

学 位 論 文 の 要 旨

Studies on the anti-disease function of vitamin B6 (ビタミン B6 の疾病予防作用に関する研究)

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Vitamin B6 is a water soluble vitamin and the active form, pyridoxal 5'-phosphate (PLP), acts as a necessary cofactor for more than 100 enzymatic reactions. There is growing epidemiological evidence for vitamin B6 acting as a protective factor against cancers, inflammatory bowel disease and cardiovascular disease. Recent evidence suggest that inadequate intake of vitamin B6 is prevalent in the US and Japan. It has been suggested that the anti-tumor effect of dietary vitamin B6 is, at least, partially ascribed to lowering colon cell proliferation, oxidative stress, inflammation, angiogenesis, epithelium cell damage and DNA damage. However, the molecular mechanisms involved in the anti-tumor effect of vitamin B6 are not yet clearly understood. Previous studies have suggested that the anti-cardiovascular disease effect of vitamin B6 might be partially ascribed to its anti-inflammatory function. However, the mechanisms are still unclear. The objective of this study was to investigate the anti-disease function of vitamin B6 in cell culture and animal studies.

The preliminary study using DNA microarray analysis was conducted to examine the expression of genes in colon carcinoma HT29 cells exposed to

pyridoxal (PL: 500 μ M) for 24 h. Expression of several genes relating to diseases, including cystatin A (CSTA), cyclin-dependent kinase inhibitor 1A (p21), insulin-like growth factor binding protein 1 (IGFBP1), growth arrest and DNA-damage-inducible beta (GADD45B), vitamin D receptor (VDR), DNA-damage-inducible transcript 3 (DDIT3) and NADPH oxidase 1 (NOX1), were altered by PL. The upregulation of mRNA expression of CSTA, p21, IGFBP1, GADD45B, VDR and DDIT3 (anti-disease factors), and downregulation of mRNA expression of NOX1 (disease promoting factor) were confirmed by real-time PCR in HT29 cells treated with PL (500 μ M) for 24 h.

In the first study, I mainly focused on the effect of vitamin B6 on gene and protein expression of IGFBP1. IGFBP1 is mainly produced in the liver and secreted from the liver. IGFBP1 has been reported to be a tumor suppressor via binding to insulin growth factors (IGFs), modulating their actions. IGFBP1 is also known to be a hepatoprotective factor. Expression of IGFBP1 is elevated by fasting and exercise, and suppressed by insulin. Recent study further suggested that IGFBP1 has a protective role in the development of cardiovascular disease. Thus, experiments were performed to examine the effect of vitamin B6 on the expression of IGFBP1 in hepatocarcinoma HepG2 cells. Adding PL (500 μ M) markedly elevated the mRNA expression of IGFBP1 in HepG2 cells at 6 h, 12 h and 24 h, whereas other vitamins (500 μ M), including pyridoxal 5'-phosphate (PLP), pyridoxine (PN) and pyridoxamine (PM), caused no such effect. PL caused a dose-dependent increase in mRNA expression of IGFBP1 in HepG2 cells. The protein expression of IGFBP1 in the cell lysate and culture medium was also markedly elevated in the presence of PL in a time-dependent manner. PL elevated the expression of p-ERK1 (active form of ERK1) and the p-c-Jun proteins (a down stream factor of ERK). Furthermore, the elevation in mRNA and protein of IGFBP1 by PL was suppressed by PD98059, an ERK inhibitor. Treatment of cycloheximide, a protein synthesis inhibitor, completely inhibited the elevation in protein expression of IGFBP1 by PL, suggesting that the higher protein expression of IGFBP1 induced by PL is dependent on the protein synthesis. Thus, the possibility of the suppressed degradation of IGFBP1 protein by

PL treatment appears to be negated. Furthermore, PL stimulated the mRNA expression of IGFBP3 and IGFBP4 in a time- and dose-dependent manner in HepG2 cells. Adding of the ERK inhibitor suppressed the stimulation of the gene expression of IGFBP3 and IGFBP4 by PL. These results suggest that PL can induce the expressions of IGFBP1, IGFBP3 and IGFBP4 in hepatoma cells via a mechanism involving the ERK pathway. Higher expression of these IGFBPs by vitamin B6, together with their inhibitory effects on cell proliferation, suggests the anti-tumor effect of vitamin B6 via elevating IGFBPs.

Growing evidence suggests that dietary vitamin B6 is linked to inhibiting tumorigenesis in the colon. Importantly, our group has demonstrated that high dietary vitamin B6 suppressed colon epithelium cell proliferation, which may contribute to suppressing the colon tumorigenesis by vitamin B6. However, the molecular mechanisms involved in this process are not yet understood. In the second study, the upregulation in mRNA expression of p21 was confirmed in colon carcinoma HT29 cells exposed to PL by real-time PCR analysis. p21 is known as cyclin-dependent kinase inhibitor 1, and functions as a negative regulator of cell cycle progression. The expression of this gene is tightly controlled by the tumor suppressor protein p53. p21 has been suggested to be a tumor suppressor *in vitro* and *in vivo* in several studies. The effect of PL on the gene expression of p21 in other cell lines was analyzed. The results showed that PL elevated the gene expression of p21 in Caco2 cells, HEK293T cells and HepG2 cells. Adding PL significantly elevated the mRNA expression of p21 in both HT29 cells and HepG2 cells at 24 h, whereas other vitamers, including PLP, PN and PM, caused no such effect. In order to understand the mechanism of higher mRNA expression of p21 by PL, the p53 activation was examined (the upstream factor for p21 mRNA transcription) in both HT29 cells and HepG2 cells. The results indicated that PL caused higher protein level of p-p53 (active form of p53) of whole cell lysate and nucleus in these two cell lines.

To understand the effect of vitamin B6 on the p53 activation and mRNA expression of p21 *in vivo*, two groups of mice fed with different vitamin B6 diets for

5 weeks were investigated. Western blot analysis showed that vitamin B6 deficient diet caused a trend of lower protein expression of p-p53 compared with normal vitamin B6 diet. Real-time PCR analysis indicated that the mRNA expression of p21 in colon was also significantly reduced by vitamin B6 deficient diet compare to that of the normal vitamin B6 diet. These results suggest an important role of vitamin B6 in elevating protein of p-p53 and mRNA of p21 in the cancer cell lines and colon of mice. Taken together, my study implies the anti-tumor effect of vitamin B6 via elevating p-p53 and p21.

In conclusion, the first study showed that PL caused upregulation of mRNA expression of CYSTA, p21, IGFBP1, GADD45B, VDR and DDIT3, and downregulation of mRNA expression of NOX1 in HT29 cells and HepG2 cells. As for the expression of IGFBPs, only PL of the vitamers caused marked elevation of the mRNA and protein expression of IGFBP1 and mRNA expression of IGFBP3 and IGFBP4 in HepG2 cells. The mechanistic study suggests that PL can induce expression of these IGFBPs in HepG2 cells via a mechanism involving ERK pathway. The second study provided evidence that PL elevated the mRNA of p21 and p-p53 protein in both HT29 cells and HepG2 cells. The significant elevation in the mRNA of p21 was found in the mice fed with the normal vitamin B6 diet compared with that of vitamin B6 deficient diet. Taken together, these findings suggest the novel mechanisms of the anti-disease function of vitamin B6 through the modulation of IGFBPs and p21.