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Magainin, isolated from Xenopus laevis, induced Apoptosis in Human Cervical Carcinoma Cells

Mukesh Bansal*, Santosh Gupta, Umesh S. Pal and Abhishek Pal

Pacific College of Pharmacy, Udaipur, Rajasthan, India.

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Abstract

Magainins are a family of potent antimicrobial cationic peptides that possess antimicrobial activity, anti-tumour activity, although the anticancer mechanism is still unclear. We investigated the effects of Magainins on apoptosis induction in human cervical carcinoma (HeLa) cells. Lapachone, an anticancer drug, was used as a positive control. The results demonstrated that Magainins inhibited proliferation of HeLa cells in a dose-dependent manner and had greater efficacy than that of lapachone. IC_{50} values of the test compound ranged from 1.2±0.1 to 5.5±0.86 µM for 2 to 24 h time periods.

Keywords: *Magainin, Human Cervical Carcinoma Cells, Apoptosis*

INTRODUCTION

Magainins are potent antimicrobial cationic peptides originally isolated from the skin of African clawed frog, Xenopus laevis. They possess a broad range of antimicrobial activity against bacteria, fungi, and protozoa[1-4]. Magainins have also been reported to be cytotoxic for tumor cells [5] but are non-haemolytic and noncytotoxic against nontumor cells. Hence, there is considerable interest in developing magainins or their analogues as therapeutic agents.

Cervical cancer is one of the most commonly diagnosed cancers in woman worldwide and is the first leading cause of cancer-related deaths in Thai women [6]. The major cause of mortality associated with this disease is the metastasis of cancer cells that fails to respond to chemotherapeutic drugs [7]. Novel preventive and therapeutic strategies are therefore urgently needed to decrease the mortality associated with this malignancy. Natural products have been found to be a potential source of novel anticancer drugs over the decades and have much contributed to cancer chemotherapy such as etoposide, camptothecin, paclitaxel, etc. [8]. In addition, these chemotherapeutic agents have been reported to exert their antitumor effects by inducing apoptosis [9]. Thus, chemical agents with strong apoptosis-inducing activity but minimal toxicity can be expected to have a potential utility as anticancer drugs. Till date, the potential anticancer activity of this drug has not been elucidated. In the present study, effects of Magainins on induction of apoptosis in human cervical carcinoma (HeLa) cells were investigated *via* mitochondria dependent pathway. Magainins could be a candidate for apoptosis-inducing cancer chemotherapeutic agents in human cervical carcinoma.

MATERIALS AND METHODS

Preparation of the drugs: The Magainins used for this study were isolated from Xenopus laevis. Lapachone purchased from Sigma-Aldrich Chemical Co. and dissolved in DMSO as a stock solution at 10 µM concentration and stored at 20°C until used.

Cell culture: Human cervical carcinoma (HeLa) cells were cultured in modified Eagle's medium (MEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml of penicillin and 100 mg/ml of streptomycin sulfate. The cells were maintained at 80% subconfluence in a humidified atmosphere of 5% CO_2 at 37°C.

Cell Proliferation Assay : The effect of magainins on the cell viability/proliferation was determined by MTT colorimetric assay[10] in this method HeLa cells were seeded into a 96-well culture plate at a density of 3×10^3 cells/well in 200µl of MEM containing 10% FBS. After 24h incubation, the cells were treated with various concentrations of Magainins (0-30µM) for the selected times (2, 4, 8, 12, 24, and 48 h). Lapachone (an anti-cancer drug) and 0.1% DMSO were used as positive and negative controls respectively. At the end of incubation, 20µl MTT solutions (5 mg/ml in PBS) was added to each well and further incubated at 37 °C for 3 h. After centrifugation at 1400 rpm for 5 min at 4 °C, the medium was aspirated and the formazan product in each well was solubilized with 100ml DMSO. Each

