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Review

In Vitro Anticancer Activities of B_6 Vitamers: A Mini-review

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Abstract. *Vitamin B_6 compounds, including pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated forms, have been investigated with regard to their cancer preventive and therapeutic effects through epidemiological, in vivo, and in vitro studies. In particular, in vitro studies in cancer cells have evaluated the effects of several B_6 vitamers such as pyridoxine, pyridoxal, pyridoxamine, and pyridoxal-5'-phosphate, which is a bioactive form of vitamin B_6. However, the anticancer activity and concentration required to influence cancer cells vary among B_6 vitamers. In this review, the various in vitro effects of vitamin B_6 compounds on cancer cells are presented and discussed.*

Vitamin B_6, a group of water-soluble vitamins, encompasses several compounds including pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), and their phosphorylated forms: pyridoxine-5'-phosphate (PNP), pyridoxal-5'-phosphate (PLP), and pyridoxamine-5'-phosphate (PMP). The unphosphorylated B_6 vitamers are phosphorylated by pyridoxal kinase (PDXK), after which PNP and PMP are converted to PLP by PMP oxidase (PNPO) (Figure 1) (1). PLP serves as a coenzyme in processes such as amino acid metabolism (2). In addition, PLP has inhibitory effects on DNA/RNA polymerase (3, 4), topoisomerase (5), and angiogenesis (6, 7), and exerts antioxidative (8) and immune effects (9, 10).

Vitamin B_6 is found in various foods; PN is abundant in grains, nuts, vegetables, and bananas, whereas PL and PM are found in fish, meat, eggs, and milk (11). The unphosphorylated forms are absorbed in the jejunum and ileum by passive diffusion. On the other hand, the phosphorylated forms are absorbed after dephosphorylation by alkaline phosphatase in the small intestine (12, 13). An appropriate intake of vitamin B_6 is important for human health, and lack thereof associates with irritability and seizures in infants, nervous system disorders, dermatitis, cardiovascular disease, and cancer (13-16).

The relationship between vitamin B_6 and cancer has been investigated from the perspective of cancer prevention and therapy. For example, epidemiological associations between vitamin B_6 intake/blood PLP concentration and cancer risk have been evaluated in various cancers (13, 14, 17). Although some studies have shown that sufficient vitamin B_6 intake decreases cancer risk, others did not confirm this relationship. Contrasting results have also been obtained in *in vivo* animal studies; for instance, vitamin B_6 administration (*via* diet or subcutaneous injection) has been reported to both suppress and enhance tumor development or have no effect (10, 18-20). However, vitamin B_6 has been reported to enhance cell proliferation of blood and spleen lymphocytes (10, 21), and the accompanying activation of the immune response is considered one of the reasons for the discrepancies. Additionally, the epidemiological studies on vitamin B_6 were performed in conditions involving other vitamins and/or micronutrients. Furthermore, the positive effects of vitamin B_6 may be observed only in specific cancers or conditions. Thus, the controversial results must be reconciled.

In vitro, the anticancer effects of vitamin B_6, including PN, PL, PM, and PLP, have also been evaluated in various cancer cells for the development of cancer therapies. However, the activity and effective concentration varied among B_6 vitamers, and to our knowledge, there is no report of the differences among B_6 vitamers based on published *in vitro* cancer research. In this review, we focus on the different effects of vitamin B_6 compounds on cancer cells.

Effects of Vitamin B_6 on Cancer Cells *In Vitro*

The effects of vitamin B_6 on cancer cells *in vitro* have been reported to include inhibition of cell proliferation, enhancement of chemotherapy, and alterations in gene

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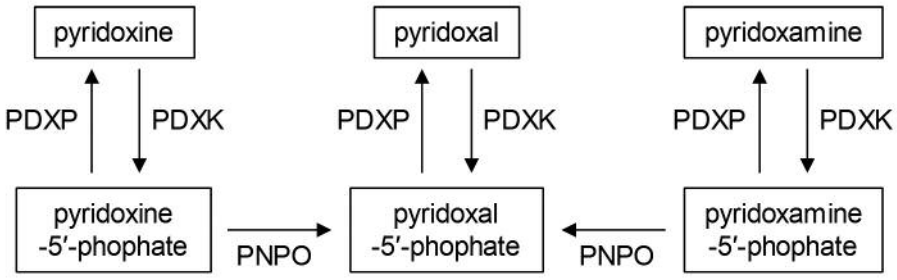

Figure 1. *Metabolic pathway of vitamin B6.*

expression (14, 22). However, these studies have investigated multiple B_6 vitamers.

