

Capsaicin-induced cell death in a human gastric adenocarcinoma cell line

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Abstract

AIM: Capsaicin, a pungent ingredient found in red pepper, has long been used in spices, food additives, and drugs. Cell death induced by the binding of capsaicin was examined in a human gastric adenocarcinoma cell line (AGS cells).

METHODS: By using XTT-based cytotoxicity assay, flow cytometry using the TUNEL method, and quantitation of DNA fragmentation, both cell death and DNA fragmentation were detected in AGS cells treated with capsaicin. By using Western blotting methods, capsaicin reduced the expression of Bcl-2, the antiapoptotic protein, in AGS cells in a concentration-dependent manner.

RESULTS: After incubation of AGS cells with capsaicin for 24 h, cell viability decreased significantly in a dose-dependent manner. After incubation of AGS cells with capsaicin for 24 h, apoptotic bodies also significantly increased, and were again correlated with the dose of capsaicin. When the concentration of capsaicin was 1 mmol/L, the amount of DNA fragments also increased. Similar results were also in the lower traces.

CONCLUSION: These results suggest that capsaicin-induced cell death might be via a Bcl-2 sensitive apoptotic pathway. Therefore, capsaicin might induce protection from gastric cancer.

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Key words: Capsaicin; Human gastric adenocarcinoma;

Apoptosis; Bcl-2

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INTRODUCTION

Carcinoma of the stomach is currently the leading cause of cancer-related death among the Chinese. The two most likely factors for such a high incidence of stomach cancer has been thought to be either *Helicobacter pylori*^[1] or diet. Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the main ingredient of hot chili peppers, has long been used in spices, food additives, and drugs^[2]. Capsaicin has been shown to protect the gastric mucosa of animals and humans against various kinds of damage^[3-6]. It is generally considered that capsaicin's effect results from the activation of sensory afferent neurons in the stomach, and is mediated by various physiological functions, such as mucosal blood flow^[7-10], mucus secretion^[11], and bicarbonate secretions^[12]. Vanilloid receptor subtype 1 (VR1), a receptor responsible for capsaicin action, has been cloned^[13] and demonstrated to be located in both neural and non-neural cells^[14]. VR1 has also been found to be expressed peripherally in gastric mucosal epithelial cells, playing a role in cell protection^[15]. Recently, a series of studies have demonstrated that capsaicin inhibits mutagenicity and DNA binding of some chemical carcinogens, possibly by suppressing their metabolic activation^[16-18]. With cells in culture, capsaicin-inhibited proliferation of HeLa, ovarian carcinoma, and mammary adenocarcinoma by decreasing NADH oxidase activity^[19]. Capsaicin can also alter the expression of tumor forming-related genes by mediating the overexpression of *p53* and/or *c-myc* genes in a Korean stomach cancer cell line^[20]. Capsaicin was found to induce apoptosis in T cells by increasing the reactive oxygen species and by a subsequent mitochondrial transmembrane potential^[21]. In this report, we have examined the underlying mechanism by which capsaicin induces apoptotic cell death in a human gastric adenocarcinoma cell line (AGS).

MATERIALS AND METHODS

Cell line

A human gastric adenocarcinoma cell line (AGS) was

obtained from American Type Culture Collection. The cells were maintained in RPMI 1640 medium (Life Technologies, Inc., Melbourne, Australia) supplemented with 10% heat-inactivated fetal bovine serum with penicillin (100 U/mL) and streptomycin (100 µg/mL). Cultures in 75- or 25-mL culture flasks were incubated at 37 °C in a humidified gas mixture containing 50 mL/L CO₂ balanced with air.

XTT-based cytotoxicity assay

Unlabeled AGS cells (1×10^4 /well) were distributed on to a 96-well flat-bottomed plate. Appropriate numbers of cells were added, resulting in triplicate wells, and a final volume of 100 µL of RPMI medium with 10% bovine serum was obtained. After incubation of AGS cells for 24 h, the medium was then replaced with serum-free RPMI medium containing capsaicin (0.05 µmol/L-10 mmol/L) at 37 °C for 24 h. The quantity of viable cell was determined using a cell proliferation ELISA kit from Boehringer Mannheim (No. 1465015), according to the recommendations of the supplier. Each sample was tested in triplicate.

