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Vitamin B6 inhibits the growth of human pancreatic carcinoma

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Abstract

Vitamin B6 serves as a coenzyme and is essential for the metabolism of amino acids. The human pancreatic carcinoma cell line PANC-1 was treated with pyridoxine (at concentrations of 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 mM) or pyridoxal (0.01, 0.05, 0.1, 0.5, 1.0 and 2.5 mM). The viable cell count was determined daily for six days using the MTT assay. The experiment was repeated three times. Each control and each concentration tested was performed in triplicate. All comparisons were analyzed by ANOVA with Tukey HSD. At doses of 2.5 mM and higher, PN significantly inhibited the growth in a dose-dependent manner ($p<0.05$). Treatment with PL (0.5, 1.0 and 2.5 mM) significantly inhibited growth from the second day ($p<0.05$). We have demonstrated the inhibitory effect of PL and PN on a human pancreatic cancer cell line. Further studies are needed to assess the impact of megadose vitamin treatment *in vivo.*

Keywords: Vitamin B6; Pyridoxal; Pyridoxine; Pancreatic cancer

1. Introduction

In North America pancreatic adenocarcinoma is the second most common gastrointestinal malignancy with an incidence of 29,000 new cases per year [1]. The 5-year survival from the disease is extremely poor, even with state of the art treatment. For these reasons pancreatic cancer is an important clinical and biological challenge.

Three primary forms of vitamin B6 occur in nature: pyridoxine (PN), pyridoxal (PL) and

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pyridoxamine. The biologically active form is pyridoxal-5'-phosphate (PLP) [2]. Vitamin B6 is an essential nutrient which is required for amino acid metabolism.

High doses of PN or PL inhibit the growth of melanoma and hepatoma cells in culture [3–6]. *In vivo,* injected pharmacological doses inhibited the growth of melanoma cells implanted into mice [7]. In the diet, supplemental vitamin B6 can suppress growth of tumors borne by mice [8]. A vitamin B6 supplemented diet may inhibit tumorigenesis in certain colon cancer models [9].

Vitamin B6 is a realistic possibility for intervention in cancer. A multivitamin regimen including high doses of PN (100 mg PN per day) reduced the risk of recurrence of transitional cell carcinoma of the bladder [10].

In this study we examined the effect of PN and PL on the growth of pancreatic cancer cells *in vitro.*

2. Materials and methods

2.1. Materials

PN, PL, sodium bicarbonate and HEPES were purchased from Sigma Chemical Co. (St. Louis, MO). Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS) and 0.05% trypsin/0.53 mM EDTA was purchased from GIBCO BRL (Gaithersburg, MD). The concentration of vitamin B6 in the FBS was 2.3 ng/mL (commercial assay by the Nichols Institute, CA). The TACS MTT (3-4,5-dimethylthiazolyl-2-2,5-diphenyltetrazolium bromide) cell proliferation assay kit was obtained from Trevigen Inc (Gaithersburg, MD).

2.2. Cell culture

The human pancreatic carcinoma cell line PANC-1 was purchased from American Type Culture Collection (ATCC, Manassas, VA). Tumor cells were maintained at 37 ^{o}C in a humidified 5% CO_2 atmosphere in DMEM containing 10% FBS, 1.5g/L sodium bicarbonate and 10 mM HEPES buffer.When the cells reached 80% confluence they were harvested using trypsin/EDTA for seeding of the plates.

2.3. Treatment with vitamin B6

Cells were seeded in 96-well plates at 5,000 cells per well in a volume of 100 μL medium. After 18 hours incubation the medium was removed and replaced with: 1. Fresh medium (control); or 2. Medium containing PN at concentrations of 0.1, 0.5, 1.0, 2.5, 5.0 or 10.0 mM; or 3. Medium containing PL at concentrations of 0.01, 0.05, 0.1, 0.5, 1.0 or 2.5 mM. Every well was drained and refreshed with new solution daily. Each experiment was carried out in triplicate. The whole experiment was repeated three times.

2.4. Cell proliferation assay

The relative viable cell number was assayed using the MTT assay, according to the manufacturer's instructions. This method is well-validated for this cell line and is known to correlate with thymidine incorporation [11]. The plates were assayed on the day of seeding the plates and daily for six consecutive days. The plates were read using a microplate spectrophotometer set to 570 nm (reference 650 nm). Linearity of the relationship between the cell number counted by Neubauer hemocytometer and absorbance at 570 nm was confirmed ($R^2=0.991$).