Vitamin B_6 and cell proliferation. Vitamin B_6 has been demonstrated to suppress cell proliferation of B16 and B16F10 murine melanoma cells and M21-HPB human melanoma cells. The effect of PL was considerably stronger than that of PN (18, 23, 24). In B16 melanoma cells, 5 mM PN was necessary to achieve activity similar to that of 0.5 mM PL. When 0.5 mM of different B_6 vitamers was added to M21-HPB melanoma cells, PL was shown to exert a substantially stronger effect than PN. In our previous study on B16F10 cells, PL significantly suppressed cell proliferation, whereas PN showed a weak activity (24). In this review, we found that PLP and PM showed modest and weak antiproliferative activity, respectively, against these cells (Figure 2A). Similar results were obtained in HepG2 hepatoma cells and MKN45 gastric cancer cells (Figure 2B, C). The marked inhibition of cell growth by PL has also been demonstrated in HepG2 cells, MCF-7 human breast cancer cells, FRM feline breast cancer cells, and PANC-1 human pancreatic cancer cells (25-28). The strong activity of PL has been partly attributed to increased intracellular PLP, which is an active form of vitamin B_6, because the intracellular PLP concentration in M21-HPB cells was higher following PL treatment than PN treatment (10). In the conversion of unphosphorylated vitamin B_6 to PLP, PL is phosphorylated by PDXK, whereas PN and PM are phosphorylated by PDXK and then metabolized by PNPO. PL is more easily metabolized than PN and PM, and thus PL treatment may increase the intracellular PLP concentration. However, Kanouchi *et al*. demonstrated that enhancement of liposaccharide-induced COX-2 expression by PL, compared to other B_6 vitamers, in RAW264.7 mouse macrophages was correlated with the interaction between PL and the cell membrane, not the intracellular PLP concentration (29). Additionally, ours and other studies have demonstrated that the activity of PN was lower than that of PL in many cancer cell types, but the effect of PN on FRM cells was similar to that of PL (27). The mechanisms underlying the activity of B_6 vitamers could vary among cancer types and/or species.

Vitamin B_6 and chemotherapy. Combination of PN and cisplatin enhanced the cytotoxic effects of cisplatin in A549 human non-small cell lung cancer (NSCLC) cells through increasing the intracellular accumulation of cisplatin compared with cisplatin treatment alone (22, 30). Galluzzi *et al*. have demonstrated that expression of PDXK is important for the efficacy of the combination therapy with cisplatin and PN (22, 30). Expression of PDXK was positively correlated with disease-free and overall survival of patients with cisplatin-treated NSCLC, based on immunohistochemical analyses. Further research to clarify the association between vitamin B_6 and chemotherapy in various cancers is expected.

Vitamin B_6 and gene expression. Treatment of HT29 human colon cancer cells, LoVo human colon adenocarcinoma cells, and HepG2 cells with 500 μM PL resulted in enhanced expression of the cyclin kinase inhibitor p21 gene through tumor suppressor gene p53, compared with other vitamin B_6 compounds (PN, PM, and PLP) (31). p21 has been known to inhibit cell cycle (32). In addition, PL (500 μM) treatment up-regulated insulin-like growth factor binding protein 1 (IGFBP-1) in HT29 and HepG2 cells (25). Moreover, MCF-7 cells exhibited up-regulated IGFBP-3 gene expression through p53 following treatment with 0.5 mM PL or 10 mM PN (26, 33). IGFBP-1 and IGFBP-3 are generally considered to play a tumor-suppressive role in cancer (34-36). However, the relationship between gene up-regulation and cell growth inhibition by PL is unclear; thus, further studies are necessary to identify the underlying mechanisms.

