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Original article

Aspirin plus sorafenib potentiates cisplatin cytotoxicity in resistant head and neck cancer cells through xCT inhibition

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ABSTRACT

The nonsteroidal anti-inflammatory drug aspirin and the multikinase inhibitor sorafenib have both shown experimental and clinical anticancer activities. The present study investigated whether aspirin and sorafenib synergize to potentiate cisplatin treatment in resistant head and neck cancer (HNC) cells. The effects of aspirin, sorafenib and cisplatin, and combinations thereof were assessed by measuring cell viability, death, glutathione (GSH) and reactive oxygen species (ROS) levels, protein and mRNA expression, genetic inhibition and overexpression of cystine–glutamate antiporter (xCT) and tumor xenograft mouse models. Even at low concentrations, aspirin plus sorafenib synergized to induce cell death of cisplatin-resistant HNC cells. The combination of aspirin and sorafenib induced xCT inhibition, GSH depletion, and ROS accumulation in cancer cells. Genetic and pharmacological inhibition of xCT potentiated the cytotoxic effects of aspirin plus sorafenib; this effect was diminished by xCT overexpression. Low-dose aspirin plus sorafenib enhanced the cytotoxicity of cisplatin in resistant HNC cells through xCT inhibition and oxidant and DNA damage. The *in vivo* effects of aspirin plus sorafenib on cisplatin therapy were also confirmed in resistant HNC xenograft models, in terms of growth inhibition, GSH depletion, and increased γH2AX formation and apoptosis in tumors. Aspirin and sorafenib synergize to potentiate the cytotoxicity of cisplatin in resistant HNC cells. This therapeutic strategy may help to eliminate resistant HNC.

1. Introduction

Head and neck cancer (HNC) is the eighth most common cancer worldwide, with more than half a million new cases diagnosed each year [1]. More than 90% of HNCs are squamous cell carcinoma, commonly arising in the upper aerodigestive tract, including the pharynx, larynx, and oral/nasal cavity. Organ-preserving chemoradiotherapy protocols with radiotherapy and systemic chemotherapy are increasingly used in the primary definitive treatment of HNC [2]. Cisplatin is used as a first-line agent in primary and postoperative chemoradiotherapy combined with other chemotherapeutic agents against HNC [3]. Cisplatin binds with high affinity to nuclear DNA, causing a DNA damage response and inducing apoptosis in cancer cells [4]. During the last few decades, overall survival rates of patients with HNC have not changed substantially, which may be the result of cancers developing resistance to therapies, including radiotherapy and chemotherapy including cisplatin [5].

Oncogene addition and inactivation of tumor suppressor genes that are frequently found in HNC result in cell deregulation because of increased cellular stress, contributing to resistance to cancer therapy [6–8]. Cancer cells commonly contain increased antioxidant defense mechanisms for coping with high levels of oxidative stress [9], which render cancer cells insensitive to chemoradiotherapy [10]. Reactive oxygen species (ROS) levels in cancer cells are characteristically higher than those in normal cells, rendering cancer cells more vulnerable to damage by further ROS insults and creating a potential therapeutic target in resistant cancer cells [11].

Acetylsalicylic acid (aspirin), a nonsteroidal anti-inflammatory drug (NSAID), has been used for relieving inflammation and pain [12]. Increasing evidence suggests that aspirin decreases cancer incidence, mortality, and the risk of cancer metastasis [13,14]. Furthermore, aspirin suppresses the growth and metastasis of cancer cells through the inhibition of nuclear factor (NF)-κB and myeloid cell leukemia 1 (Mcl-1) proteins [15,16]. Sorafenib is a multikinase inhibitor targeting the RAF–MEK–ERK pathway and receptor tyrosine kinases [17]. It is currently used in patients with kidney and liver cancers. The targets

