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Capsaicin synergizes with camptothecin to induce increased apoptosis in human small cell lung cancers via the calpain pathway

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ABSTRACT

Small cell lung cancer (SCLC) is characterized by excellent initial response to chemotherapy and radiation therapy with a majority of the patients showing tumor shrinkage and even remission. However, the challenge with SCLC therapy is that patients inevitably relapse and subsequently do not respond to the first line treatment. Recent clinical studies have investigated the possibility of camptothecin-based combination therapy as first line treatment for SCLC patients. Conventionally, camptothecin is used for recurrent SCLC and has poor survival outcomes. Therefore, drugs which can improve the therapeutic index of camptothecin should be valuable for SCLC therapy. Extensive evidence shows that nutritional compounds like capsaicin (the spicy compound of chili peppers) can improve the anti-cancer activity of chemotherapeutic drugs in both cell lines and animal models. Statistical analysis shows that capsaicin synergizes with camptothecin to enhance apoptosis of human SCLC cells. The synergistic activity of camptothecin and capsaicin is observed in both classical and variant SCLC cell lines and, *in vivo*, in human SCLC tumors xenotransplanted on chicken chorioallantoic membrane (CAM) models. The synergistic activity of capsaicin and camptothecin are mediated by elevation of intracellular calcium and the calpain pathway. Our data foster hope for novel nutrition based combination therapies in SCLC.

1. Introduction

Small cell lung cancer (SCLC) accounts for about 15–20% of all lung cancer cases and is the most aggressive type of lung cancers [1,2]. Cisplatin or carboplatin in combination with etoposide is the standard of care for SCLC patients. Although this regimen initially works very well in SCLC patients with a response rate of greater than 80%, the disease inevitably relapses within a year, at which point the tumor is non-responsive to cisplatin-based combination therapies [3]. Another drawback with the cisplatin-etoposide regimen is its toxicity, which may render SCLC patients more susceptible to adverse symptoms upon subsequent treatments [4]. Patients with recurrent SCLC have very limited options, as the only standard chemotherapy with an FDA-approved drug, camptothecin (Fig. 1a), has an objective response rate of approximately 3% and little or no survival benefit [5]. Clinical trials have explored the possibility of camptothecin-based combination regimens for standard of care therapy for SCLC patients [4]. Therefore, agents which can increase the therapeutic efficacy of camptothecin may improve the outcomes of SCLC therapy. Several convergent studies have shown that dietary compounds can sensitize neoplastic cells to the apoptotic effects of chemotherapeutic drugs [6,7]. Our published data show that capsaicin (the spicy compound of chili peppers; Fig. 1b) can induce robust apoptosis in human SCLC cells in cell culture and mouse models [8,9]. Therefore, we conjectured that low doses of capsaicin (where it does not cause cell death) may sensitize human SCLC cells to the apoptotic activity of camptothecin and its derivatives.

Abbreviations: SCLC, small cell lung cancer; CAM, chorioallantoic membrane; CI, combination index; STR, short tandem repeat; RPMI, Roswell Park Memorial Institute; EDTA, ethylene diamine tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SPF, specific pathogen-free.

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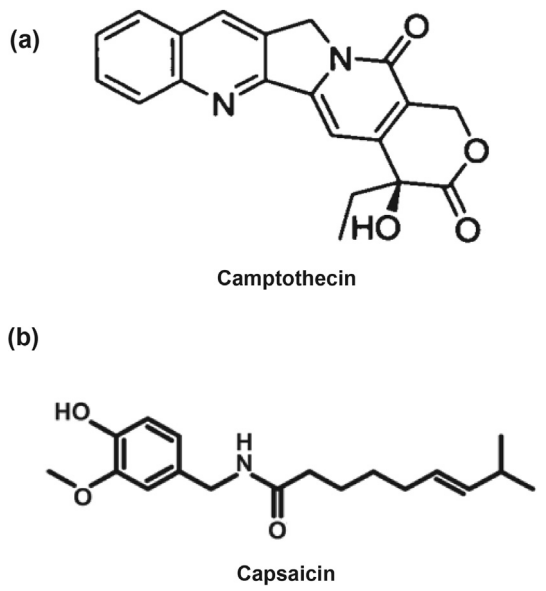

Fig. 1. Structure of camptothecin (a) and capsaicin (b).

