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A combination of *p*-coumaric acid and metformin enhances the cytotoxicity of carboplatin and epirubicin in the gastric cancer cell line AGS

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ABSTRACT

Gastric cancer is a deadly disease with low survival rates. The chemotherapeutic drugs used to treat gastric cancer are expensive and cause several side effects. Here, treatments involving natural compounds, established therapeutic agents, or a combination of both groups may present a fascinating alternative. Among the compounds with such promising anticancer potential are p-coumaric acid (pCA) and metformin (Met). They may resolve the problem of drug resistance as well. As a result, the present study investigated the chemotherapeutic cytotoxicity-enhancing effects of the combination of pCA and Met in the human gastric cell line AGS. Cytotoxicity of carboplatin and epirubicin was assessed in single treatments and in combination with 0.2 mM pCA and 0.8 mM Met. MTT-based viability measurements demonstrated that both carboplatin and epirubicin decreased the survival of AGS cells in a concentration-dependent fashion after 48 h of incubation. Compared to the single treatments, the combination treatments had significantly higher cytotoxicity. pCA and Met enhanced carboplatin and epirubicin cytotoxicity at concentrations significantly lower than their IC_{50} values. Collectively, these findings suggest that pCA and metformin (Met) warrant further investigation as promising therapeutic candidates for overcoming drug resistance in gastric cancer and could introduce new ways to fight against drug resistance in gastric cancer models.

Keywords: *p*-Coumaric acid; Metformin; Carboplatin; Epirubicin; Gastric cancer

INTRODUCTION

Gastric cancer is one of the leading causes of cancer-related deaths worldwide. Despite gradual declines in death rates over recent decades in some high-income countries, the sheer number of annual deaths continues to rise in many low- and middle-income nations, making gastric cancer one of the few common malignancies that still resists substantial mortality reduction [1]. Despite progress in treatments, gastric cancer patients have low survival rates because the disease typically remains silent until it has advanced beyond curability. Even with refined surgeries, improved chemotherapy, and newer targeted or immunotherapies, most patients are diagnosed only after the cancer has spread regionally or metastasized, at which point long-term survival is rare. While early-stage patients may achieve remission, the majority face a prognosis of months, not years. This makes gastric cancer a malignancy for which therapeutic advances have yet to meaningfully improve overall survival, underscoring that

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without widespread screening for early detection, even the best treatments will yield persistently low survival rates. [2]. Gastric cancer incidence shows significant variation in different geographical regions. The highest rates are consistently observed in East Asia, Eastern Europe, and parts of South America, while incidence is much lower in North America and Africa. However, recent projections indicate that the global burden is shifting: although East Asia still accounts for the largest number of cases, the most rapid increases in the coming decades are expected in sub-Saharan Africa, driven by population ageing and demographic transitions. These regional differences reflect a complex interplay of *H. pylori* infection prevalence, dietary habits (especially high salt intake), and access to screening programmes [3].

Carboplatin [4] and epirubicin [5] are chemotherapeutic agents used to fight against gastric cancer. However, the treatments for gastric cancer are expensive and cause several side effects [6]. Furthermore, chemotherapeutic drug resistance is another limitation increasing the complexity of gastric cancer treatment. As a result, even when patients initially respond to standard regimens, many eventually experience disease progression due to acquired resistance, leading to treatment failure and poor clinical outcomes [7]. As a result, treatments involving natural compounds, established therapeutic agents, or a combination of both groups can potentially overcome these limitations. Natural compounds offer advantages including low systemic toxicity, multi-targeted mechanisms, and the ability to modulate drug resistance pathways. When used alongside conventional chemotherapeutic agents, these natural products can enhance drug uptake into tumor cells, inhibit efflux pumps, and restore apoptotic signaling. Moreover, combining natural compounds with established agents can reduce the required dose of chemotherapy, thereby minimizing adverse effects while maintaining or even improving efficacy. In many preclinical and some clinical studies, these integrated strategies have demonstrated higher anticancer effects compared to either approach alone, including better suppression of tumor growth, reduced metastasis, and prolonged survival in gastric cancer models [8, 9].

