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THE EVALUATION OF BIOLOGICAL EFFECT OF CYTOTOXIC PEPTIDES ON TUMOR CELL LINES

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This study evaluated the cytotoxic effect produced by Magainin II, Cecropin A and Cecropin B on four tumor cell lines. The cytotoxic effect of the Magainin II, Cecropin A and Cecropin B by the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] viability test was evaluated. Peptides used did not show the same cytotoxic effect on all cell lines tested; which means that the cytotoxic effect of tested peptides depends on the tumor cell used in this experiment. Cytotoxic effect was more pronounced with increasing concentration of peptide. The antimicrobial peptides used in our experiment had variable potential on A549 human alveolar carcinoma cells.

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Keywords: MTT, culture cell lines, cytotoxic peptides, citostasis

1. Introduction

Although there have been many advances in cancer treatment, cancer remains a major source of morbidity and mortality throughout the world. Cancer is a class of diseases characterized by uncontrolled division of a group of cells with the ability to invade other tissues in the body, either by direct growth into adjacent tissue (invasion) or cell migration to distant places in the body (metastasis). Although localized cancers can often be successfully treated by surgery and/or radiation therapy, chemotherapy remains the usual treatment for advanced or metastatic disease [1].

A growing number of studies have shown that some of the cationic peptides (AMPs), exhibit a broad spectrum of cytotoxic activity against cancer cells. A significant number of these peptides have been shown to have anticancer and antiviral activities [1]. Cytotoxic peptides are relatively small cationic molecules consisting of amino acids, extracted from natural sources [2]. Cecropins were first isolated from the hemolymph of the giant silk moth *Hyalophora cecropia* [3]. Magainins are positively charged peptides that were originally isolated from frog skin (*Xenopus laevis*) [4].

The evaluation of cytotoxic effect was achieved by the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] viability test.

In the present study we evaluated of cytotoxic effect produced by Magainin, Cecropin A and B on four tumor cell lines.

It was used a negative control (cells without peptide) to make a comparison beween cells treated and untreated peptide, and a positive control (Sindroxocin) used in chemotherapy. This drug has a known cytotoxic potential.

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2. Methods

2.1. Peptides, cell lines and cell culture

Lyophilized Magainin II, Cecropin A and Cecropin B were purchased from Sigma. In this study we used four adherent cell lines: MDA-MB231 (Disease: mammary adenocarcinoma; Morphology: epithelial), HT-29 (Disease: colorectal adenocarcinoma; Morphology: epithelial), A549 (Disease: human alveolar carcinoma; Morphology: epithelial) and M14K (Disease: human mezoteliom; Morphology: mesothelial). Cells were cultured under standard conditions.

Four cell lines were cultured in RPMI 1640 medium (Sigma Aldrich), supplemented with 10% fetal bovine serum (FBS, GIBCO), 1% antibiotic antimycotic solution (Sigma) at 37 ^{0}C in a humidified atmosphere of 5% CO_2. The culture medium was changed every 2nd day and the cells at 100% confluence were passaged at a 1:3 split ratio [5-7].

For cytotoxicity and proliferation assays, cells were seeded in 96-well microtiter plates in a total volume of 200 µl. After adding the cytotoxic peptid, cells were incubated for 48 hours.

2.2. Cell cytotoxicity assay

In order to evaluate the cytotoxicity and cell proliferation using MTT assay. The MTT assay is a colorimetric test for measuring the activity of enzymes that reduce MTT to formazan, giving a purple color. MTT test is used for studies of viability, cytotoxicity and cell proliferation [8]. For dissolve the formazan crystals formed was used as solvent dimethyl sulfoxide (DMSO). The absorbance of this colored solution was determined with a TECAN SUNRISE™ microplate reader for spectrophotometric at a test wavelength of 570 nm and a reference wavelength of 620 nm.

We used 96-well culture plate with flat bottom and 10^4 cells in a volume of 200 µl/well. We used 20 µM, 40 µM and 80 µM concentrations for Magainin and 10 µM, 20 µM and 50 µM for Cecropin A and Cecopin B. Sindroxocin was used in concentrations of 25 nM and 200 nM. It has a high toxicity compared with the peptides used.

3. Results

For each tumor cell line the experiments was made in triplicate. Cells were incubated for 48 hours with various concentrations of Magainin II, Cecropin A and Cecropin B. Absorbance of the colored formazan was determined with a TECAN SUNRISE™ microplate reader for spectrophotometric (readings were made at 570/620 nm wavelength).

In this study was calculated the percentage of citostasis (stop proliferation and development cell of ascending to cell death).

