Proton pump inhibitors as chemosensitizer: New indication for a well-known medication

Christiane Matuszcak¹, Kirsten Lindner¹, Jörg Haier², Richard Hummel¹

¹Department of General and Visceral Surgery, Muenster University Hospital, Muenster, Germany
²Comprehensive Cancer Centre, University of Muenster, 48149 Muenster, Germany

Correspondence: Richard Hummel
E-mail: richard.hummel@uni-muenster.de
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In the last decades, resistance to chemotherapy has become a major problem in the treatment of different tumors, resulting in unfavorable therapeutic success and worse patient survival. Among other mechanisms of multidrug resistance, in-vitro studies demonstrated that tumors present changes in microenvironment that lead to pH alterations, and acidic tumor microenvironment modulates sensitivity to chemotherapy, cancer progression and metastasis in cancer cell lines. Currently, various potential therapeutic strategies targeting intracellular pH regulation and metabolic interactions in tumor cells are under investigation. Proton pump inhibitors (PPIs) are one of the most promising therapeutic approaches in this context - due in particular to their worldwide clinical use, good availability and low side effect profile. PPIs influence pH balance, and recent studies have shown increased chemosensitivity of tumor cells and enhanced cytotoxicity of chemotherapeutic drugs after treatment with PPIs. This brief review discusses the use of PPIs as chemosensitizer in tumors in-vitro and in-vivo on the basis of the current literature.

Keywords: proton pump inhibitor; PPI; chemoresistance; chemosensitizer


Introduction

Drug resistance of tumors is a major problem in chemotherapy treatment. Two main categories of drug resistance were described: acquired/adaptive or primary/innate drug resistance. Acquired drug resistance develops during or briefly after chemotherapy application, and was reported for example in adult acute leukemia, Non-Hodgkin's lymphoma and breast and ovarian cancers [1]. Primary resistance on the other hand describes an existing resistance before the start of the chemotherapy, and this type of resistance was observed for example in non-small cell lung cancer, kidney, pancreas, stomach and cancers of the colon [1]. The resistance of cancer cells towards several different drugs is called multidrug resistance (MDR) [2].

However, both, innate and/or adaptive resistance, critically limit treatment success and remain a key challenge for clinicians [3].

A number of different mechanisms can affect levels of drug resistance in cancer cells, including a hypoxic tumor microenvironment leading to an extracellular acidification outside of the cells (extracellular pH (pHe)) and a cellular interior alkalization (pHi) [2,4]. Currently, several potential strategies to target intra-/extracellular pH are under investigation. One very promising option with regards to its potential influence on drug resistance in tumor cells could be the use of so called proton pump inhibitors (PPIs) that disrupt intra-/extracellular pH gradients. PPIs are one of the most promising therapeutic approaches - especially due to their
Mechanisms of drug resistance in cancer cells and the role of disrupted pH gradient

The mechanisms of tumor cells to overcome toxicity of chemotherapeutic drugs are multifarious and include for example [5]: Drug inactivation; Drug target alteration; DNA damage repair; Increased drug efflux and reduced influx; Regulation by cancer stem cells; Epigenetic regulation; Epithelial-Mesenchymal Transition; Cell-death inhibition.

In this context the altered pH gradient across the cell membrane in cancer cells - extracellular acidification and alkalization intracellular - supports several of the above mentioned drug resistance mechanisms resulting for example in enhanced drug efflux (by drug enrichment in acidic vesicles, vesicular transport to the cell membrane and drug discharge in the extra cellular space [3,4]), diminished drug influx (impaired uptake of weakly basic chemotherapeutic drugs) or cell cycle arrest (restricted mTOR complex 1 activation with inhibition of protein synthesis and G0 and G1 cell cycle arrest) [6].