Abbreviations: HNC, head and neck cancer; GSH, glutathione; ROS, reactive oxygen species; DCF-DA, 2′,7′-dichlorofluorescein diacetate; MTT, 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide; siRNA, short interfering RNA; xCT, cystine–glutamate antiporter; IC_{50}, half maximal inhibitory concentration; Mcl-1, myeloid cell leukemia 1; NSAID, nonsteroidal anti-inflammatory drug; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling

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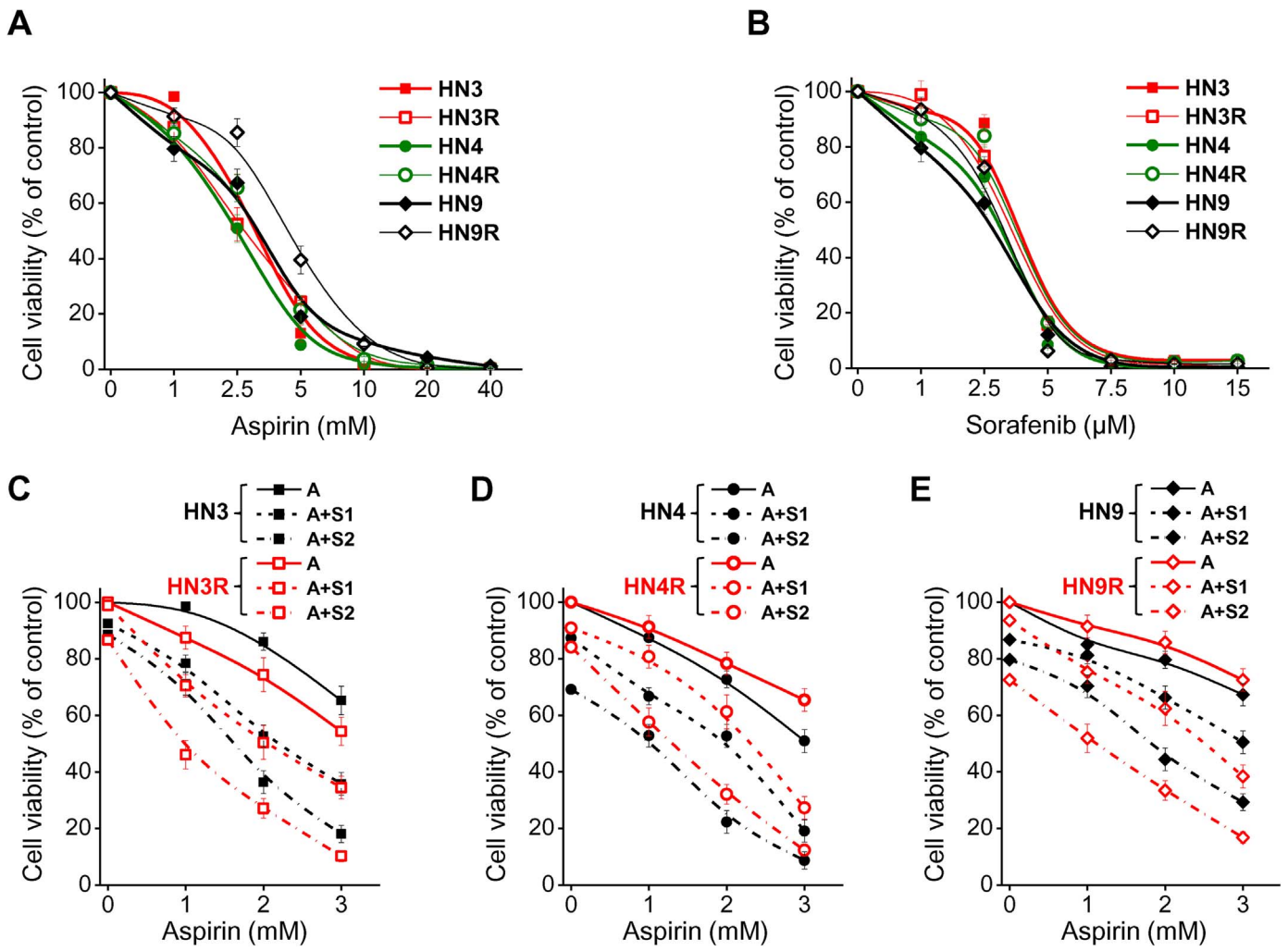