Percentage of citostasis was calculated as:

$$(1\text{-}A) \times 100 = \%\ \text{citostasis}$$

where:

A = is the reporting of cells absorbance treated with peptide and absorbance of untreated cells.

Peptides used did not show the same cytotoxic effect on all cell lines tested; which means that the cytotoxic effect of tested peptides depends on the tumor cell used in the experiment.

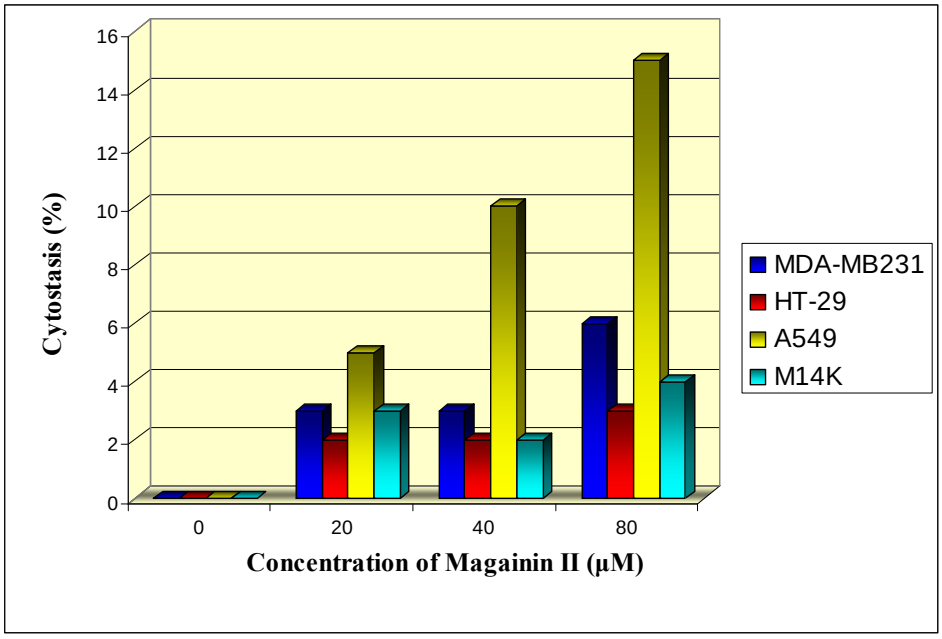

Fig. 1. Cytotoxic effect on cell lines MDA-MB231, HT-29, A549 and M14K at 48h of incubation cells with 20 µM, 40 µM and 80 µM Magainin II concentrations. Control cells were cultured without Magainin II. The absorbance of the control cells represented 100% cell proliferation.

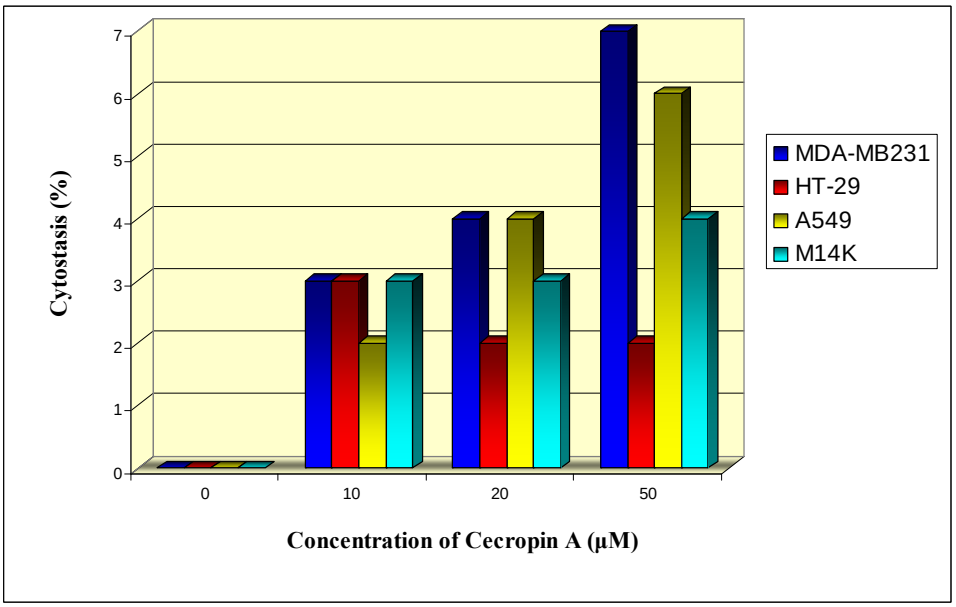

Fig. 2. Cytotoxic effect on cell lines MDA-MB231, HT-29, A549 and M14K at 48h of incubation cells with 10 µM, 20 µM and 50 µM Cecropin A concentrations. Control cells were cultured without Cecropin A. The absorbance of the control cells represented 100% cell proliferation.