Conclusion

In this review, we presented and discussed the *in vitro* activities of B_6 vitamers in cancer cells. The effects of PL on cancer cells were the strongest among B_6 vitamers and were exerted at lower concentrations compared to other B_6 vitamers. Vitamin B_6 compounds should be carefully selected in future *in vitro* cancer studies; for example, PL may be selected for a study first owing to its pronounced effects, or B_6 vitamer combinations may be tested

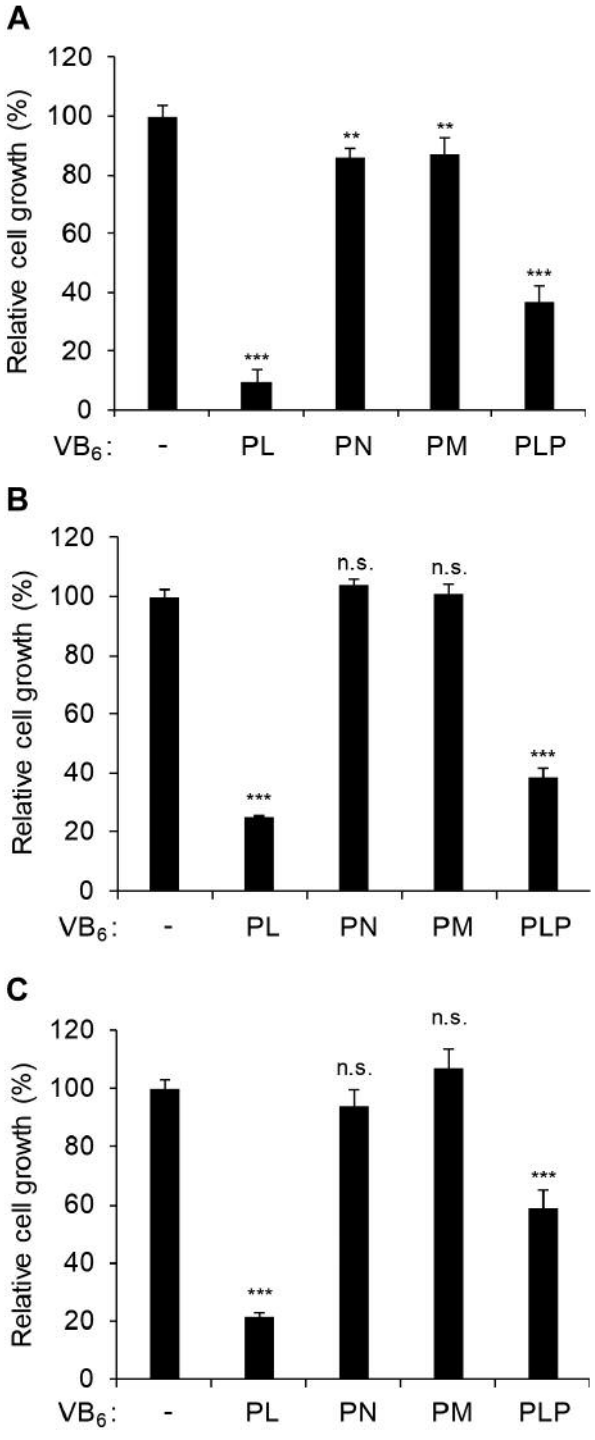

Figure 2. *Effects of vitamin B6 compounds on cell proliferation. (A) B16F10 cells, (B) HepG2 cells, and (C) MKN45 cells. B16F10 cells (0.5×10^5 cells/ml) and Hep G2 cells (1×10^5 cells/ml) were cultured in DMEM. MKN45 cells (1×10^5 cells/ml) were cultured in RPMI medium. The cells were treated with 500 μM B6 vitamer (PL, PN, PM, or PLP) for 72 h. Vitamin B6 (–) indicates culture in DMEM without vitamin B6; RPMI medium contained 5 μM PN. Cell proliferation was analyzed by cell counting or Cell Counting Kit-8. *p<0.05, **p<0.01, ***p<0.001 compared with vitamin B6 (–) by Dunnett's test. n.s.: Not significant.*

simultaneously. Nonetheless, it is expected that this review will help researchers understand the roles of vitamin B_6 in cancer treatment.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors' Contributions

TM designed the study and wrote the final version of the manuscript. YS supervised the study.

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