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Original Research

Dietary phosphate supplementation delays the onset of iron deficiency anemia and affects iron status in rats

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ABSTRACT

Inorganic phosphate (Pi) plays critical roles in bone metabolism and is an essential component of 2,3-diphosphoglycerate (2,3-DPG). It has been reported that animals fed a low-iron diet modulate Pi metabolism, whereas the effect of dietary Pi on iron metabolism, particularly in iron deficiency anemia (IDA), is not fully understood. In this study, we hypothesized the presence of a link between Pi and iron metabolism and tested the hypothesis by investigating the effects of dietary Pi on iron status and IDA. Wistar rats aged 4 weeks were randomly assigned to 1 of 4 experimental dietary groups: normal iron content (Con Fe) + 0.5% Pi, low-iron (Low Fe) + 0.5% Pi, Con Fe + 1.5% Pi, and Low Fe + 1.5% Pi. Rats fed the 1.5% Pi diet for 14 days, but not for 28 days, maintained their anemia state and plasma erythropoietin concentrations within the reference range, even under conditions of low iron. In addition, plasma concentrations of 2,3-DPG were significantly increased by the 1.5% Pi diets and were positively correlated with plasma Pi concentration ($r = 0.779$; $P < .001$). Dietary Pi regulated the messenger RNA expression of iron-regulated genes, including divalent metal transporter 1, duodenal cytochrome B, and hepcidin. Furthermore, iron concentration in liver tissues was increased by the 1.5% Pi in Con Fe diet. These results suggest that dietary Pi supplementation delays the onset of IDA and increases plasma 2,3-DPG concentration, followed by modulation of the expression of iron-regulated genes.

Abbreviations: 2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; Con Fe, normal iron content; EPO, erythropoietin; DcytB, duodenal cytochrome B; DMT1, divalent metal transporter 1; FGF23, fibroblast growth factor 23; Hb, hemoglobin; IDA, iron deficiency anemia; iFGF23, intact fibroblast growth factor 23; iPTH, intact parathyroid hormone; Low Fe, low iron; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; mRNA, messenger RNA; Pi, inorganic phosphate; PTH, parathyroid hormone; RBC, red blood cell; Tf, transferrin.

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1. Introduction

Inorganic phosphate (Pi) is an essential nutrient in the processes of glycolysis, gluconeogenesis, mineral metabolism, and other diverse cellular functions that involve intermediary metabolism and energy transfer mechanisms [1,2]. Serum Pi concentration is maintained through a complex interplay between intestinal absorption, exchange with intracellular and bone storage pools, and renal tubular reabsorption [3,4]. Absorption of Pi in the intestine and its transport in the kidney are mediated by several sodium-dependent phosphate cotransporters [4-6]. Parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and 1α,25-dihydroxyvitamin D_3 are hormones that are well recognized for their roles in regulating Pi metabolism [7]. We have previously reported the effects of Pi intake on circulating FGF23 and PTH as well as vascular endothelial function in human and animal studies [8-10].

Iron deficiency anemia (IDA) is characterized by a reduction or absence of iron stores; low serum concentrations of iron, hemoglobin (Hb), and ferritin; decreased hematocrit; increased platelet count; reduced transferrin (Tf) saturation; and a marked increase in total iron-binding capacity [11,12]. Total body iron stores are maintained through dietary absorption by duodenal enterocytes. Dietary nonheme iron exists mainly in ferric form (Fe^{3+}). Fe^{3+} must be reduced by apical membrane–bound ferric reductase activity via duodenal cytochrome B (DcytB) [13,14]. Duodenal cytochrome B is a candidate enterocyte ferric iron reductase, but its physiological importance is not clear. Reduced iron (Fe^{2+}) then enters the enterocytes via the divalent metal transporter 1 (DMT1) located in the apical membrane of enterocytes [15-17]. Furthermore, the control of iron absorption is regulated by hepcidin, a polypeptide hormone secreted by the liver in response to a high concentration of iron in the body [18,19]. Hepcidin binds to ferroportin, an iron exporter on the surface of nucleated cells, leading to its internalization and degradation, thereby inhibiting iron export from intracellular pools [20].