From the analysis we found that after 48 h of incubation tumor cells with Magainin II we obtained a different cytotoxic effect for cell lines studied. After 48 h of incubation of tumor cells with magainin II was observed an increase in the percentage of citostasis of the increase of Magainin II concentration. Magainin II showed cytotoxic effect on A549 and MDA-MB231 cell lines for all concentrations taken it, inhibiting development and cell proliferation. The most relevant effect is for cell line A549 (15%) (Fig. 1.).

For MDA-MB231 cells incubation at 48h with Cecropin A was observed in figure 2 that this peptide had a cytotoxic effect, a much higher percentage of citostasis (7%) for the biggest concentrations of peptide who were added to the culture cell lines. For the second cell line (A549) evolution to cell death was evident of increasing peptide concentration, the absorbance values was smaller compared with the absorbance values for negative control (culture cell line without peptide).

Compared with results for Cecropin A, it was found that Magainin II was more pronounced cytotoxic effect on alveolar carcinoma cells (A549) to concentrations of 50µM (citostasis 15%). At 20 µM Cecropin A concentration was found that only a small number of tumor cells tend to cell death, it was observed from similar absorbances values with and without peptide.

After 48 hours of incubation of M14K cells with Cecropin A, was observed an insignificat effects of the citostasis percentage.

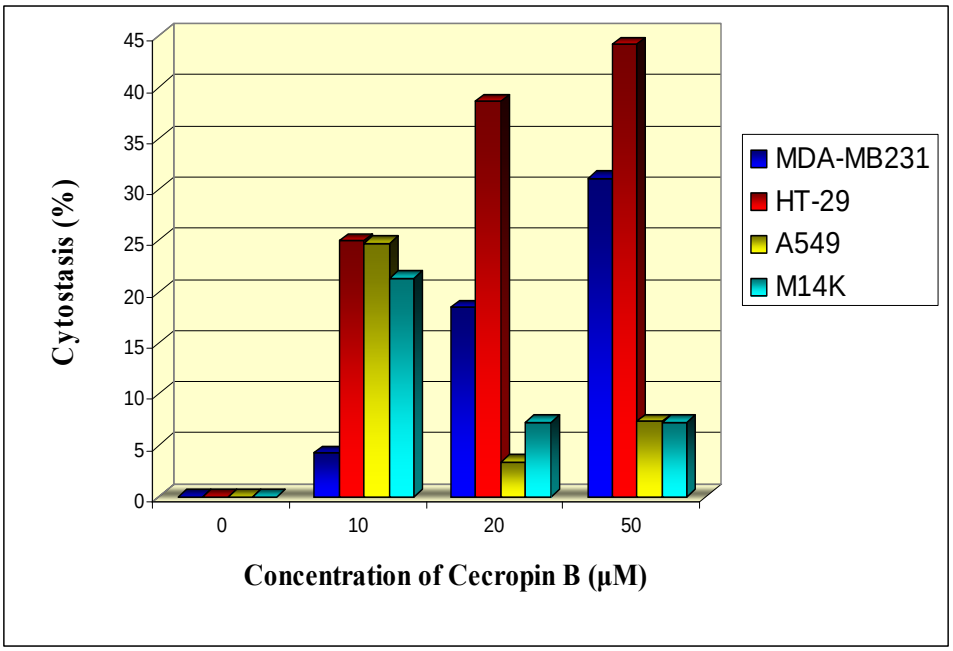

Fig. 3. Cytotoxic effect on cell lines MDA-MB231, HT-29, A549 and M14K at 48h of incubation cells with 10 µM, 20 µM and 50 µM Cecropin B concentrations. Control cells were cultured without Cecropin B. The absorbance of the control cells represented 100% cell proliferation.

The A549 cells incubated with Cecropin B of different concentrations was noted the highest percentage of citostasis (10%) for 50 µM concentration, therefore cells stop growing and proliferation. Cecropin B presented a cytotoxic effect only for A549 cell line (Fig. 3).

For cell line A549 was obtained a relevant percentage of citostasis (24.7 %) only for 10 µM Cecropin B peptide concentration. Citostasis percentage increases with increasing of peptide concentration in M14K cell line case (Fig. 3.).