Background of pH alteration in cancer cells

In normal tissues, pH is regulated in a very tight range to ensure basic cell functions including cellular metabolism, membrane permeability, ATP maintenance, enzyme activity, apoptotic mechanisms and cell proliferation [7]. This seems different in tumor tissue: instead of normal tissues were the pH range is from 7.2 to 7.5, in different tumors over the last decade have demonstrated that the pH ranges from 5.7 to 7.3 [8]. The main cause of tumor microenvironment acidification is the Warburg effect [9]. This describes the observation that glycolytic metabolism is stimulated in cancer cells what generates increased amounts of lactic and carbonic acids [10]. The overexpression of lactic acid by malignant tumors results in proton accumulation in the cytoplasm what then leads to an acidic microenvironment due to for example overexpression of proton pumps by tumor cells in order to avoid intracellular acidification [9]. In general, pH alteration in tumor cells is caused by a number of metabolic changes and pH-regulatory proteins, and correlates with tumor invasion and metastatic potential [11].

Mechanisms that contribute to the regulation of pH include hypoxia-inducible factor (HIF), Na+/H+-exchanges (NHE1), carbonic anhydrases (CAIX, CAXII, CAII), bicarbonate transporters (NBC), proton-glucose and amino-acid transporter (Glut1, LAT1), anion exchange proteins (AE1), monocarboxylate transporters (MCT1, MCT4), the voltage-gated proton channel Hv15 and the hydrogen/potassium adenosine triphosphatase enzyme (H⁺/K⁺-ATPase) [11,12]. Figure 1 presents an overview about
these different mechanisms and their impact on pH, including potential therapeutic approaches to target pH homeostasis in cancer cells.

Especially the last aspect of H⁺/K⁺-ATPases seems to play a crucial role in tumor’s control of pH homeostasis. Expression and activity of H⁺/K⁺-ATPases are enhanced in certain human tumors [7]. H⁺/K⁺-ATPases hydrolyze ATP into ADP+P and energy which is necessary to pump intracellular protons into the extracellular space [3]. In multidrug resistance cell lines and in response to anticancer drugs an overexpression of the gene encoding the H⁺/K⁺-ATPase subunit C was described. In addition, an inhibition of the C subunit expression by small interfering RNA (siRNA) resulted in an increased sensitivity of adriamycin resistant breast cancer cells (MCF7/ADR) to cytotoxic agents [2].

Recent studies demonstrated that H⁺/K⁺-ATPase were also involved in tumor invasion and multidrug resistance in oral squamous cell carcinoma [13,14], hepatocellular carcinoma [15], pancreatic cancer [16], prostate cancer [17] and esophageal cancer [18]. A review by Spugnini et al. furthermore summarized characteristics of the most important proton pumps found in mammalian cells which included vacuolar H⁺-ATPases (V-ATPase; expressed on membranes of acidic organelles) and Na⁺/H⁺-ATPase (expressed ubiquitously on plasma membranes) and the mentioned H⁺/K⁺-ATPase. The authors showed that all of these pumps were over-expressed and/or hyperfunctional in malignant tumors [19]. Most interestingly from a clinical point of few: these ATPases are potential targets for proton pump inhibitors.

Proton pump inhibitors: mechanism and background informations

Proton pump inhibitors induce a reduced gastric acid production in gastric cells regardless of the stimulus for acid secretion [20, 21].

PPIs are administered as neutrally charged drugs. Like all prazoles, they have a benzimidazole basic structure. After resorption in the small bowel, PPIs reach the parietal cells in the stomach. There, in acidic intracellular compartments, PPIs were protonated resulting in the active form of these drugs. This active form binds covalently and irreversibly to cysteine molecules of H⁺/K⁺-ATPase in activated gastric parietal cells, thus deactivating the proton pumps by forming disulfide bonds [19,21]. Thereby, PPIs were shown to reduce gastric acid secretion by up to 99% [21].

The overall oral bio-availability of PPIs is very high: 89% for esomeprazole, 80-90% for lansoprazole and 77% for pantoprazole. The main actors of the metabolism of PPIs are the cytochromes P450 (CYP) enzymes in the liver, in main cases by CYP 2C19 and CYP 3A4. Generally, the compatibility of PPIs is very high and the incidence of short-term side effects like headache, nausea, diarrhea, abdominal pain, fatigue or dizziness is low [22]. Occurrence and severity of adverse effects are similar for all members of this drug family [8,23]. Long-term use of PPIs has been less studied, what makes a final statement difficult [24]. Main indications for the use of PPIs are treatment of e.g. gastroesophageal reflux disease (GERD) or gastritis [11,25].

