Title page

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Authors names and the Affiliations and Addresses

Author/s: Family A[1], Family B[1], Family C[2]

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1. Cancer Research Center,Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Surgery,Shohada Hospital,Shahid Beheshti University of Medical Sciences,Tehran,Iran (Font: Tw Cen MT,Size:12,Left)

Corresponding Author's Contact Informations

Corresponding author: Name Family MD/PhD/MSc

Degree

Tel:(#98)2122744090

Email: info@ijcp.ir

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Abstract page

Original Articles should have a structured abstract (Background, Methods, Results, Conclusions), Should be equal or less than 250 words in length

Abstract

Background: Chemotherapy by using agents such as etoposide is a common way for inhibition of tumors. This treatment is accompanied by many undesirable side effects. Calprotectin is an abundant protein in the neutrophil cytosol, it has growth-inhibitory and apoptosis-inducing activities against various cell types such as tumor cells. In this study to introduce calprotectin as a suitable substitute anticancer, its growth inhibitory effect on human gastric adenocarcinoma cell line (AGS) and human foreskin fibroblast cells (HFFF) is compared to etoposide effect on these two cell lines.

Methods: Calprotectin was purified from human neutrophil by chromatography methods. AGS and HFFF cell lines were used. These cells were maintained in RPMI 1640 medium supplemented with 10% FCS in a humidified incubator (37 °C & 5% CO2). AGS cells (10000 cells per well) were exposed to the different concentrations of calprotectin and etoposide for 24 and 48 h. MTT assay was used for evaluation of cytotoxicity.

Results: Results indicate that calprotectin has more potent anticancer activity in comparison to the etoposide but it has nearly similar inhibitory effect on the proliferation of fibroblast cells.

Conclusion: Since calprotectin affect about 20 times more than etoposide on cancer cells, it can be concluded that it is a suitable candidate to be studied as anti cancer drug.

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3 to 5 Keywords

Key words: Keyword 1, Keyword 2, Keyword 3, Keyword 4, Keyword 5

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Introduction (Font: Tw Cen MT, Size: 12, Left)

Calprotectin is a heterodimeric protein complex with zinc and calcium binding capacity that predominantly found in cytosolic component of neutrophils [1-4]. Calprotectin exhibits growth inhibitory and apoptosis inducing activity against some normal and a broad spectrum of tumor cells with different origins i.e., MM46 mouse mammary carcinoma, MH-134 mouse hepatoma, EL-4 mouse thymoma, L-929 mouse fibrosarcoma, B16 mouse melanoma, J774.1 mouse macrophage-like cells, Ros17/2.8, rat osteosarcoma, MCF-7 human mammary adenocarcinoma and MOLT-4 human leukemia cells [1,2,5,6]. Although several reports suggest that cell death inducing activity of calprotectin is due to exclusion of zinc from target cells [7,8], and may obey single target-single hit theory via binding to its receptor [9]. The recent study indicated that the final target of calprotectin may be DNA [10]. By the way, previous studies demonstrate that calprotectin inhibits the activity of casein kinase II, which is involved in the phosphorylation of several enzymes including topoisomerase I and II [11, 12].

The citation in the text should be identified by numbers in square brackets and before .

Materials and Methods (Font: Tw Cen MT, Size: 12, Left)

Dithiothreitol (DTT) and lymphoprep were obtained from Merck and Amersham Company respectively. Fetal calf serum (FCS) was obtained from Gibco and Seromed-Germany at 2005. RPMI 1640 medium, penicillin, streptomycin, trypan blue (TB) and MTT (dimythylthiazol diphenyl tetrazolium bromide) all were purchased from Sigma Chemical Co and were of analytical grade. Flask, tubes and culture plates were obtained from Griner- Germany. Other chemicals used in this study were purchased from Sigma Chemical Co. All solutions were made in deionized double distilled water (Table 1).

Cell line. Human fetal foreskin fibroblast(HFFF-PI6, NCBI: C-170) and gastric adenocarcinoma cell line (AGS, NCBI: C-131) was obtained from National Cell Bank of Iran, Pasteur Institute of Iran. These cells were maintained in RPMI 1640 medium supplemented with 10% FCS in a humidified incubator (37 °C & 5% CO_2).

Calprotectin purification. Human neutrophil were prepared from leukocyte-rich blood fractions (buffy coat) according to the reported method [23, 24]. Method of purification of human calprotectin was described previously [24]. The purification procedure included preparation of human granulocytes, ammonium sulfate precipitation, and anion-exchange chromatography (Q sepharose and SP sepharose) and resulted in the copurification of MRP8, and MRP14. Individual proteins were separated by either preparative isoelectric focusing or preparative SDS-PAGE. The procedure was carried out in the course of 4 days and yielded several milligrams of essentially pure P6, MRP8, and MRP14 in either native or denatured form (Fig2).

The citation of the Table & Figures in text should be in Parenthesis as the example

Results (Font: Tw Cen MT, Size: 12, Left)

Etoposide is a chemical agent that is used widely in chemotherapy treatment of cancer [26-30]. In order to analysis of anticancer property of calprotectin , the effect of various concentrations of calprotectin and etoposide (as positive control)at 24 and 48 h incubation times is studied on the AGS cell lines (Fig1). The two agents inhibit cell proliferation significantly in all stages of experiment except for 1.025 µM concentration at 24 h time of incubation ($P<0.001$by one-way ANOVA). Since a suitable anticancer drug is one that has the least effect on the proliferation rate of normal cells, in the resemble experiment, the effect of calprotectin and etoposide was studied on HFFF (as a normal cell line) (Fig 2). The obtained results except for 1.025 µM concentration at 24 h incubation time, are statistically significant ($P<0.001$ by one-way ANOVA).

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Discussion (Font: Tw Cen MT, Size: 12, Left)

Anticancer property of calprotectin on the various cell lines was studied. According to the previous studies that reported anticancer property of calprotectin on the various cell lines, there are three important points regard to the effect of a drug on the human body including potency, mechanism and side effects. The wide ranges of in vitro studies are published in order to description of mechanism of calprotectin effect on the various cancer cell lines [2, 3]. It was also reported that calprotectin inhibits efficiently growth of AGS cell line in a dose and time dependence pathway [31]. In the present study we compared the ability of calprotectin in inhibition the growth of AGS cell line with etoposide (as a common drug in cancer chemotrapy).

As it is illustrated in the Fig 1, etoposide has a strong cytotoxic effect on the AGS cell line since viability of the cells decreases continuously during incubation time. Furthermore, we used LC50 parameter to determine the level of cytotoxicity of calprotectin in comparison with etoposide. The LC50 values of calprotectin after 24 and 48 h of incubation times were obtained 3.96 and 1.58 μM respectively. However the LC50 values of the same incubation time for etoposide were acquired 76.35 and 41.9 μM respectively.

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The financial support from Clinical Proteomics Research Center of Shahid Beheshti Medical University, Skin Research Center of Shahid Beheshti Medical University and Asre-Novin Institute of Research and Industrial Services is gratefully acknowledged.

Conflicts of Interest (Font: Tw Cen MT, Size: 12 Left)

The authors have no conflict of interests in this article.

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Author's Contribution (Font: Tw Cen MT, Size: 12 Left)

AM designed the study, analyzed the data and wrote the paper.SD contributed to the data entry, literature review and writing-up process.SA and ADR contributed to the study design and analysis…..

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Authors Name (s): ATIEH AKBARI, ZAHRA RAZZAGHI, FATEMEH HOMAEE, MARYAM KHAYAMZADEH, MOHAMMAD MOVAHEDI, MOHAMMAD ESMAEIL AKBARI

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