Detection of apoptotic cells by flow cytometry using TUNEL method

For demonstration of apoptosis, TUNEL assay was performed with an *in situ* cell death detection kit (POD, Boehringer Mannheim) according to the manufacturer's recommendations. After incubation of AGS cells for 24 h, cells were analyzed by Epics Elite ESP flow cytometer. Apoptosis was analyzed using the Wincycle Software (Coulter). Adherent cells were harvested and stained in hypotonic fluorochrome solution (propidium iodide 50 µg/mL in sodium citrate plus 0.1% Triton X-100, Sigma). Apoptotic nuclei were identified as a subgenomic DNA peak and were distinguished from cell debris on the basis of both forward light scatter and fluorescence of propidium iodide.

Quantitation of DNA fragmentation

Apoptosis was also evaluated with a cell death ELISA kit (Boehringer Mannheim, Indianapolis, IN, USA) which utilizes a monoclonal antibody against histone to detect DNA fragments in the cytosolic fraction of lysed cells. Cells treated with or without capsaicin were harvested and lysed according to the manufacturer's instructions. The samples were transferred into 96-well dishes coated with a mouse monoclonal antibody against histone. After incubation and washing, anti-DNA-peroxidase was added to the wells. The reaction was developed with the substrate supplied by the manufacturer and the absorbance of the wells was read at 410 nm. The ratio of the absorbance of the treated cells to the untreated cells was calculated as an enrichment factor, which provides a qualitative assessment of apoptosis. Each sample was tested in triplicate.

Western blotting

After incubation of AGS cells for 24 h, about 10^7 cells were washed in PBS and solubilized buffer (50 mmol/L Tris-HCl pH 7.4, 125 mmol/L NaCl, 0.1% NP-40, 5 mmol/L NaF, 1 mmol/L PMSF, 1 ng/mL leupeptin, 10 ng/mL soybean trypsin inhibitor, 1 ng/mL aprotinin, 10 ng/mL N-tosyl-L-phenylalanyl chloromethyl ketone) for 60 min on

ice. Lysates were centrifuged at 2 500 *g* for 5 min. Protein concentration was determined by means of the Bradford protein assay (BioRad Lab., Richmond, CA, USA) using bovine serum albumin as the standard. Thirty micrograms of protein was resolved by electrophoresis on 10% polyacrylamide gels, electrotransferred to a polyvinylidene difluoride filter (Millipore, Bedford, MA, USA), and then blotted with mouse monoclonal antibody for Bcl-2 (1:500 dilution). Blots were developed with peroxidase-labeled anti-mouse IgG (1:400 dilution) using a Lumi-Light Plus Western Blotting Kit (Boehringer Mannheim).

Statistical analysis

All values in the text and figures are expressed as mean ± SE. Statistical differences were evaluated by Student's *t*-test in unpaired samples, by paired *t*-test in paired samples, or by Wilcoxon's sum of ranks test. When evaluating multiple values, the Bonferroni correction was used. Probability values less than 0.05 were considered to be significant in all experiments. Analysis of the data and plotting of the figures were done with the aid of software (SigmaStat and SigmaPlot, Version 5.0, Jandel, USA; PHARM/PCS, Version 4.2, MCS, USA) run on an IBM PC-AT computer. Protein blot images were captured by an Imaging Densitometer with the aid of software (Bio-ID, V.97 software for Windows 95, Vilber Laurmat, France). Comparisons were made only between averaged values of bands within the same gel.

RESULTS

Based on the XTT assay, DNA fragmentation and flow cytometric analysis, induction of apoptosis by capsaicin was reconfirmed in stomach tumor cell line AGS. In this study, after incubation of AGS cells with capsaicin for 24 h, cell viability decreased significantly in a dose-dependent manner (Figure 1A). This reduction in cell viability induced by treatment with capsaicin was ascribed to apoptotic DNA fragmentation, and the amounts of fragmented DNA were also dependent on the dose of capsaicin (Figure 1B). After incubation of AGS cells with capsaicin for 24 h, apoptotic bodies also significantly increased, and were again correlated with the dose of capsaicin. The upper traces of Figure 2 show capsaicin-induced, concentration-dependent positive TUNEL staining. When the concentration of capsaicin was 1 mmol/L, the amount of DNA fragments also increased. Similar results were also in the lower traces of Figure 2, where apoptotic bodies increased due to the application of capsaicin. Figure 3 shows that the expression of Bcl-2, the antiapoptotic protein, was significantly reduced in AGS cells by capsaicin in a concentration-dependent manner.

