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Original Research Article

Esomeprazole and pantoprazole enhance the antiproliferative effects of cisplatin on the human neuroblastoma SH-SY5Y cell line

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ABSTRACT

Background: Proton pump inhibitors (PPIs) largely used a drug to treat gastroesophageal disease such as gastric ulcers. Moreover, in recent years, several studies suggest that PPIs have an important anti-cancer effect in monotherapy and or combination with chemotherapy. The aim of this study was to investigate whether esomeprazole and pantoprazole exhibit anti-cancer effect alone or could enhance chemosensitivity on the human neuroblastoma cell line SH-SY5Y to cisplatin.
Methods: The human neuroblastoma SH-SY5Y cells were cultured and treated with different concentrations of esomeprazole, pantoprazole, and cisplatin alone. Also, these cells exposed to cisplatin+ esomeprazole and cisplatin + pantoprazole combinations, respectively and incubated 24 h. The antiproliferative activities of the (PPIs) alone or in a combination of cisplatin was evaluated using the XTT colorimetric assay.
Results: According to experimental data, neither PPIs showed no cytotoxicity on the human neuroblastoma cell line SH-SY5Y at all concentrations. However, when combined with cisplatin separately, they were found to have significant antiproliferative effects on the human neuroblastoma SH-SY5Y cell lines when compared to cell lines treated with cisplatin alone ($p<0.05$).
Conclusions: Taken together, the inhibition of V-ATPase via esomeprazole and pantoprazole might enhance the chemosensitivity of cisplatin on the human neuroblastoma cell line SH-SY5Y. However, further studies are needed to be able to utilize PPIs in human neuroblastoma cells.

Keywords: Cisplatin, Esomeprazole, Human neuroblastoma SH-SY5Y cell line, Pantoprazole, V-ATPase

INTRODUCTION

Neuroblastoma is the most commonly extracranial solid malignant tumor of childhood. It's an embryonal tumor of the sympathetic nervous system and accounts for 7%-8% of all pediatric malignancies. Half of the cases occur below the age of three years. It happens less frequently every year. This solid tumor is in adult life is well known but may be seen very rare. Male: female ratio is equally.[1] Neuroblastoma originates from primitive neuroblasts of the embryonic neural crest and therefore can occur within the sympathetic nervous system anywhere. The most common site of tumor occurs within the abdomen. About 50% of these tumors originate from the adrenal medulla. Neuroblastoma may be seen on paravertebrally regions such as the neck, chest, and pelvis, it may cause neurological findings to occur due to the pressure on the spinal cord throughout the foramen and vertebral body.[2] Diagnosis is the important principal criterion for planning the treatment policy. Other related factors are age and biological features should be considered into account when planning treatment. Patients with stage 1 and stage

2A disease are usually suitable for curative surgery. Chemotherapy is strictly provided, which comprises vincristine, cyclophosphamide, etoposide, and cisplatin. In these patients, it is preferable to use chemotherapy as the first treatment in order to reduce the tumor mass, to remove it more fully and to make the operation more secure.[1] Cisplatin is a widely used chemosensitive therapeutic agent for the treatment of neuroblastoma.

Platinum compounds are intensely effective anti-cancer agents. Of this group used in clinical practice cisplatin [cis-diaminedichloroplatin (II), CDDP] is still among the first-line therapeutic agents for certain solid tumors. The most limitations in the use of cisplatin are nephrotoxicity and systemic neurotoxicity. The hydration of patients treated with cisplatin and the use of diuretics greatly reduces the effect of the drug on renal function, and currently, neurotoxicity continues to be the important dose-limiting side effects. Cisplatin may be combined with different medical agents to increase the efficacy and anticancer effects to treat malignant tumors.[3]

