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SHORT COMMUNICATION

Pyridoxine to protect from oxaliplatin-induced neurotoxicity without compromising antitumour effect

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Abstract
Purpose Oxaliplatin (OHP) in combination with 5-fluorouracil/leucovorin (FOLFOX) is clinically used as front-line therapy in patients with advanced colorectal carcinoma (CRC), with response rates ranging from 46 to 71%. This combination is now considered a standard treatment for metastatic CRC and also in the post-operative adjuvant setting. Reversible, cumulative, peripheral sensory neuropathy is the principal dose-limiting toxicity of OHP therapy. Pyridoxine (vitamin B6) has been shown to reduce cisplatin and fluoropyrimidine-related neurotoxicity but its administration with OHP has not yet been studied. Low doses of pyridoxine are free of side effects; it can be given orally. If pyridoxine administration with oxaliplatin has no adverse effect on OHP cytotoxicity effects, it will be a simple and cost-effective way to minimise OHP-induced neurotoxicity.
Methods In vitro simultaneous combination of OHP and pyridoxine was studied in 6 CRC cell lines (HT29, Widr, SW480, HCT116, H630 and SW1116), in an ovarian cancer cell line (A2780) and its cisplatin-resistant subline (ADDP) and in an oestrogen-dependent breast cancer cell line (MCF-7). Three fixed concentrations of pyridoxine: 1, 10 and 25 μM were combined with varying concentrations of OHP, and the growth inhibitory effects were evaluated using the MTT cell growth assay.
Results Oxaliplatin induced consistent cytotoxicity in all cell lines with GI_{50} values between 0.23 and 7.6 μM. Addition of pyridoxine at concentrations of 1–25 μM does not affect OHP cytotoxicity.
Conclusions Administration of pyridoxine, at concentrations extending across possible therapeutic plasma levels in humans, does not antagonise OHP antitumour effects in a range of relevant tumour cell lines. This study provides a foundation for clinical studies to test whether pyridoxine can minimise OHP-related neurotoxicity, and clinicians can be confident that pyridoxine is very unlikely to reverse the antitumour effects of OHP, as seems to be the case with Ca/Mg infusions. This could prove to be a cost-effective way to minimise OHP-related neurotoxicity, allowing more effective less toxic treatment and better outcomes in patients.

Keywords Oxaliplatin · Pyridoxine · Neurotoxicity · Colorectal cancer · Cell lines · MTT

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Introduction

Oxaliplatin (OHP, (trans-l)1,2-diaminocyclohexaneoxalatoplatinum(II)) is a platinum-based chemotherapeutic agent with an oxalate ligand as the leaving group and a *trans*-1,1,2-diaminocyclohexane as the transport ligand which causes intrastrand cross-links. OHP has a broad spectrum of antineoplastic activity and has demonstrated a lack of cross-resistance with other platinum compounds [1, 2]. It is active in a range of malignancies and has undergone clinical development against CRC and gastric cancer [3]. OHP is

effective against metastatic colorectal cancer as a single agent and in combination with 5-FU/leucovorin (FOLFOX) as first-line treatment [4] and in 5-FU pretreated patients [5]. This combination is now considered a standard treatment for metastatic colorectal cancer [4] and also in the adjuvant setting [6]. OHP also has single agent activity in ovarian cancer [7] and a variety of other malignancies including non-Hodgkin's lymphoma, breast cancer, mesothelioma and non-small cell lung cancer [3].

Neurotoxicity is frequently a dose-limiting toxicity of OHP [8]. Two different types of clinical neurological symptoms occur as a consequence of OHP treatment. A transient peripheral sensory neuropathy can occur, manifesting as paresthesias and dysesthesia in the extremities exacerbated by exposure to cold and sometimes accompanied by muscle contraction of the extremities or the jaw. It is always reversible, dose-limiting and does not require discontinuation of therapy. A chronic syndrome manifesting as deep sensory loss, functional impairment and ataxia, usually correlated with the cumulative dose of OHP, occurs late [9] and is generally reversible, but may last for several months [10]. The major site of damage appears to be the dorsal root ganglia (DRG) which is consistent with the platinum accumulation studies in humans [11].