A growing body of evidence has demonstrated that p-coumaric acid (pCA) and metformin (Met) possess significant antitumor activity. pCA is a phenolic compound found in fruits, vegetables, and cereals. It has shown significant anticancer properties in various cancer models, including gastric cancer. These effects are mediated through multiple mechanisms such as induction of apoptosis, cell cycle arrest, inhibition of epithelial-mesenchymal transition (EMT), and modulation of microRNA expression. Recent studies have also revealed that pCA can trigger ferroptosis, a novel form of regulated cell death, and downregulate telomerase activity. Furthermore, pCA enhances the effectiveness of conventional chemotherapeutic agents and helps overcome drug resistance. Synthetic derivatives of pCA have been developed as dual inhibitors of growth factor receptors, highlighting its potential as a lead compound for anticancer therapy. [10-13]. Although metformin is primarily prescribed for the management of type 2 diabetes, accumulating evidence indicates that it also exerts anticancer effects in multiple tumor types, such as gastric, ovarian, and endometrial cancers [14-17].

Metformin has been shown to enhance the cytotoxic effects of the anticancer drug carboplatin in cases of ovarian cancer [18, 19]. lung cancer [20], breast cancer [21], and cervical cancer [22]. Similarly, it can improve the cytotoxic effects of epirubicin on thyroid cancer [23] and breast cancer stem cells [24]. On the other hand, as an established anticancer agent, pCA might improve the cytotoxic effects of these chemotherapeutic agents. Our previous investigation has proved the anticancer effects of Met and pCA co-treatment on the gastric cancer cell line AGS [25]. Accordingly, the present study was designed to evaluate whether the combined administration of p-coumaric acid (pCA) and metformin (Met) could potentiate the cytotoxic effects of carboplatin and epirubicin in AGS gastric cancer cells.

MATERIALS AND METHODS

Reagents and Cell Culture: Human gastric cell line AGS (Pasteur Institute, Iran) was cultured in RPMI 1640 culture medium (BioIdea, Iran). The culture medium was enriched with

10% (v/v) heat-inactivated fetal bovine serum (FBS; Gibco, USA) and supplemented with penicillin and streptomycin, each at a final concentration of 100 U/mL (BioIdea, Iran). *p*-Coumaric acid (Sigma, USA) was dissolved in DMSO (Shellmax, China). Metformin (Santa Cruz Biotechnology, USA) was dissolved in RPMI 1640 culture medium (BioIdea, Iran). Carboplatin (Mylan, USA) and epirubicin (Ebewe, Austria) were dissolved in the RPMI 1640 culture medium (BioIdea, Iran). RPMI 1640 culture medium (BioIdea, Iran) was used as the diluent for the preparation of all three treatment agents. MTT was the product of Roche (Switzerland). All experiments were done in triplicate.

MTT-Based Cell Viability Analysis: MTT test determined the cytotoxic effects of carboplatin, epirubicin, and their combinations with pCA and Met on the AGS cell line. AGS cells were plated at a density of 5×10^3 cells per well in 96-well culture plates and maintained for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. After 24 hours, treatments with 3-fold increasing concentrations of carboplatin (0.41-100 μM), and 3-fold increasing concentrations of epirubicin (0.037-9 μM), and the combinations of 3-fold increasing concentrations of the two drugs with 0.2 mM pCA and 0.8 mM Met were performed for 48 hours. Each concentration was examined in triplicate. Cell viability was subsequently determined using the MTT colorimetric assay. Following incubation with the MTT reagent for 4 h, absorbance was measured at 595 nm using a microplate ELISA reader. Cell viability was calculated according to the equation below:

$$\text{Viability \%} = 100 \times OD595_{test} / OD595_{control}$$

Statistical Analysis: All experiments were done in triplicate. The data is presented as mean ± standard error of the mean (SEM). All statistical computations were conducted in SPSS version 16.0 (SPSS Inc., USA). Group comparisons were analyzed using the independent *t*-test, whereas correlations were assessed with Spearman's rank correlation test. Statistical significance was defined as $p < 0.05$. The MTT curves were plotted using Microsoft Office Excel 2013 software. After obtaining the MTT test results, GraphPad Prism Software Version 10 was used to determine the IC_{50} values for carboplatin and epirubicin.