The extracellular acidity is the distinctive feature of malignant tumors that play an important role in the selection of more aggressive cells that invade to survive in the invasion, metastasis, drug resistance, and a very hostile micro-environmental state. One of the most important mechanisms that enable tumor cells to survive in the acidic state is some proton exchangers, including vacuolar ATPase (V-ATPase). V-ATPase, on the one hand, removes H + from the cytosol from the inner vesicles, while leaving H + out of the cells, thereby contributing to extracellular acidification (pHe) and cytosolic alkalization (pHi); again, a characteristic feature of malignant tumors. Tumor cells use proton pumps to prevent a lytic enzyme activation step that inevitably leads to self-digestion. Preclinical evidence has shown that an anti-acidic approach based on buffering molecules or inhibitors of proton exchangers can enhance the effectiveness of chemotherapeutics. However, may also induce a direct antitumor effect, which results in tumor cell death by inhibiting H + clearance of malignant cells. More evidence also supports the role of tumor acidity as a tumor escape mechanism. Actually, proton pump inhibition enhances the effect of immunotherapy and spontaneous anti-tumor immune response. Although these are the features described for malignancies, extracellular acidity has proven to play a key factor in the resistance of cancer cells to chemotherapeutics.[4]

PPIs that inhibits the H+/K+ ATPase function and the strongest gastric acid secretion in clinical use are omeprazole, lansoprazole, pantoprazole, rabeprazole, and esomeprazole. Pantoprazole and esomeprazole have been widely used in previous experimental cancer studies. It has been shown that PPIs effectively increase the efficacy of chemotherapy and are well tolerated in studies with in vivo and in vitro cell cultures.[5-8]

Based on the above information, in the present study, we aimed to investigate whether esomeprazole and pantoprazole could enhance chemosensitivity of to cisplatin. To the best of our knowledge, this is the first study to investigate the effects of esomeprazole and pantoprazole alone and combine with cisplatin on the human neuroblastoma SH-SY5Y cell lines.

METHODS

In-vitro cytotoxicity assay

Cell culture

The cytotoxicity of the esomeprazole/pantoprazole alone and combine with cisplatin was tested against human neuroblastoma SH-SY5Y cell line (ATCC CRL-2266). The cells were cultured in RPMI-1640 (Gibco Thermo Fisher Scientific) containing 10% FBS, 1% L-glutamine, 100IU/mL penicillin and 10mg/mL streptomycin (Gibco Thermo Fisher Scientific) in 25cm^2 polystyrene flasks and maintained in a humidified atmosphere with 5% CO_2 at 37°C. Growth and morphology were monitored and the cells were passaged when they had reached almost 85% confluence.

Cell viability assay

Cell viability was evaluated using the XTT (2,3- bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay (Roche Diagnostic, Germany) against the human neuroblastoma SH-SY5Y cells. PPIs and cisplatin were dissolved in RPMI-1640 and stock solutions were prepared. Then these stocks were diluted in RPMI-1640 and various concentrations were prepared prior to treatment. The human neuroblastoma SH-SY5Y cells were seeded in 96-well plates at a density of 1 × 104, cells per well in 100µl colorless RPMI-1640 medium and the cells were treated with the PPIs alone (ranging from 1 to 1000µM) or combined with cisplatin at 10µM (this concentration is the IC50 value determined for cisplatin in this study) concentrations and incubated for 24 h. At the end of the incubation period, for cytotoxicity, 50µL XTT labeling mixture was added to each well and then the plates were incubated at 37°C for 4h. Finally, the absorbance of XTT-formazan was measured using a microplate (ELISA) reader at 450nm against the control. All experiments were performed in three independent experiments and the cell viability was expressed in % related to control (100% of viability).

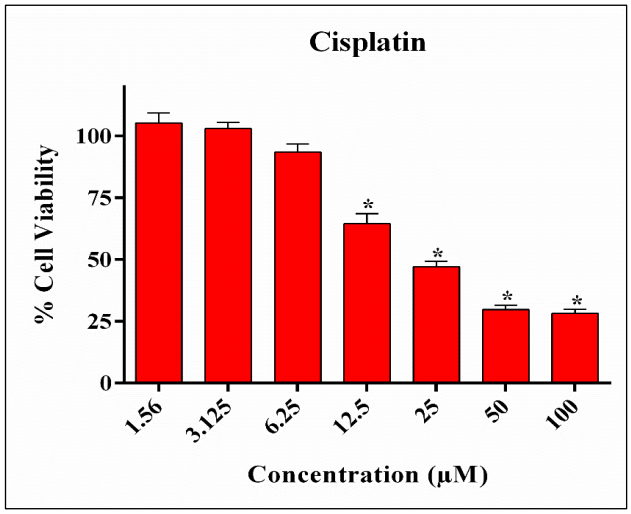
Statistical analysis

Statistical analysis was carried out using GraphPad Prism 7 version and all data are expressed as mean±SEM. Groups were compared statistically using general linear models of analysis of variance (ANOVA) followed by Tukey test. P values less than or equal to 0.05 were considered to be statistically significant.

