation. Tissues were immediately dissected and frozen on dry ice prior to storage at −80 °C.

2.4. Recycling study

In a subsequent experiment, four male Hartley guinea pigs (5 weeks old, 400 g bodyweight) (Charles River Laboratories, Kisslegg, Germany), four male Spraque Dawley rats (7 weeks old, 200 g bodyweight (Taconic, Ejby, Denmark)) and four male black6 (B6NTac) mice (7 weeks old, 23 g bodyweight (Taconic, Ejby, Denmark)) were euthanized as described above following one week of equilibration and blood, liver and intestinal samples (sectioned from the middle third of the jejunum) were obtained. Blood samples were immediately centrifuged (5 min at 2000 × *g*, 4 °C) and erythrocytes (RBCs) were isolated and washed three times in 10 volumes of PBS. Subsequently, RBCs were diluted to 25% with PBS and used in the recycling experiments are described below. Tissues were gently rinsed and blotted dry and subsequently homogenized in nine volumes of PBS and used directly in recycling experiments as outlined below.

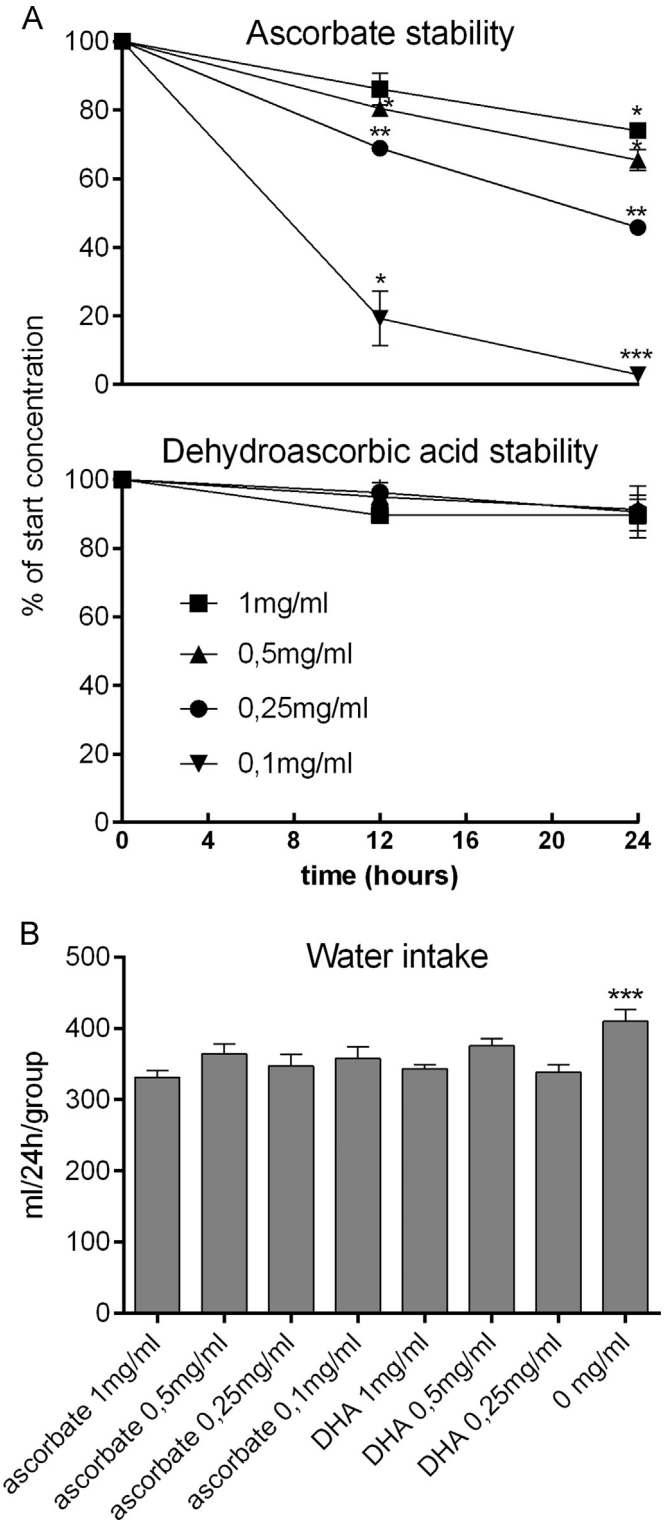

Fig. 1. Stability of drinking solutions and water intake. In A, the stability of ascorbate and DHA, respectively, are presented over time. The solutions were freshly made and kept in drinking bottles in the animal facility. Concentrations were normalized to that of the initial preparation. B shows the daily water intake within each group. Data is presented as mean ± SEM. $^{*}=p\leq 0.05$; $^{**}=p\leq 0.01$; $^{***}=p\leq 0.001$ by one-way ANOVA with Bonferroni correction for multiple comparisons.

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