The cellular mechanism of OHP neurotoxicity is unclear. OHP may directly or indirectly affect neuronal voltage-gated sodium (Na+) channels through a pathway involving calcium chelation by its metabolite oxalate [12, 13]. Various strategies have been studied for preventing neurotoxicity. Ca/Mg infusions seem to reduce incidence and intensity of acute OHP-induced symptoms and might delay cumulative neuropathy, as shown in a non-randomised retrospective study on a cohort of 161 patients treated with OHP and 5-FU/leucovorin for advanced CRC, with and without Ca/Mg infusions. About 20% of patients in the Ca/Mg group had chronic neuropathy *versus* 45% without Ca/Mg ($P = 0.003$), while tumour response rate was similar in CRC in both groups [12]. However, a more recent randomised trial (CONCEPT trial) was suspended as it indicated that Ca/Mg infusions may be associated with a reduced response rate (17 vs. 33%) to OHP treatment [14]. Antiepileptic drugs like carbamazepine, gabapentin, venlafaxine, amifostine; alpha-lipoic acid; and glutathione have also demonstrated some activity in the prophylaxis and treatment of OHP-induced acute neuropathy [15, 16]. However, randomised trials demonstrating a prophylactic or therapeutic effect on OHP's cumulative neurotoxicity are still lacking. In a recent RCT, oral glutamine was found to significantly reduce the incidence and severity of peripheral neuropathy of metastatic CRC patients receiving OHP without affecting response and survival [17].

Pyridoxine (vitamin B6), a water soluble B-complex vitamin, is necessary for the proper functioning of over 70 different enzymes that participate in a variety of cellular processes including amino acid metabolism and synthesis of neurotransmitters in the brain and nerve cells. Pyridoxine deficiency can cause a number of neurological effects and raised plasma or serum homocysteine levels.

Oral pyridoxine administration has been reported to significantly reduce the neurotoxicity of cisplatin-hexamethylmelamine regimens in patients with ovarian epithelial cancer [18]. However, pyridoxine appeared to reduce the duration of tumour response in this model. Pyridoxine is also used to ameliorate hand–foot syndrome, a side-effect of chronic fluoropyrimidine administration [19].

Hence, pyridoxine has properties suggesting it is worthy of study as a possible agent for preventing OHP-induced neurotoxicity in the clinic. Therefore, we investigated the possible negative interaction of OHP and pyridoxine in a range of human tumour cell lines.

Materials and methods

Cell culture

Six colon adenocarcinoma cell lines with a range of characteristics (HT29, Widr, SW480, H630, SW1116, HCT116), an ovarian cancer cell line A2780 and its cisplatin-resistant subline ADDP and oestrogen-dependent breast cancer cell line MCF-7 were used. Cell lines were cultured at 37°C, under air containing 5% CO_2 and passaged every 3–7 days; all cell lines were tested and found to be mycoplasma free. ADDP cells were maintained in RPMI medium containing 10% foetal bovine serum (FBS) and all other cell lines in DMEM medium containing 10% FBS. All culture medium preparations were further supplemented with penicillin/streptomycin (100 µg/ml) and glutamine (2 mM).

In vitro growth inhibition assay

For experiments, cells in logarithmic growth were transferred to 96-well plates in 100 µl medium at a density of 2,000–2,500 cells per well. On day 1 (24 h after plating), 100 µl medium with or without the test agent (50 µl single agent with 50 µl medium or 50 µl of both agents for combination) was added to each well in triplicate. Drug exposure experiments were carried out on each cell line using varying concentrations of OHP (5 nM–50 µM) or pyridoxine (0.5–500 µM) as single agents and for combinations (in triplicates) for 72 h.

Three fixed concentrations of pyridoxine 1, 10 and 25 µM, extending across a possible therapeutic plasma concentration range) were combined with a range of concentrations of OHP to rule out interaction with OHP. Growth inhibitory effects were evaluated using the MTT