2.5. Statistics

The mean value from three repetitions was calculated for each data point. All comparisons were analyzed by ANOVA with Tukey HSD using STATISTICA version 5.5 (StatSoft Inc, Tulsa, OK). Significance was defined as $p<0.05$.

3. Results

3.1. Pyridoxine

At 2.5 mM and higher, PN inhibited the growth of the cells compared to control ($p<0.05$). This effect was apparent from the fourth day of treatment for 5 and 10 mM concentrations. No significant difference was observed between control cells and cells treated at a concentration lower than 2.5 mM. (Fig. 1). The IC_{50} (Inhibitory $Concentration_{50}$) for PN was 5 mM (Fig. 2).

3.2. Pyridoxal

At 0.5mM and higher the inhibitory effect of PL was demonstrated from the second day ($p<0.05$). No significant effect on growth was observed for PL at concentrations of 0.1 and 0.01 mM (Fig. 1). The IC_{50} for PL was 0.3 mM (Fig. 2).

4. Discussion

In this study we report that the PL and PN forms of vitamin B6 inhibit the proliferation of the human pancreatic cancer cell line, PANC-1. The IC_{50} for PN was 5 mM and for PL it was 0.3 mM.

To our knowledge this is the first report of the sensitivity of pancreatic cancer cells to high dose vitamin B6 treatment.

There is epidemiological evidence of an association between serum vitamin B6 levels and pancreatic cancer. In a recent case-control study of male smokers, the odds ratio for

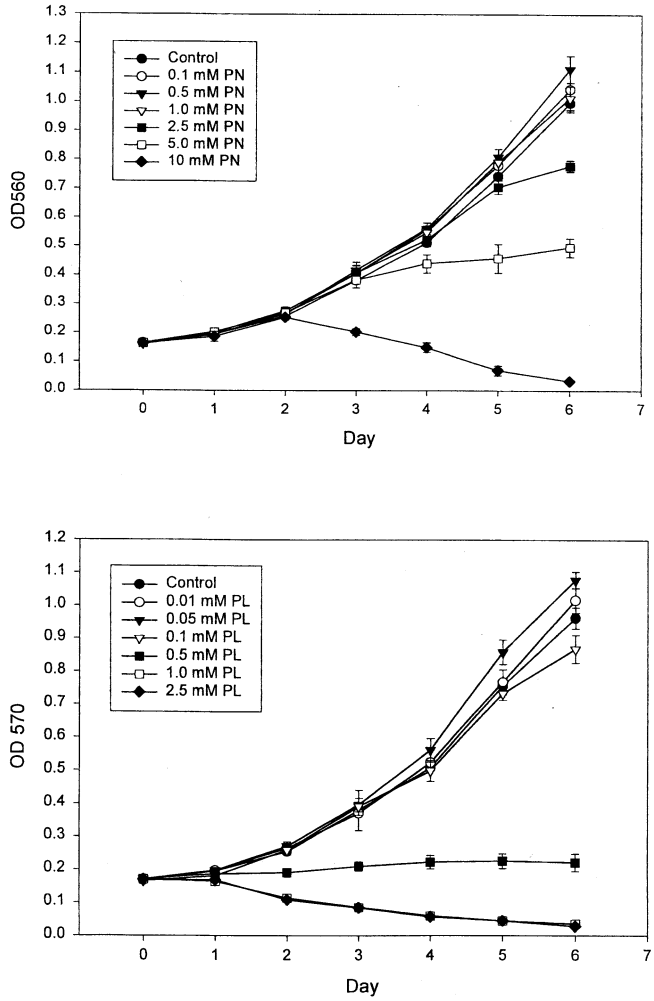

Fig. 1. The number of viable cells is represented by the OD_{50} data from the MTT assay. The mean value from three repetitions is plotted against the number of days of treatment. The cells were seeded on day 0. Upper: The effect of pyridoxine treatment on the growth of PANC-1 cells. Lower: The effect of pyridoxal on growth of PANC-1. * $p<0.05$.

pancreatic cancer was 0.43 for those with PLP levels falling in the highest tertile, compared to those in the lowest. This difference was statistically significant [12].