DISCUSSION

Apoptosis, or programmed cell death, is a natural form of cell death controlled by a constitutively expressed machinery that induces condensation of the nucleoplasm and cytoplasm, blebbing of cytoplasmic membranes, and fragmentation of the cell into apoptotic bodies that are rapidly recognized and eliminated by adjacent cells^[22-24]. Induction of apoptosis by the vanilloid compound capsaicin has been reported in several studies. Vanilloid compounds

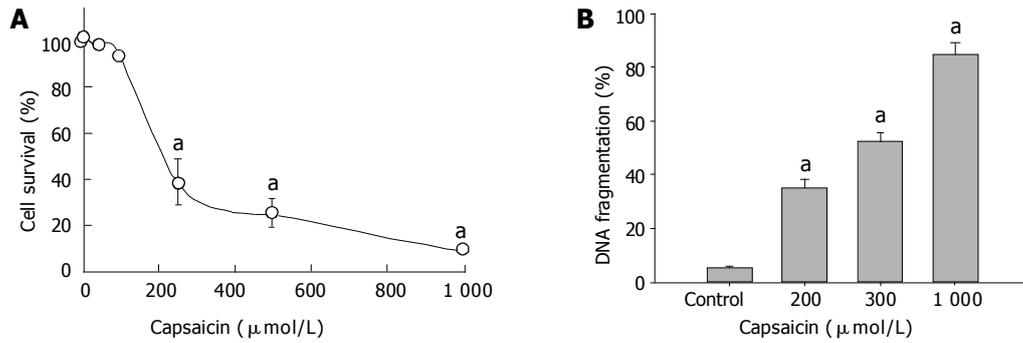


Figure 1 A: Effect of capsaicin on cell survival in the cultured gastric cancer cell line, AGS. Survival was analyzed by a cell proliferation ELISA kit from Boehringer Mannheim. The results are the mean \pm SE of three experiments. ^a P <0.05 vs solvent control; **B:** Effect of capsaicin on the amount of DNA fragmentation in the cultured gastric cancer cell line, AGS. The amount of fragmented DNA produced was determined with an ELISA kit from Boehringer Mannheim. The results are the mean \pm SEM of three experiments. ^a P <0.05 vs solvent control.