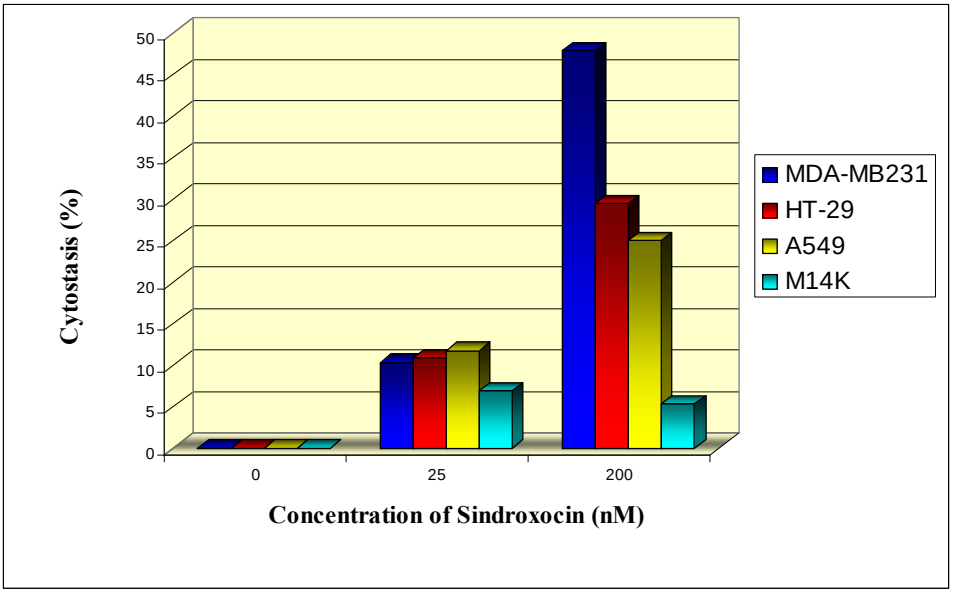

Fig. 4. Cytotoxic effect on cell lines MDA-MB231, HT-29, A549 and M14K at 48h of incubation cells with Sindroxocin (positive control) -25 nM and 200 nM. Control cells were cultured without Sindroxocin. The absorbance of the control cells represented 100% cell proliferation.

Sindroxocin is a citostatic used frequently in cancer chemotherapy. It is known that this drug has a very intense cytotoxic effect on tumor cells. Compared with the peptides used in this study, Sindroxocin had a very intense cytotoxic effect a thousand times smaller concentrations, so it could be used as a positive control in our experiment. Citostasis induced of Sindroxocin was more intense (10.28 % at a concentration of 25 nM and respectively 47.89 % for a concentration of 200 nM in the cellular medium) compared with the results obtained for cytotoxic peptides used in this experiment.

Sindroxocin had a similar effect for HT-29 and MDA-MB cells, but different for A549 cells. With increasing concentration of Sindroxocin increase the percentage of citostasis, a greater number of cells is going to cell death. These results lead us to conclusion that Sindroxocin inhibits cell proliferation in different mode depending on the type of cancer. For M14K cell line not observed a significant cytotoxic effect of Sindroxocin. A general conclusion about the action a Sindroxocin on various cell types is that action differently depending on the type of tumor cell.

4. Discussion

Peptides may be considered as a new family of anticancer drugs, and therefore, the current study of their biological activity and mechanisms of action acquires a crucial importance [9].

Cecropin A synthesized of many insects is one of the most extensively studied peptide [10]. Besides the well-known antimicrobial properties, recent studies have demonstrated the antitumor specific activity for both cecropins (Cecropin A and Cecropin B) against leukemia, lymphoma and colon carcinoma cell lines [11, 12] as pulmonar cancer and gastric cancer cells [13].

After 48 h of incubation of tumor cells with magainin II, an increase in the percentage of citostasis of the increase of Magainin II concentration was observed for A549 and MDA-MB231 cell lines.

For Cecropin A was obtained a cytotoxic effect with citostasis (6-10 %) at MDA-MB231 and A549 cell lines. Cecropin B had a cytotoxic effect only for A549 cell line. A

more relevant cytotoxic effect was obtained for A549 cell line because absorbance value at this concentration is smaller than the negative control (no added peptide).

Our study confirms the results obtained from the literature according to which the cytotoxic effect is more pronounced with increasing concentration of peptide [3, 14, 15]. Recent studies have reported an antitumor effect of Magainin II against various tumor cell lines [16].

5. Conclusion

We concluded that in this study, Magainin II had a more intense cytotoxic effect compared with Cecropin A and B against tumor cell lines studied. From this study we can say that the three types of peptide used had a similar cytotoxic effect on A549 cell line.

Acknowledgements

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