Recent studies have provided evidence of a relationship between iron and Pi metabolism. Iron deficiency stimulates the expression of hypoxia-inducible factor 1 α, leading to an increase in the transcription of *Fgf23* [21]. In addition, duodenal *Npt 2b* (Slc34a2), which primarily mediates intestinal Pi absorption, was down-regulated in rats by a low-iron diet [22]. A low-iron diet has also been shown to induce hypophosphatemia in neonatal wild-type mice [23]. On the other hand, hypophosphatemia caused by a Pi imbalance reduced red blood cell (RBC) adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) contents [24,25]. 2,3-Diphosphoglycerate is an erythrocyte-specific metabolite that decreases the oxygen-binding affinity of Hb and thereby induces oxygen release from Hb [26-29]. A strong correlation has been reported between the serum Pi and 2,3-DPG concentrations [30]. However, little is understood about the effect of Pi supplementation on 2,3-DPG concentration in IDA. Acute hemolytic anemia was associated with serum Pi concentrations less than 1.0 mg/dL, whereas parenteral Pi administration corrected the hemolytic anemia and restored low 2,3-DPG and ATP concentrations to a reference range [31]. Based on these studies, it was clear that a low-iron diet could modulate Pi metabolism and that Pi administration corrected hemolytic anemia, whereas the effects of dietary Pi on iron metabolism and IDA, in particular, are not fully understood.

We hypothesized that Pi administration may also correct IDA and tested the hypothesis by determining the effect of dietary Pi supplementation on diet-induced IDA in rats. Young rats were used to ensure induction of anemia.

2. Methods and materials

2.1. Animals

Male Wistar rats aged 4 weeks (Japan SLC, Inc, Shizuoka, Japan), weighing approximately 80 g, were individually caged in a climate-controlled room (23°C ± 1°C, humidity 70%-75%; specific pathogen free) with a 12-hour light/12-hour dark cycle. Rats of equivalent mean body weight were distributed among 4 dietary treatment groups and given free access to food and deionized water. At the end of the experiment, all rats were killed by exsanguination after being anesthetized, and blood and tissue samples were collected for analysis. Tissue samples were stored at –80°C until analysis. The breeding and handling of all animals in this study were approved by the Animal Experimentation Committee of the University of Tokushima.

2.2. Diets

The compositions of the experimental diets are shown in Table 1. The rats were divided into 4 experimental groups of 5 rats per group. Each group was fed 1 of the 4 different diets for 14 days: normal iron content (Con Fe) + 0.5% Pi, low iron (Low Fe) + 0.5% Pi, Con Fe + 1.5% Pi, and Low Fe + 1.5% Pi. The experimental diets were based on the AIN-93G formulation [32]. The basal diet was supplemented with iron citrate ($FeC_6H_5O_7$) and potassium dihydrogenphosphate (KH_2PO_4) (Wako Pure Chemical Industries Co, Osaka, Japan). The Con Fe diet contained 250 ppm iron and the Low Fe diet contained 5 ppm iron. For the long-term experiment, rats were fed the same experimental diets for 28 days.

2.3. Blood parameters

For biochemical analysis, blood was collected into tubes containing heparin or EDTA-2K after puncture of inferior vena cava. Concentrations of Pi, calcium (Ca), and iron were measured using assay kits (Wako Pure Chemical Industries Co) [33]. To calculate the degree of Tf saturation, plasma total iron-binding capacity was determined using an unsaturated iron-binding capacity kit (AKJ Global Technology Co, Chiba, Japan). Red blood cell count and Hb concentration were measured at 14 and 28 days using a blood corpuscle analyzer (Mitsubishi Chemical Medicine, Tokyo, Japan). The concentrations in plasma of intact PTH (iPTH), intact FGF23 (iFGF23), and 2,3-DPG were determined using the Rat Intact PTH ELISA Kit (Immutopics, San Clemente, CA, USA), the FGF23 ELISA Kit (Kinos, Tokyo, Japan), and the Rat 2,3-diphosphoglycerate ELISA Kit (Cusabio Biotech, Hubei, China), respectively [33]. Concentrations of plasma erythropoietin (EPO) were measured using the radioimmunoassay 2-antibody method (SRL Co, Tokyo, Japan).