**PPIs as chemosensitizer - an overview about the current literature**

ATPase of tumor cells could be an interesting target for cancer therapeutics because of the typical acidic microenvironment in tumors and the subsequent effects as described above that result in an increased pH in acidic vesicles such as endosomes, thereby inhibiting the accumulation of basic cytotoxic agents and additionally leading indirectly to apoptosis of cancer cells [20, 23]. By inhibiting drug sequestration, a treatment with ATPase-targeting PPIs for example could alter intracellular drug distribution, thus allowing higher drug levels in the nucleus with increased cytotoxicity. Furthermore, PPIs may facilitate drug distribution by decreased drug efflux of the cell [20].

Until now, there is only limited data available on the potential use of PPIs as chemosensitizer in solid tumors, and most studies focus on in-vitro experiments. Quar et al. for example found in daunomycin resistant kidney cancer cells of the mouse, which were pretreated with concanamycin A (CCMA) an inhibitor of V-ATPase that the daunomycin concentration increased in nucleo-cytoplasmic compartments [25]. In addition, CCA A pretreatment in resistant kidney cancer cells had no impact on the cellular efflux but induced a intra cellular reallocation of the drug [25]. The authors suggested that drugs, like PPIs and CCM A, which regulate the intracellular pH followed by enhanced intra cellular accumulation of weak-base chemotherapeutics could invert the anthracycline resistance in MDR cells - generally described in case of an expanded acidic lysosomal compartment [25]. Luciani and his coworkers showed in different cell lines including melanoma, colon adenocarcinoma and lymphoma cells, that pretreatment with PPIs enhanced resistance in tumor cell lines to cisplatin, 5-fluorouracil and vinblastine with an up to 2 logs reduced median lethal concentration [26]. They also postulated that a pretreatment with PPIs induced increased cytoplasmic retention of the cytotoxic drugs [26]. Chen et al. and Huang et al. investigated whether PPIs enhance cytotoxicity of
Table 1. Overview about the current literature about PPIs as chemosensitizer in-vitro. ↑: increase, ↓: decrease

<table>
<thead>
<tr>
<th>Proton pump inhibitor (PPI)</th>
<th>Tumor type</th>
<th>PPI-dose</th>
<th>Chemotherapy</th>
<th>Effect of PPI</th>
<th>Year</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concanamycin A</td>
<td>kidneys cancer cells (mouse)</td>
<td>20 nM</td>
<td>Daunomycin</td>
<td>drug sensitivity ↑</td>
<td>2003</td>
<td>[25]</td>
</tr>
<tr>
<td>Omeprazole, Esomeprazole, Pantoprazole</td>
<td>melanomas, colon adenocarcinomas, lymphomas</td>
<td>1 µg/ml</td>
<td>Cisplatin, 5-Fluorouracil, Vinblastine</td>
<td>drug sensitivity ↑ epH ↓</td>
<td>2004</td>
<td>[26]</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>gastric cancer cells</td>
<td>10 µg/ml</td>
<td>Cisplatin, 5-Fluorouracil</td>
<td>cell growth ↓ apoptosis ↑ invasion ↓</td>
<td>2008</td>
<td>[22]</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>sarcoma cancer cells</td>
<td>200 µM</td>
<td>Doxorubicin</td>
<td>ipH ↑ drug uptake ↑ drug sensitivity ↑</td>
<td>2013</td>
<td>[20]</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>osteosarcoma cancer cells</td>
<td>60 µM</td>
<td>Cisplatin</td>
<td>drug sensitivity ↑</td>
<td>2013</td>
<td>[9]</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>gastric cancer cells</td>
<td>1,53 - 13,52 µg/ml</td>
<td>Cisplatin</td>
<td>drug sensitivity ↑ apoptosis ↑</td>
<td>2013</td>
<td>[7]</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>breast cancer cells</td>
<td>70 µM</td>
<td>Doxorubicin</td>
<td>drug sensitivity ↑</td>
<td>2014</td>
<td>[28]</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>Esophageal cancer cells</td>
<td>200-250 µM</td>
<td>Cisplatin 5-Fluorouracil</td>
<td>drug sensitivity ↑ adhesion ↓ migration ↓ ipH/epH ↑</td>
<td>2014</td>
<td>[18]</td>
</tr>
</tbody>
</table>