RESULTS AND DISCUSSION

MTT assay results demonstrated that exposure of AGS gastric cancer cells to carboplatin for 48 h produced a concentration-dependent reduction in cell viability. As the concentration of carboplatin increased across a range of low to high micromolar values, a corresponding decrease in cell viability was observed, indicating that higher doses of the drug led to greater metabolic suppression and cell death. This clear concentration-dependent relationship confirms that the antiproliferative effect of carboplatin on AGS cells is not merely an all-or-none phenomenon but rather a graded response that correlates strongly ($r = -0.874$, $df = 19$, $p < 0.001$) with drug dosage over the 48-hour exposure period (Fig. 1). The AGS cell line was co-treated with 3-fold increasing concentrations of carboplatin (0.41-100 μM), along with 0.2 mM pCA and 0.8 mM Met. The result indicated that, except for the last concentration of carboplatin (100 μM), combination treatments had significantly higher ($p < 0.01$, or $p < 0.001$) cytotoxic effects than single treatment with carboplatin (Fig. 1). According to our previous work [25], the half-maximal inhibitory concentration (IC_{50}) was determined to be 3.36 mM for p-coumaric acid (pCA) and 70.40 mM for metformin (Met). In addition, the IC_{50} value of carboplatin was 12.44 μM. Therefore, pCA and Met, at concentrations radically lower than their IC_{50} values, enhanced the cytotoxic effect of carboplatin on the AGS cell line. This was true for the first five concentrations of carboplatin. The results obtained in this part are comparable to similar investigations reporting the enhanced cytotoxicity of carboplatin in the presence of metformin and some plant phenolic compounds like curcumin (26). However, this was not the case for the

concentration of 100 µM of carboplatin. At this concentration, the combination treatment did not produce a statistically significant difference compared with the corresponding single-agent treatment (p = 0.719). This was not in accordance with the results reported about the significantly higher cytotoxic effects of combination treatments [26]. The observed incompatibility could be due to some unknown interactions between the three anticancer agents in the presence of high-concentration carboplatin.