A survey of literature shows that capsaicin increases the therapeutic index of several anti-cancer treatments. The administration of capsaicin increased the therapeutic efficacy of radiation in prostate cancer. Monofunctional platinum-based drugs, like LH5, showed increased apoptotic activity in combination with capsaicin [10]. Similarly, the treatment of stomach cancer cells with a combination of cisplatin and capsaicin caused greater apoptosis than either of these agents given singly [11,12]. A similar effect was also observed when capsaicin was given in combination with the doxorubicin analog pirarubicin [13]. The present manuscript investigates for the first time the anti-cancer activity of the combination of capsaicin and camptothecin. We show that low doses of capsaicin (where it does not cause any apoptosis) synergizes with camptothecin to induce high levels of cellular apoptosis in human SCLCs. We confirmed the synergistic apoptotic activity of capsaicin and camptothecin in classical human SCLC cell lines (NCI-H69 and DMS 114), as well as the variant human SCLC cell line NCI-H82. Another innovative feature about our study is that we have analyzed the synergistic interaction between these two drugs by the Chou-Talalay isobologram method [14,15].

The apoptotic activity of capsaicin-camptothecin combination was confirmed using two independent apoptosis assays. Subsequently, we show that the combination of capsaicin and camptothecin enhances apoptosis (compared to these agents given alone) *in vivo*, in human SCLC tumors xenotransplanted on chicken chorioallantoic membranes (CAM) [16]. We also examined the signaling pathways underlying the combinatorial synergistic apoptotic activity of capsaicin and camptothecin. We found that the synergistic apoptotic activity capsaicin and camptothecin was mediated by elevation of intracellular calcium and activation of the calpain pathway both in cell culture and in chicken CAM models. The results of our studies may lead to improved treatment regimens for SCLC.

2. Materials and methods

2.1. Reagents

Camptothecin, capsaicin, BAPTA-AM (1,2-*Bis*(2-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis(acetoxymethyl ester) and calpeptin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All cell culture reagents, including RPMI-1640, FBS, Trypsin-EDTA, and HEPES, were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Sodium pyruvate, glucose, and penicillin-streptomycin solutions were obtained from Corning (NY, USA).

2.2. Cell culture

The human SCLC cell lines NCI-H82, NCI-H69 (hereafter referred to as H82 and H69) and DMS 114 were purchased from ATCC (Manassas, VA). The ATCC used Short Tandem Repeat (STR) profiling for authentication of these cells. H69 and H82 were cultured in RPMI-1640 supplemented with 2 mM glutamine, 4.5 g/L glucose, 100 units/ml penicillin, 100 units/ml streptomycin and 10% fetal bovine serum (FBS). DMS 114 was cultured in RPMI-1640 containing with 2 mM glutamine, 25 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, 100 units/ml penicillin, 100 units/ml streptomycin and 10% FBS. All cell lines were maintained in a 37 °C humidified incubator with 5% carbon dioxide (NuAire Laboratory Equipment, Plymouth MN).

2.3. Preparation of lysates

Cell lysates were made using detergent-based lysis protocol as described previously [17]. Cells were harvested and washed three times with cold PBS. Cells were then lysed with M2 lysis buffer (20 mM Tris, pH 7.6, 0.5% IGEPAL-CA-630, 250 mM NaCl, 3 mM EGTA, 3 mM EDTA, 4 μM DTT, 5 mM PMSF, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 25 μg/ml leupeptin, 5 μg/ml pepstatin, 5 μg/ml aprotinin, and 25 μg/ml trypsin-chymotrypsin inhibitor) and the lysates were prepared as detailed elsewhere [17]. The protein concentration of the lysate was measured using Bradford Reagent (Bio-Rad Laboratories, Hercules, CA, USA).

2.4. Measurement of caspase-3 activity

DMS 114 human SCLC cells were cultured to 80% confluence as described above. On the day of the experiment, the medium of the cells was changed to RPMI medium containing 1% FBS. Subsequently, cells were treated with the indicated concentrations of the relevant drugs for 24 h at 37 °C. A few of the drug treatments involved treating the human DMS 114 cells with both camptothecin and capsaicin. In these cases, capsaicin was added 45 min before camptothecin and then the cells were incubated for 24 h at 37 °C.

Cell lysates were made using the Caspase-3 Activity Kit (EMD Millipore Corporation, Billerica, MA, USA). The protein concentration of the lysate was measured using Bradford Reagent (Bio-Rad Laboratories, Hercules, CA, USA). An aliquot of the cell lysate containing one hundred micrograms of protein was used for the measurement of caspase-3 activity, according to the manufacturer's protocol.

Each sample was measured in triplicate and the whole experiment was repeated three times using independent sets of cell lysates. Caspase-3 Activity in untreated lysates was considered to be equal to 1, and the activity observed in treated lysates was calculated as fold increase relative to the untreated control sample. The experimental procedure was identical in H69 and H82 cells.

2.5. Cell death ELISA assay

DMS 114 human SCLC cells were cultured to 80% confluence in T-75 tissue culture flasks (Nunc, Roskilde, Denmark). On the day of the experiment, the medium of the cells was changed to RPMI medium containing 1% FBS. The DMS 114 human SCLC cells were treated with the indicated concentration of the appropriate drug for 24 h at 37 °C. A few experiments involved treating the human

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