Table 2. Overview about the current literature about with PPIs as chemosensitizer in-vivo. ↑: increase, ↓: decrease

<table>
<thead>
<tr>
<th>Proton pump inhibitor (PPI)</th>
<th>Tumor type</th>
<th>PPI-dose</th>
<th>Chemotherapy</th>
<th>Effect of PPI</th>
<th>Year</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole, Esomeprazole, Pantoprazole</td>
<td>mice with melanomas, colon adenocarcinomas</td>
<td>75 mg/kg</td>
<td>Cisplatin</td>
<td>tumor growth ↓</td>
<td>2004</td>
<td>[26]</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>mice with breast cancer tumors</td>
<td>100-300 mg/kg</td>
<td>Doxorubicin</td>
<td>tumor growth ↓</td>
<td>2013</td>
<td>[20]</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>dogs+cats with spontaneous occurring tumors</td>
<td>5 mg/kg</td>
<td>various</td>
<td>tumor growth ↓</td>
<td>2011</td>
<td>[19]</td>
</tr>
</tbody>
</table>

anti-tumor drugs by pH reduction in human gastric adenocarcinoma cell lines by inhibiting V-ATPases [7,27]. They found, that a treatment with PPIs in cancer cells reduced the V-ATPase levels in a dose dependent manner resulting intracellular in a decreased uptake of protons and an inversion of transmembrane pH gradient, but with no pH-effect on epithelial cells [7,27]. Additionally, Chen et al. revealed that the greatest cytotoxic effect of chemotherapeutic drugs on gastric tumor cells was achieved 24 hours after a PPI pretreatment with significantly increased early and total apoptosis rates [27]. Patel et al. found in sarcoma cell lines that a pantoprazole concentration of about 200 µM increased pH and lead to an enhanced nuclear uptake of doxorubicin [20]. In addition, a pretreatment with pantoprazole increased tissue penetration of doxorubicin in sarcoma cells [20]. The research group of Ferrari et al. showed that PPI sensitized human osteosarcoma cell lines to cisplatin treatment [9], additionally the pretreatment with esomeprazole suppressed growth of triple-negative breast cancer cells in a dose-dependent manner via increasing intracellular acidification and doxorubicin effects [28]. Most interestingly, esomeprazole had no significant effects on non-cancerous breast epithelial cells [28]. For esophageal cancer cell lines, Lindner et al. reported that after pretreatment with esomeprazole overall tumor cell survival was significantly reduced in a dose-dependent manner, as well as tumor cell adhesion and tumor cell migration. Furthermore, the cytotoxic effects of cisplatin and 5-FU were augmented [18]. Table 1 provides an overview about these data on in-vitro studies in the current literature.

With regards to a potential implementation of PPIs as anticancer drugs in clinical settings, a few authors took this work one step further and investigated the effect of PPI treatment on chemoresistance in cancer in-vivo (please see table 2 for details).

Luciani et al. found in nude mice with melanomas or colon adenocarcinomas that oral pretreatment with omeprazole induced tumor sensitivity to cisplatin [26]. Also, Patel et al. could show in mice with induced breast cancer xenografts that single or multiple dosage of pantoprazole - given prior to doxorubicin treatment - led to a further delay in cellular growth [20]. In addition, Spungini et al. found in 7 cats and 27 dogs with spontaneous occurring tumors that the PPI lansoprazole achieved a partial or complete response to conventional chemotherapy (67.6% of all animals) with good tolerability. Only four dogs developed typical side effects such as vomiting and/or diarrhea due to gastric hypochlorhydria [19]. The authors concluded from their
Table 3. Overview about the current studies on humans dealing with PPIs as potential chemosensitizer in different tumor types.