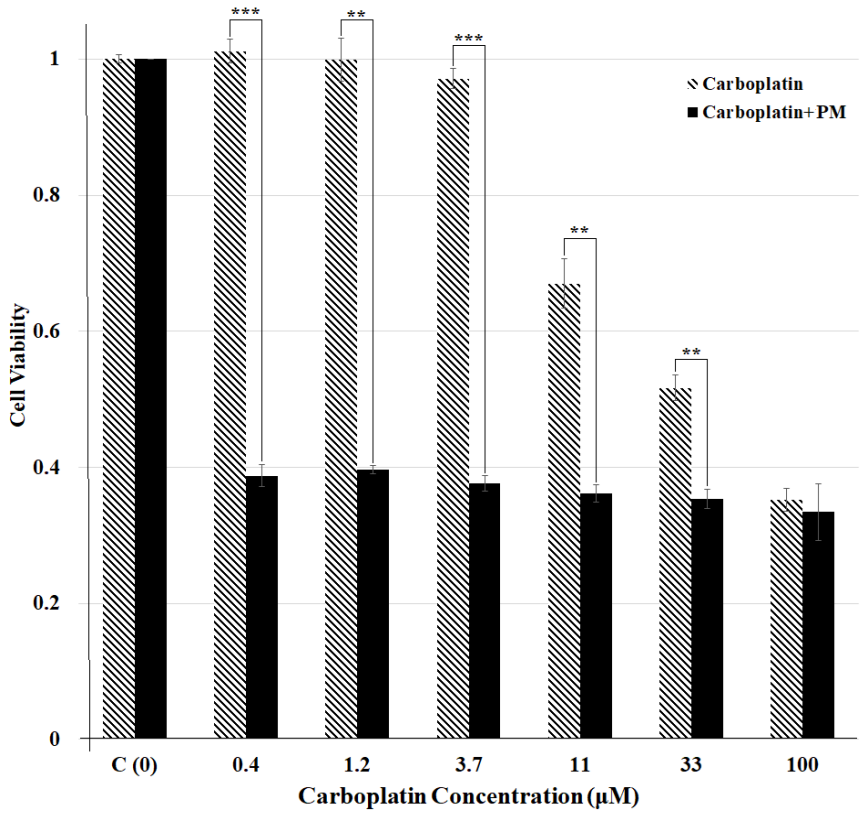

Figure 1: The results of the MTT assay in the AGS cell line treated with carboplatin and carboplatin + *p*-coumaric acid + metformin. The data shown represents the AGS cell line viability (mean ± SEM) for three independent repeats after treating with carboplatin and the combination of carboplatin, *p*-coumaric acid, and metformin. Independent *t*-test was the basis of statistical analyses. ** and *** show significant differences between single and combination treatments at $p < 0.01$ and $p < 0.001$, respectively. Carboplatin + PM represents combination treatments with carboplatin, 0.2 mM *p*-coumaric acid, and 0.8 mM metformin.

Epirubicin, like carboplatin, produced a concentration-dependent reduction in the viability of AGS gastric cancer cells (r = -0.622, df = 19, p = 0.003). Exposure of AGS cells to increasing concentrations of epirubicin over 48 hours resulted in a progressive reduction in cell viability, mirroring the pattern observed with carboplatin. At lower doses, epirubicin caused moderate growth inhibition, whereas higher concentrations led to a marked decline in metabolic activity and cell survival. The dose-response relationship followed a consistent trend, with the most pronounced cytotoxic effects occurring at the upper end of the concentration range. This finding indicates that epirubicin, similar to carboplatin, suppresses AGS cell proliferation in a concentration-dependent manner, further supporting the notion that both chemotherapeutic agents share a common feature of dose-driven anticancer activity in this gastric cancer model (Fig. 2). Co-treatment with 3-fold increasing concentrations of epirubicin (0.037-9 µM) and the combination of 0.2 mM pCA and 0.8 mM Met had a significantly higher ($p < 0.01$, or $p < 0.001$) cytotoxic effect on the AGS cell line than the single treatments with epirubicin. The IC_{50} value for epirubicin was 0.26 µM. Therefore, once again, it could be said that pCA and Met, at concentrations much lower than their IC_{50} values, could significantly improve the cytotoxic

effects of epirubicin on the human gastric cancer cell line AGS. Unlike carboplatin (with insignificant differences at the final concentration), combination treatments had significantly higher cytotoxic effects at all epirubicin concentrations. It is compatible with the results of studies proving the boosted cytotoxicity of epirubicin in the presence of metformin and some plant phenolic compounds like curcumin [26].