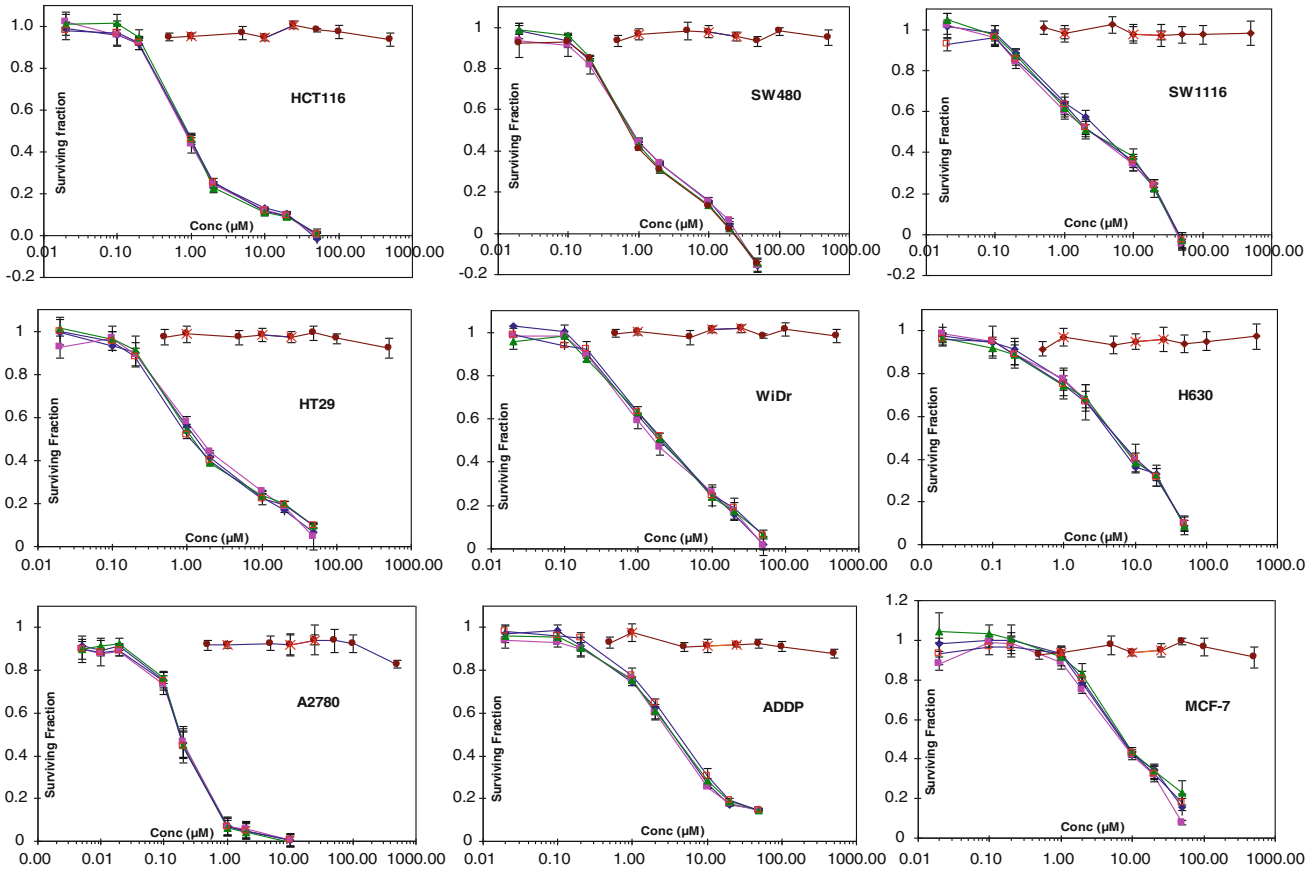

Fig. 1 Representative growth response curves for cell lines treated for 72 h with varying concentrations of OHP alone (*filled diamond*), in combination with fixed concentration of 25 μM pyridoxine (*filled square*), in combination with fixed concentration of 10 μM pyridoxine (*filled triangle*), in combination with fixed concentration of 1 μM pyridoxine (*open square*) and varying concentrations of pyridoxine alone (*multiple symbol*). The three concentrations of pyridoxine used for combinations are represented on the pyridoxine alone curve by the symbol (*open circle*). Values are means ±SEM

(3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyl-tetrazolium bromide) cell growth assay [20] and absorbance read at 540 nm. Control cell growth was exponential during the whole incubation period. GI_{50}, calculated as the drug concentration at which cell growth was inhibited by 50% compared to untreated control cells, was determined for both single agents and for combinations in each cell line. Mean surviving fraction (SF) ± SEM values (minimum of 3 experiment values) were drawn for concentration data points (Fig. 1).

Results

Pyridoxine alone from 0.5 to 500 μM had no significant cytotoxic effect on any cell line (Fig. 1) except for A2780 cells, in which the highest concentration lowered surviving fraction to 0.83. Oxaliplatin induced consistent cytotoxicity in all cell lines with GI_{50} values between 0.23 and 7.6 μM (Table 1). Addition of pyridoxine at 1.0–25 μM did not affect OHP cytotoxicity as GI_{50} is unaffected and growth response curves for OHP with or without pyridoxine overlap for all these cell lines (Fig. 1).

Table 1 GI_{50} values for 72-h oxaliplatin exposure to cell lines

Cell lines	Mean GI_{50} ± SEM for oxaliplatin (μM)
A2780	0.23 ± 0.04
SW480	0.85 ± 0.06
HCT116	0.98 ± 0.11
HT29	1.38 ± 0.15
WiDr	2.30 ± 0.20
ADDP	3.50 ± 0.50
SW1116	4.0 ± 0.89
H630	5.60 ± 1.17
MCF-7	7.60 ± 0.24

Conclusions

These in vitro studies show that administration of pyridoxine, at concentrations extending across possible therapeutic plasma levels in humans, does not antagonise OHP antitumour effects in a range of relevant tumour cell lines. This study provides a foundation for clinical studies to test whether pyridoxine can minimise OHP-related neurotoxicity, and clinicians can be confident that pyridoxine is very unlikely to reverse the antitumour effects of OHP, as seems to be the case with Ca/Mg infusions. Further confidence for a positive outcome of a clinical trial could be provided by in vitro studies in a peripheral neuron model. Demonstration that pyridoxine effectively prevents or minimises neurotoxicity could significantly extend the clinical utility of OHP.

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Conflict of interest None.

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