RESULTS

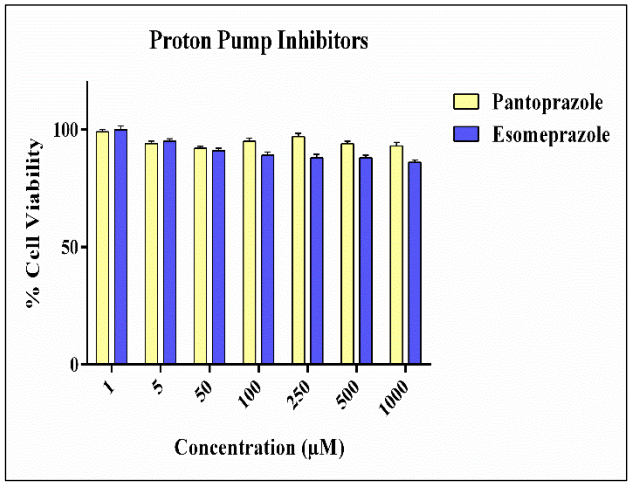
Inhibition of cell proliferation

In the present study, XTT cell proliferation assay was performed to assess the antiproliferative effects of the PPIs alone and combined with cisplatin on the human neuroblastoma SH-SY5Y cell line for 24h. At first, to determine the cytotoxicity of PPIs alone, exponentially growing cells were treated with increasing concentrations of esomeprazole and pantoprazole and incubated for 24h. Then, to determine the IC50 value of cisplatin on the human neuroblastoma SH-SY5Y cells, cisplatin was administrated on SH-SY5Y cells at various concentrations and incubated for 24h (Figure 1).



Data are expressed as mean±SEM in three experiments and the differences are identified as * from control p<0.05

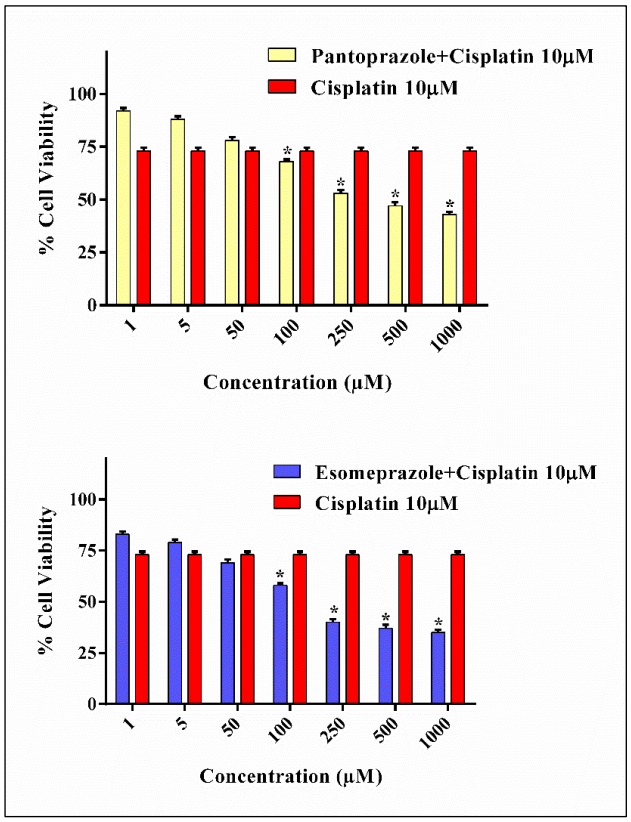
Figure 1: Antiproliferative activity of cisplatin on SH-SY5Y cells.



The cells were treated with different concentrations of pantoprazole and esomeprazole ranged between 1 and 1000 µM and any cytotoxicity was determined by XTT assay

Figure 2: The effects of pantoprazole and esomeprazole on viability of SH-SY5Y cells.