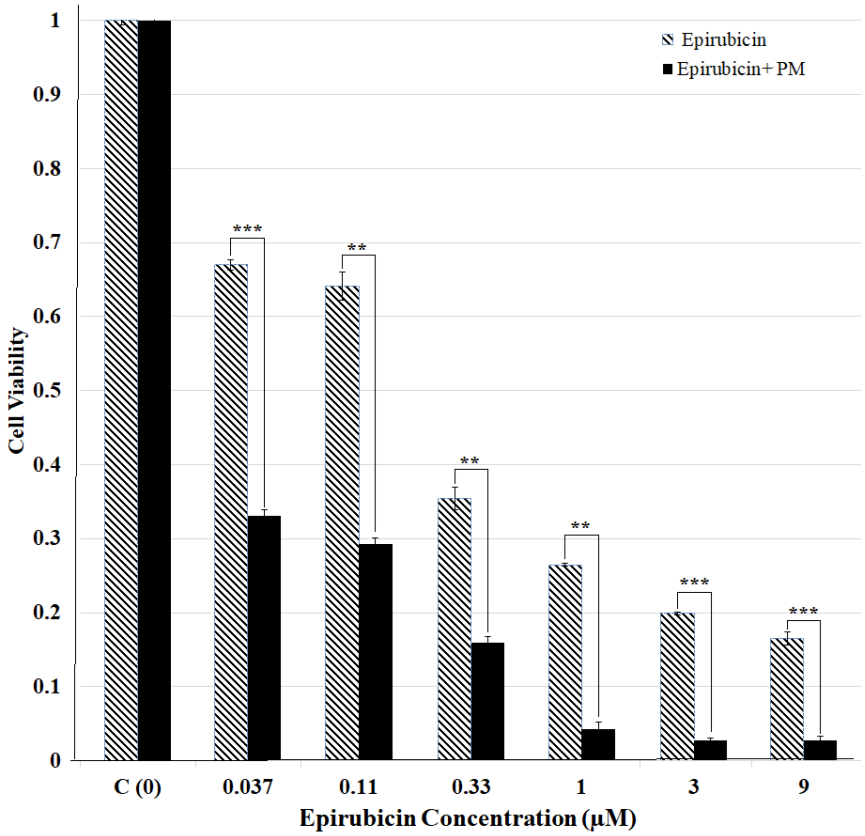

Figure 2: The results of the MTT assay in the AGS cell line treated with epirubicin and epirubicin + *p*-coumaric acid + metformin. The data shown represents the AGS cell line viability (mean ± SEM) for three independent repeats after treatment with epirubicin and the combination of epirubicin, *p*-coumaric acid, and metformin. Independent *t*-test was the basis of statistical analyses. ** and *** show significant differences between single and combination treatments at $p < 0.01$ and $p < 0.001$, respectively. Epirubicin + PM represents combination treatments with epirubicin, 0.2 mM *p*-coumaric acid, and 0.8 mM metformin.

Gastric cancer is a significant public health issue for which there are different therapeutic options, including chemotherapy. However, chemotherapy suffers from some limitations, such as severe side effects and drug resistance [6]. Here, treatments using natural anticancer compounds, established anticancer, non-chemotherapeutic drugs, or a combination of both groups can resolve the problems related to different cancers, like gastric cancer [8, 9]. In conclusion, the results of the present study showed that the two anticancer agents, *p*-coumaric acid and metformin, could augment the cytotoxic effects of carboplatin and epirubicin in a gastric cancer cell culture model. These findings may sugest the potential anticancer effects of *p*-coumaric acid and metformin. Besides, it could be concluded that *p*-coumaric acid and metformin could be candidates to solve the problem of anticancer drug resistance in gastric cancer models. The ability of these two agents to enhance the cytotoxic effects of conventional chemotherapeutic drugs such as carboplatin and epirubicin suggests that they may interfere with key mechanisms underlying drug resistance, including reduced drug uptake, increased drug efflux, activation of survival signaling pathways, and impaired apoptotic responses. By modulating these resistance pathways, *p*-coumaric acid and metformin could potentially restore

sensitivity in cancer cells that have become refractory to standard treatments. Moreover, their low toxicity profile relative to conventional chemotherapeutics makes them attractive adjuvants that could be combined with existing regimens without adding significant adverse effects. While further validation in resistant cell lines and animal models is necessary, the present findings provide a rationale for considering these natural compound-drug combinations as a strategy to overcome or delay the emergence of chemoresistance in gastric cancer therapy. Collectively, these findings suggest that p-coumaric acid and metformin warrant further investigation, including in vivo studies, as potential adjuvant agents for overcoming drug resistance in gastric cancer. Unfortunately, technical limitations made it impossible to conduct confirmatory analyses like flow cytometry. These analyses could shed more light on the desirable effects mentioned in the present study. After such complementary studies, these compounds may set the stage for introducing new ways to fight against drug resistance in gastric cancer models.

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Conflict of Interest: The authors have no conflicts of interest.

Authors' Contribution: YSH conducted the experiment and data analysis and wrote the original draft. EZ assisted in data analysis and writing the original draft. IS designed the research and supervised, writing and editing the manuscript.

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