<table>
<thead>
<tr>
<th>Proton pump inhibitor (PPI)</th>
<th>Tumor type</th>
<th>PPI-dose</th>
<th>Chemotherapy</th>
<th>Year</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoprazole</td>
<td>humans with gastric lymphoma</td>
<td>2 x 40 mg</td>
<td>Doxorubicin, Cyclophosphamide, Vincristine, Prednisone, Mitoxantrone, Chlormbucil</td>
<td>2005</td>
<td>[29]</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>humans with resectable non-metastatic osteosarcoma</td>
<td>60 mg/day</td>
<td>Methotrexat, Cisplatin, Adriamycin</td>
<td>2013</td>
<td>[9]</td>
</tr>
</tbody>
</table>

experiments that high dose treatment with proton pump inhibitors might reverse chemotherapy resistance and was in general well tolerated in the majority of the treated animals [19]. Ferrari and coworkers showed in nude mice with osteosarcoma and in patients with resectable non-metastatic osteosarcoma that the chemotherapy effect was after pretreated with esomeprazole - represented by portion of tumor necrosis [9].

At this stage, there are two highly interesting clinical trials in progress that investigate the effect of combined application of PPIs and chemotherapy treatment on cancer treatment (see table 3). The first trial aims to evaluate the impact and compatibility of high-dose proton pump inhibitor (esomeprazole 200 mg/day) treatment combined with chemotherapy (docetaxel and cisplatin in various doses) in metastatic breast cancer [29]. The second clinical trial assesses the effectiveness and safety of a combined treatment with pantoprazole and docetaxel (with prednisone) in metastatic castration-resistant prostate cancer patients [30]. The main objectives of these trials are the assessment of the activity and safety of a treatment with PPI and chemotherapy and an evaluation of the pharmacokinetic interactions of the two drugs. We are very looking forward to the results of these two currently ongoing prospective clinical trials as they might have a decisive impact on potential use of PPIs in combination with chemotherapeutic drugs in the future.

Limitations of treatment with PPIs as chemosensitizer

However, until yet, there are several limitations that have to be addressed before using PPIs as first line or additive drug in cancer treatment. Firstly, although the different PPIs share a common structure and mode of action, the different substances differ with regards to their exact clinical pharmacology or physical and chemical properties. Secondly, the application route (oral, intra-venous) is crucial for the sensitizing effect of PPIs on chemotherapy and might impact on the bio-availability. For example, the structural stability of omeprazole various according to the pH: at acidic conditions (pH 1-3) the half-life is only 2 min, compared to around 20 hours at pH7. Thirdly, doses of PPIs are varying considerably between studies and are ranging between 20 nM and 250 µM in-vitro respectively between 5 mg/kg and up to 300 mg/kg in-vivo. However, all studies reported that PPI treatment is feasible with limited side-effects and a high rate of compliance regardless of the doses used. Fourthly, until now there are no data available which evaluate high dose PPI treatment combined with chemotherapy in humans, and two first clinical trials are currently on the way. Finally, the impact of long-term use of high doses of PPIs has not yet been studied thoroughly.

Conclusion

Multidrug resistance in solid tumors is a major cause for failure of chemotherapy treatment. One important aspect of drug resistance is the acid microenvironment in the tumor. Proton pump inhibitors may present a potential solution for this problem. In-vitro experiments demonstrated that PPIs positively impact on sensitivity towards chemotherapy in different tumors. Additionally, first in-vivo data reported good tolerability of PPI treatment in animals and humans undergoing chemotherapy.

Altogether, these results highly suggest that the use of PPIs in combination with chemotherapy might positively affect sensitivity towards a number of different chemotherapeutic drugs. Further clinical trials are needed to evaluate whether and - if so - in which dose and application form PPIs could be safely administered either as first-line treatment or additive therapy in cancer patients.

Conflicting interest

The authors have declared that no competing interests exist.

Author contribution

Christiane Matuszcak, Kirsten Lindner and Richard Hummel have been involved in drafting the manuscript. Christiane Matuszcak and Kirsten Lindner contributed equally. Jörg Haier revised it critically for important intellectual content.

References

1. Nooter K, Stoter G. Molecular mechanisms of multidrug