This IC50 value of cisplatin was also used in combination with PPIs. According to experimental results, neither esomeprazole and pantoprazole exhibited any cytotoxicity on the human neuroblastoma SH-SY5Y cell line (Figure 2). Moreover, we were interested if esomeprazole and pantoprazole might affect the sensitivity of the human neuroblastoma SH-SY5Y cells towards frequently used antineoplastic drug cisplatin. We hence treated SH-SY5Y cells with esomeprazole +cisplatin or pantoprazole+cisplatin combinations respectively at the IC50 concentrations. As presented in Figure 3, an important loss of viability was observed in both combinations especially at 50, 100, 250, 500 and 1000µM concentrations at 24h.



The differences are *p<0.05 compared with pantoprazole or esomeprazole alone.

Figure 3: The effects of different concentrations of pantoprazole/esomeprazole and cisplatin (10µM) combination on viability of SH-SY5Y cells.

These results suggested that when compared with cisplatin treatment alone, esomeprazole and pantoprazole pretreatment significantly enhanced the antiproliferative and neurotoxic effect of cisplatin in the human neuroblastoma SH-SY5Y cells.

DISCUSSION

Microenvironmental acidity becomes an important role for the new way of malignancies treatment. Actually, malignancies are characterized by genetic heterogeneity, extracellular acidity is a common phenotype of almost all

cancer types. Cancer cells up-regulate proton exchangers and transporters to survive under acidic conditions (mainly V-ATPase, Na+/H+ exchanger, monocarboxylate transporters, and carbonic anhydrases, that actively extrude excess protons, avoiding intracellular accumulation of toxic molecules. These systems also relate to non-responsiveness or resistance to chemotherapic agents, leading to the protection of cancer cells from the majority of drugs that can not enter cancer cells when protonated in the acidic tumoral environment. In fact, as it usually occurs in progress against malignancy, resistant tumors appear and proliferate following an initial response to treatment, resulting in more benign behaviors and rapid progression of the tumor.[6]

Recent studies support the use of PPI's as a new strategy against cancer as a cocktail. The antiproliferative effects of various PPI combinations are currently being evaluated for the treatment of various tumors such as pancreatic cancer, melanoma and colon cancers.[9-11]

Song et al, demonstrated in their recent study, it has been reported that esomeprazole enhanced the cytotoxicity of paclitaxel in various cervical cancer cells HeLa and INT407.[12]

In a different study, Lindner et al reported that the esomeprazole significantly inhibited tumor cell survival and increased the cytotoxic effect of cisplatin and 5-FU in esophageal cancer cells.[13]

A similar in vitro study Wang et al demonstrated that the PPI pantoprazole enhances the chemosensitivity of CRC, HT29 and RKO cells to fluorouracil (5-FU).[14]

Furthermore, to the best of our knowledge, no studies have been conducted on the investigation of the effects of PPIs and cisplatin combination on the human neuroblastoma SH-SY5Y cells.

In the present study, XTT cell proliferation assay was performed to assess the antiproliferative effects of the PPIs alone and combined with cisplatin on the human neuroblastoma SH-SY5Y cell line. We hence treated SH-SY5Y cells with esomeprazole+cisplatin or pantoprazole + cisplatin combinations, an important loss of viability was observed in both combinations. These results suggested that when compared with cisplatin treatment alone, esomeprazole and pantoprazole pretreatment significantly enhanced the antiproliferative effect of cisplatin in the human neuroblastoma SH-SY5Y cells. In this study accordingly with the previous studies, similar results were reported for the combination of esomeprazole and pantoprazole with cisplatin.

CONCLUSION

In conclusion, inhibition of the V-ATPase by using PPIs (esomeprazole and pantoprazole) enhanced the chemosensitivity of the human neuroblastoma SH-SY5Y cells to cisplatin. Our findings suggest that esomeprazole and pantoprazole may be useful as a chemosensitizer in the treatment of patients with neuroblastoma. This is the first study demonstrating that PPIs increase the antiproliferative and neurotoxic effect of cisplatin on the human neuroblastoma SH-SY5Y cell line. However, the large prospective clinical studies are needed.

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