修士論文要旨

Effects of folate-deficiency on the distribution of white blood cells and liver fat metabolism in rats

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Introduction

Folate is a critical co-enzyme that mediates single carbon transfer reactions, and essential for the synthesis of DNA and RNA. It is well known that folate-deficiency can lead to malignant anemia and neural disorder of fetus. It is also reported that folate-deficiency decreased the number of white blood cells (WBCs) in rats. The distribution of WBCs is also essential for the immune responses. However, the effects of folate-deficiency on the distribution of WBCs have not been systematically elucidated. Therefore, we investigated the effects of folate-deficieny on the circulating number of WBCs [Monocytes, granulocytes [neutrophils, eosinophils and basophils] and lymphocytes (T-, B-cells and NK cells)] in rats (**Exp. I**). In addition, folate-deficiency also causes an elevation in plasma homocysteine (Hcy) concentrations by suppression of methionine-homocysteine metabolism. Epidemiological studies also indicate that the elevation of plasma Hcy is closely associated with increased levels of triglycerides in liver. However, the mechanisms of how folate-deficiency induces lipid accumulation in liver are still unknown. Therefore, the effects of folate-deficiency on the mRNA expressions of fatty acid metabolism-related enzymes in rat liver were studied (**Exp. II**).

Materials and Methods

Exp. I: Distribution of white blood cells

Male 5 weeks old Sprague Dauley rats were divided into the Folate-deficient diet (FAD: 0.007mg folate/100 g diet) group and the control diet (20 mg folate/100 g diet) group, and were pair-fed for 8 weeks. Blood samples were collected from tail vein. Once a week, the number of white blood cells (WBCs) was analyzed by flow-cytometry. On the final day of the experiment, the fractionation and count analyses of lymphocytes were conducted by flow-cytometry. The plasma folate and Hcy concentrations were analyzed by the reagent lit of access folic acid (FOL2), and HPLC, respectively.

Exp. II: Expression of fat metabolism-related enzymes in liver

Plasma free fatty acid (FFA) and triglyceride (TG) concen trations were assayed spectrophotometrically. The mRNA expression of carnitine palmitoyl transferase-1: CPT-1, glycerol-3-phosphate acyltransferase: GPAT, diacylglycerol acyltransferase 1: DGAT1 and 2: DGAT2 was analyzed by RT- PCR.

Results and Discussion

Exp. I: Folate-deficiency for 8 weeks markedly decreased plasma folate concentrations and increased homocystein concentrations in the FAD group than in the CON group. After 5–8 weeks of the feeding, the number of total WBCs was gradually decreased. After the 7 and 8 weeks of the feeding, FAD group had a significantly lower number of neutrophils and eosinophils as compared with the CON group. In contrast, the number of basophils showed transiently tendency to increase in the FAD group, during the experimental period as compared with the CON group. The number of total lym-phocytes (A) 8 weeks after the feeding was 0.64 ($p<0.001$) times lower in the FAD group than in the CON group. The number of T-lymphocytes (B) and B-lymphocytes (C) was 0.73 times ($p<0.01$) and 0.49 times ($p<0.01$) lower in the FAD group than in the CON group, respectively.

Exp. II: Plasma TG concentration in the FAD group was relatively higher than in the CON group after 3 weeks of the feeding. After 8 weeks from the feeding, the expression of CPT-1 and DGAT2 mRNA was decreased. On the contrary, that of GPAT and DGAT1 mRNA was increased in rat liver. These results suggest that folate-deficiency mediates suppression of the import of FFA to TCA cycle in part by down-regulating CPT-1 expression and subsequent suppression of mitochondrial β-oxidation in liver. These results also show that folate-de- ficiency stimulates TG synthesis by up-regulation of GPAT and DGAT2.

Reference

Abe I, *et al*., Folate-deficiency induced cell-specific changes in the distribution of lymphocytes and granulocytes in rats. ***Environ Health and Prev Med,*** 18 (1): 78–84 (2013).