*Corresponding author; E-mail: bansalpharma1987@gmail.com

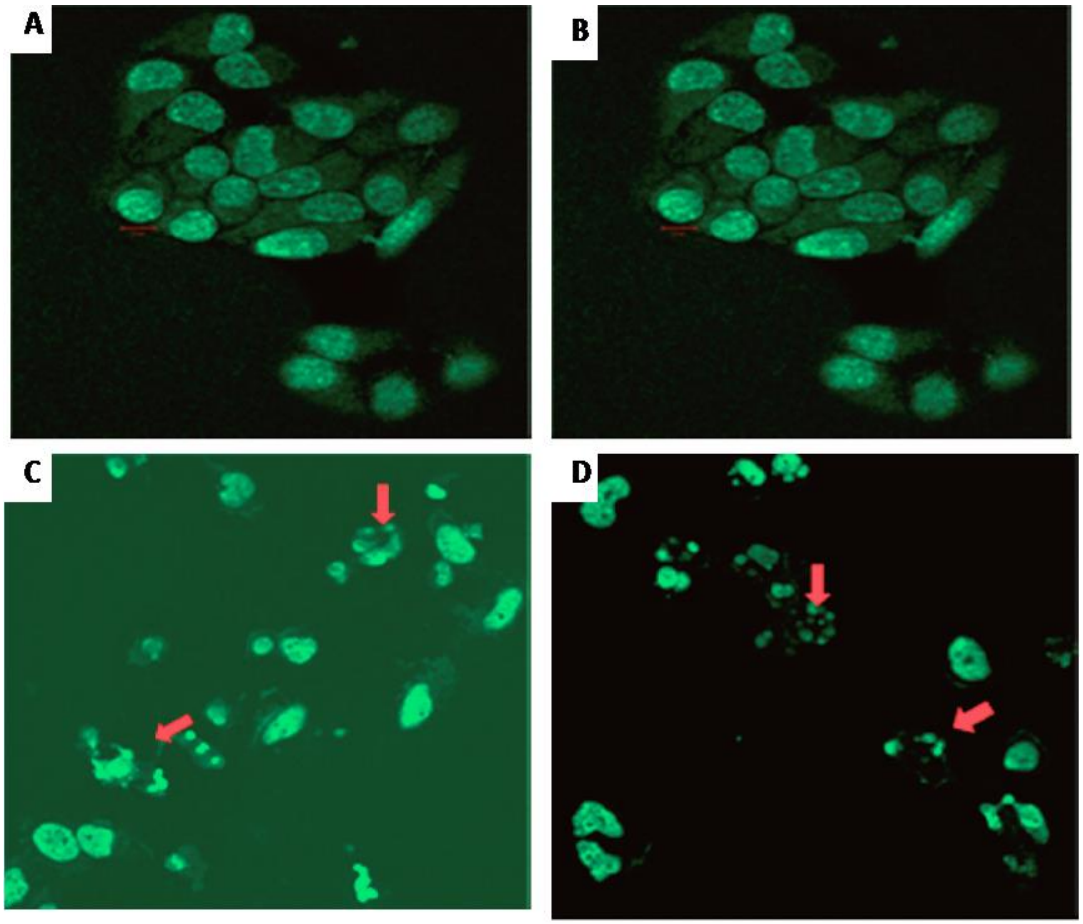

Figure 1. Treatment groups; A. Control, B. Magainin 3µM, C. Magainin 6µM and D. Magainin 10µM

concentration of drugs was repeated in six wells for three independent experiments. IC50 value was determined by plotting of percentage of cell viability *versus* drug concentrations.

Morphological examination: The apoptotic effect of Magainins on HeLa cells was analyzed by a terminal deoxynucleotidyl transferase (TdT) mediated deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) assay. The test is based on the ability of TdT to catalyze polymerization of nucleotides to free 3-OH DNA end generated during apoptosis. Briefly, HeLa cells (1×10^4 cells/well) were grown on 8-well chamber slide and treated with 0.1% DMSO or Magainins, lapachone, adriamycin (3, 6, and 10µM) for 4 and 8 h cells were fixed with 4% paraformaldehyde for 10 min and then washed three times with PBS. Nuclear DNA was denatured with a cooled ethanol/acetic acid (2 : 1) at 20°C for 5 min. Cells were rehydrated with PBS and incubated in TdT buffer at 37°C for 1 h. at the end of incubation, the reaction was blocked by the adding of stop/wash buffer and incubated with anti-digoxigenin fluorescein in dark humidified chamber at room temperature for 30 min. After washing with PBS, the cells were counterstained with 10m g/ml of DAPI and incubated at room temperature for 10 min. Finally, the slides were mounted with Perma fluor aqueous mounting medium under a glass cover slip and were then observed under a Carl Zeiss LSM 510 confocal laser scanning microscope (Thornwood, NY, U.S.A.). Fluorescein was excited by using an argon laser at 458 nm. Apoptotic cells were identified as cells with condensed and fragmented nuclei [10, 11].

RESULTS

Effect of Magainin on the Growth of HeLa Cells: The effect of Magainins on the proliferation of human cervical carcinoma (HeLa) cells was first examined. Cell survival was assessed using the MTT colorimetric assay.

As shown in Figure 1A, treatment of HeLa cells with Magainins resulted in a marked suppression of cell proliferation in a dose-dependent manner. The IC50 values of Magainins were 5.5±0.86, 4.5±0.38, 2.5± 0.37, 2.0±0.72, and 2.0±0.36 and 1.2±0.1 μM for 2, 4, 8, 12, 24 and 48 h, respectively. Magainins had greater efficacy than that of lapachone in the same time points (IC50 values of 7.0±0.44, 5.8±0.18, 5.5±0.94, 3.5±0.27, 3.0±0.27 and 2.5±0.13m M, as shown in Figure. 1B).

Induction of Apoptosis by Magainins in HeLa Cells: In order to examine whether the inhibitory effect of Magainins on the growth of HeLa cells is due to apoptosis, we confirmed the apoptotic characterizations in HeLa cells by several approaches, *e.g.,* morphological changes, DNA fragmentation, cell cycle progression, TUNEL staining assay detection, agarose gel electrophoresis, and FACScan flow cytometry. In the morphological change analysis, the cells treated with 3, 6, and 10μM Magainins for 4 and 8 h were examined by using a confocal laser scanning microscope after staining the cells with *in situ* TUNEL assay. As shown in Figure 1, marked morphological changes were observed such as becoming round in shape, membrane blebbing and apoptotic bodies; these were the characteristics of apoptotic cells.

DISCUSSION

Magainin-like analogues have been detected in human submandibular and labial salivary glands [12] and may function as innate antimicrobial agents to maintain oral health. Recent studies have demonstrated that protegrins, antimicrobial peptides derived from porcine leukocytes, possess bactericidal activity against oral anaerobic pathogens [13]. Defensins, antimicrobial peptides of human neutrophils, have exhibited a bactericidal effect on Capnocytophaga sp. but have no effect on A. actinomycetemcomitans[14]. Magainins have also been reported to be cytotoxic for tumor cells. In summary, our studies demonstrate for the first time that magainins induce apoptotic cell death by mitochondrial dependent pathway.

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