Melanoma and hepatoma cells in culture are sensitive to high dose vitamin B6 treatment [3–7,13]. DiSorbo and Litwack showed that 5 mM PN inhibits growth of hepatoma cells in culture and 10mM causes cell death [3]. Similar findings were reported for melanoma cells [4]. The concentration of PL required to show inhibition of growth was about one-tenth of that for PN [4]. This data is consistent with our findings with pancreatic cancer cell lines. Shultz et al [6] reported that human malignant melanoma cells responded to PL (at 0.25 to 0.5 mM) by slowed growth. However, PN (up to 0.5 mM) actually stimulated growth. The

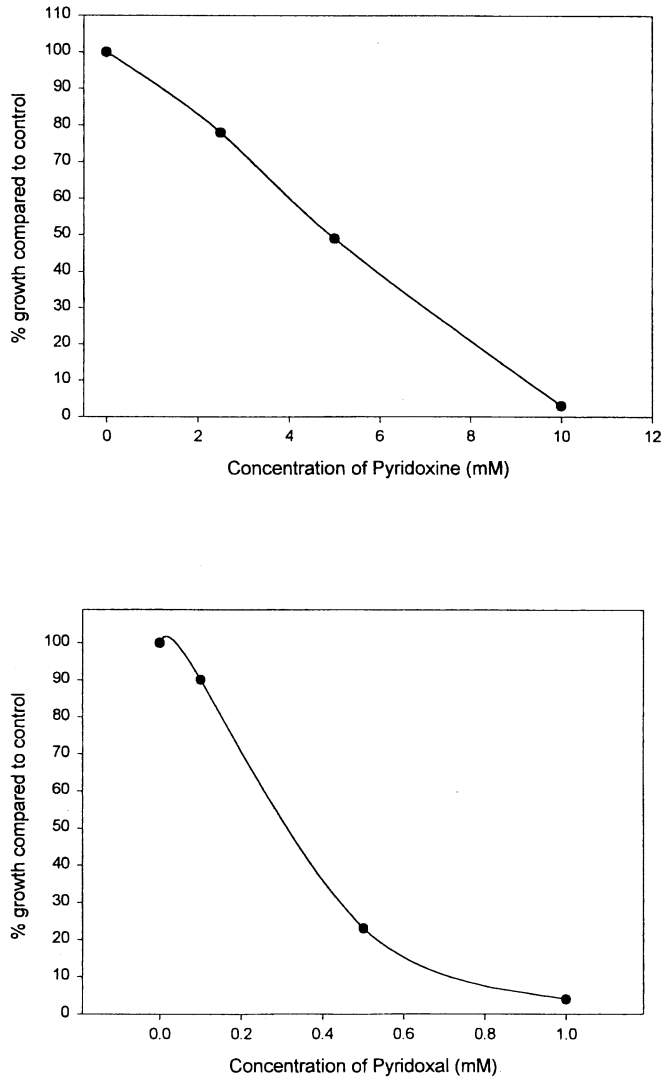

Fig. 2. The data are shown as a percentage of viable cell number after six days of treatment compared to the number of control cells on the same day. Upper: The concentration-response curve for pyridoxine. Lower: The concentration-response curve for pyridoxal.

dose of PN used was lower that the threshold dose required for inhibition of pancreatic cancer that we report.

In *vivo,* DiSorbo et al [7] tested the effect of high-dose PL on xenografted melanoma cell growth in mice. They showed regression of established subcutaneous tumors by injecting PL directly into the mass. A greater effect was seen with subcutaneous PL treatment before injection of the tumor cells.

The reported physiological range of vitamin B6 is 180-480 nM. The dose used in these studies is a pharmacological level. Mentally retarded adults given up to 350 mg PN daily

achieved a mean serum concentration of 5 μM [14]. Singh et al [15] gave 400 mg vitamin B6 daily to physically active men and reported a mean blood concentration of 0.9 μM after 12 weeks. No side effects at these doses were reported.

The mechanisms by which PN influences cancer growth are not yet elucidated. Nevertheless, plausible biological mechanisms for the action of vitamin B6 have been proposed. PLP inhibits the key enzymes RNA polymerase [16], DNA polymerase [17] and glycogen phosphorylase [18]. Thus, it is possible that the inhibition of cell proliferation in human pancreatic carcinoma is caused by reduced DNA synthesis or gene transcription, or metabolism of energy stores.

Vitamin B6 has potential for prevention and treatment for pancreatic carcinoma. Further studies are needed to evaluate the effect of this vitamin in an *in vivo* cancer model.

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