Fig. 1. Aspirin and sorafenib synergize to induce death of HNC cells. (***A***, ***B***) Cell viability of HNC cells 72 h after exposure to different concentrations of aspirin or sorafenib. (***C***, ***D***, ***E***) Cell viability after exposure to aspirin (A), sorafenib (1 μM–S1 or 2 μM–S2), or the combination of the two in cisplatin-sensitive (HN3, HN4, and HN9) or cisplatin-resistant cells (HN3R, HN4R, and HN9R). Combination indices (CIs) were < 1 in all the cell lines. The error bars represent the standard errors of the mean from three independent experiments, each performed in triplicate.

of sorafenib include the Mcl-1 and NF-κB pathways and its effects may overlap with those of other anticancer therapeutics [18,19].

The low-dose combination of aspirin and sorafenib sensitizes colon cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by targeting FLICE (caspase-8) inhibitory protein (FLIP) and Mcl-1 [20]. Combining the two drugs in low doses may sensitize cancer cells to other anticancer therapies without increased side effects, which has not been examined much in the context of targeting resistant cancers. Therefore, the present study investigated whether aspirin and sorafenib synergize to potentiate the effects of cisplatin in HNC cells. In the current study, low-dose aspirin plus sorafenib enhanced the cytotoxicity of cisplatin in resistant HNC cells through cystine–glutamate antiporter (xCT) inhibition and oxidant and DNA damage *in vitro* and in a tumor xenograft mouse model.

2. Results

2.1. Aspirin and sorafenib synergize to induce death of HNC cells

Aspirin and sorafenib alone each decreased the viability of cisplatin-sensitive and cisplatin-resistant HNC cells in a dose-dependent manner (Fig. 1**A** and **B**). Aspirin and sorafenib induced death in most cells at concentrations of < 10 mM and < 10 μM, respectively. The low dose combination of aspirin (1–3 mM) and sorafenib (1–3 μM) synergized to induce effective cell death in cisplatin-resistant HN3R, HN4R, and HN9R cells as well as their parental cisplatin-sensitive HN3, HN4, and HN9 cells (CIs < 1.0, Fig. 1**C–E**). The synergism of aspirin plus sorafenib was also confirmed in > 10 other different HNC cell lines.

2.2. The combination of aspirin and sorafenib induces xCT inhibition, GSH depletion, and ROS accumulation

Treatment with high concentrations of aspirin (5–10 mM) or sorafenib (5–10 μM) induced activation of cPARP and inhibition of p65, Mcl-1, and xCT proteins in a dose-dependent manner (Fig. 2**A**). High dose aspirin inhibited xCT gene expression and glutamate release relative to control, in cisplatin-resistant HN3R and HN4R cells (Fig. 2**B** and **C**). Aspirin (5 mM), sorafenib (5 μM), or the combination increased the cPARP level and decreased the p65, Mcl-1 and xCT proteins, 6 h later after treatment (Fig. 2**D**). Aspirin (5 mM) or sorafenib (5 μM) decreased the xCT mRNA level and glutamate release in the cisplatin-resistant HNC cells, of which the effects were larger by the treatment with sorafenib than aspirin ($P < 0.05$, Fig. 2**E** and **F**). The combination of aspirin and sorafenib greatly decreased xCT gene expression and glutamate release ($P < 0.01$).

Low concentrations of aspirin (1–2 mM) or sorafenib (1–2 μM) did not significantly inhibit cell growth or colony forming ability ($P > 0.2$), whereas the low dose combinations of the two drugs significantly