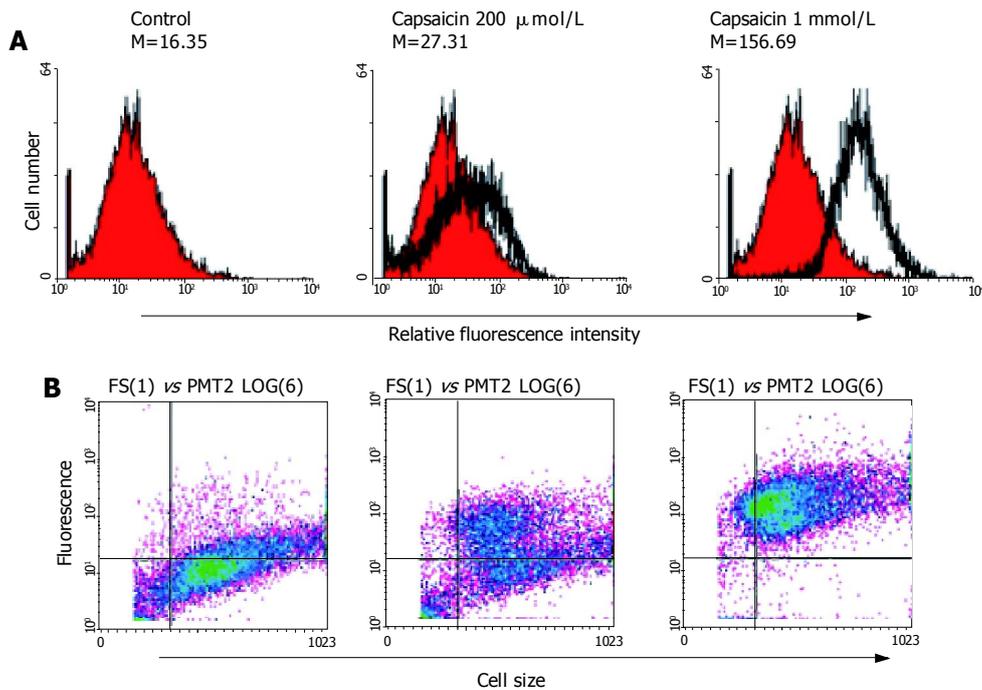


Figure 2 Flow cytometry analysis for capsaicin-induced apoptosis in AGS gastric carcinoma cells (A,B).

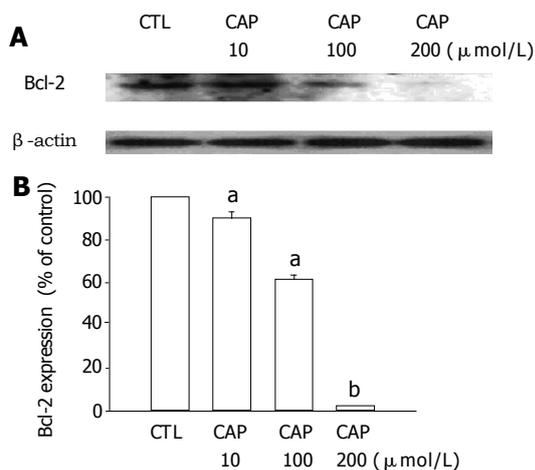


Figure 3 Effect of capsaicin (CAP) on the expression of Bcl-2 in AGS cells. Each bar represents means \pm SE of three experiments(A,B). ^a P <0.05, ^b P <0.01 vs solvent control (CTL).

are quinone analogs that inhibit the NADH-plasma membrane electron transport system and induce apoptosis in transformed cells^[21]. Capsaicin (3.5-10 $\mu\text{mol/L}$) induces apoptotic cell death in an *in vitro* Korean stomach tumor cell (SNU-1), which may possibly be mediated by overexpression of *p53* and/or *c-myc* genes, but not by overexpression of those of *c-erbB-2*, *c-jun* and *bcl-2* genes^[20], based on XTT assay, DNA fragmentation and flow cytometric analysis. In our study, induction of apoptosis by capsaicin was reconfirmed in another stomach tumor cell line, AGS. In this study, cell viability significantly decreased in a dose-dependent manner after incubation of AGS cells with capsaicin for 24 h. This reduction in cell viability induced by treatment with capsaicin was ascribed to apoptotic DNA fragmentation, and the amounts of fragmented DNA were also dependent on the dose of capsaicin. After incubation of AGS cells with capsaicin for 24 h, apoptotic bodies increased significantly in a dose-dependent manner.

We examined the role of Bcl-2 in capsaicin-induced cell death. In this study, the expression of Bcl-2, the antiapoptotic protein, was significantly reduced by capsaicin. Savill J *et al.*^[25] were the first to report that *bcl-2* can prolong cell survival. Recently, the *bcl-2* gene has emerged as a critical regulator of programmed cell death in a variety of physiological and pathological contexts^[26]. Bcl-2 has also been reported to regulate transmembrane calcium fluxes^[27,28]. It is of interest to note that when AGS cells were exposed to higher doses (10-200 $\mu\text{mol/L}$) of capsaicin, the expression of Bcl-2 protein was reduced, which differs from findings reported by Kim *et al.*^[20], and suggests that vanilloid compound-induced cell death might be via a Bcl-2 sensitive apoptotic pathway in the AGS cells.

In conclusion, capsaicin reduced the expression of Bcl-2 in a concentration-dependent manner in AGS cells, which suggests that Bcl-2 may play an important role in capsaicin-induced apoptosis.

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