Effect of melittin isolated from bee venom (Apiscerana indica) on anti-proliferation in human cancer cervix hela cells through activation of Caspase 3 and p53 protein

by Upik Miskad

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Effect of melittin isolated from bee venom (*Apiscerana* indica) on anti-proliferation in human cancer cervix hela cells through activation of Caspase 3 and p53 protein

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ABSTRACT

Melittin (MEL) is the main toxin protein constituent of bee venom isolated from A cerana with powerful anti-proliferation activity. This research aimed to evaluation effect of melittin isolated from A. cerana on anti-proliferation in human cancer cervix Hela cells through activation of p53 and caspase 3 protein using ICC method. The result show that melittin induced expression of p53 and caspase 3 as apoptotic marker on cell on HeLa cell line ($p<0.05$) and has a potential as candidate of anticancer drug in future.

Keyword: Melittin, anti-proliferation, HeLa, p53 and caspase 3

INTRODUCTION

Estimates of the worldwide incidence and mortality from 27 major cancers has been reported by GLOBOCAN. Estimated over 20 million new cancer cases expected annually as early as 2025 [1]. Ovarian cancer causes the first mortality was found in female reproduction system. A combination of surgery and chemotherapy is a best therapy for ovarian cancer less often, treatment may include radiotherapy managed by a gynecological oncologist [2].Chemotherapy using drug synthetic has many side effect that cannot be tolerated in patients. it is encouraging researchers to look for other alternatives from nature, less side effect such as melittin.

Melittin is a main toxic protein isolated for bee venom has many pharmacological activities [3]. Jo M (2012) reported anti-cancer effect of bee venom and melittin in ovarian cancer through induction of death receptors and inhibition of JAK2/STAT3 pathway[4].Subbalakshmi C (1999), Biological activities of C-terminal 15-residue synthetic fragment of melittin: design of an analog with improved antibacterial activity [5]. Dual secured nano-melittin for the safe and effective eradication of cancer cells by Cheng [6].

Doe to its pharmacological activity, this research evaluated the effect of melittin to activated p53 and caspase 3 on HeLa cell line using ICC method. The p53 tumor suppressor protein functions as a transcriptional activator that play rule in the induction of apoptosis in cell [7] and caspase 3 is crucial mediator of cell regulatory networks controlling inflammation and cell death programmed, a frequently activated death protease, catalyzing the specific cleavage of many key cellular protein.

EXPERIMENTAL SECTION

Melittin
Melittin isolated from bee venom were purchased from Sigma-Aldrich (Sigma-Aldrich Ltd, Singapore Cat. No M2272 Sigma). Human cervical line (HeLa) was obtained from Department of Parasitology, Faculty of medicine, Gadjah Mada University, Indonesia. All mediums were purchase from Sigma Aldrich Singapura. P53 and caspase3 primary antibody were purchase from Biocare, ICC reagent were purchase from Dako.

Cell culture
The human cervical cancer HeLa cell line was obtainedfrom Department of Parasitology, Gadjah Mada University Indonesia. The cells were grown and maintained in RPMI 1640 supplemented with 10% (v/v) FBS and 100 U/mL penicillin-streptomycin, incubated at 37°C in a humidified atmosphere with 5% CO_2[8- 9].

Cytotoxic Assay
Toxicity of melittin on HeLa (IC10, IC25 and IC50) was based on describe in previously [10].

Cell treated
p53 and caspase 3 were detected using ICC method. Briefly, HeLa cells were cultured overnight in a 24-well plate (covered with coverslip) and treated with various concentration of melittin (IC10, IC25 and IC50).

Analysis of p53 and Caspase 3
Cells are fixed using 70% cold methanol, passed through alcohol and washed in water. They were then rinsed with 0,2 M Tris-buffered saline pH 7.6 (TBS) and endogenous peroxidase removed by soaking in 1% hydrogen peroxide for 5 min. Cells were incubated with a blocking solution of 1:10 normal goat serum and TBS for 30 min before application of the primary antibody. This sections were incubated at room temperature 30 min with rabbit monoclonal antibody p53 (Biocare, 1:100) or rabbit polyclonal antibody caspase3 (Biocare, 1:100) for 10 min, then washed twice with TBS before a biotinylated second antibody, anti-mouse Ig antibody (1:200) was applied. Streptavidin-horseradish peroxidase conjugate was added (1:200) to all cells and incubated for 1 h before washing twice with TBS. chromogen solution containing 6 mg of diaminobenzoate (DAB) was added and color allowed to develop for 5 min before being stopped by washing in TBS. Hematoxylin and eosin (H&E)using a contra staining. All cells washed with water, 70% ethanol, 90% ethanol, absolute ethanol and xylene.Total cell number and number of positive cells were counted in each field, obtaining a mean percentage expression in each case.

RESULTS AND DISCUSSION

From current understanding, there are two pathways transducing a death signal to the apoptotic machinery: extrinsic and intrinsic pathway [11]. The “extrinsic” pathway involves trimerization of death receptors such as TNF receptor 1, inducing p53 and activated caspase 3. X-irradiation, anticancer drugs and growth factor as an apoptotic inducer, were shown to activate apoptotic cell death independent of death receptor pathways [12].

One of the candidate drug isolated of *A. cerana* is melittin. Melittin is a major peptide consisting of 26 amino acid residues ($C_{13}1H_{229}N_{39}O_{31}$) with the sequence GIGAVLKVLTTGLPALISWIKRKRQQ[13]. Recent report the cytotoxic effect of melittin on cell cancer and its mechanism such as cell cycle alterations effect, effect on proliferation and/or growth inhibition and induction of apoptotic [14]. This study aimed to evaluation effect of melittin isolated from *A. cerana* on anti-proliferation in human cancer cervix Hela cells through activation of p53 and caspase 3protein.

This study analyzed the expression of p53 and caspase 3 in HeLa cell line. Limitations of this type of Immunocytochemistry study relate to the diverse antibodies used by different authors, the lack of agreement among researchers on cutoff points for considering. Nevertheless, Immunocytochemistry analysis yields considerable data on the topographical expression of markers and the relationships of positive cells with the treated cells [15,16].

The toxicity of melittin against HeLa cell line according to Plasay. Melittin has a strong activity and it can inhibit the cell growth. The IC10, IC25 and IC50 of melittin against HeLa cell line are 0.73; 1.72 and 2.45, respectively. While the IC10, IC25,IC50 of doxorubicin as positive control are 0.034; 0.173 and 1.052, respectively[10]. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio-guided studies if it exerts an IC50 value <30 μg/mL[17].

Table 1. Expressions of p53 and caspase 3 in HeLa cell line per 100 counted cells (n= 3)

No	Sample		p53 (Percentage)	Caspase 3 (Percentage)
1	Melittin	IC10	15.52±3.82	31.07±10.17
		IC25	30.80±6.37	39.24±5.58
		IC50	27.49±6.97	41.65±12.71
2	Doxorubicin	IC10	44.82±6.44	49.61±7.95
		IC25	49.37±3.29	56.54±16.67
		IC50	56.69±1.13	53.44±7.15
3	Untreated cell		14.03±2.75	14.65±4.13

Table 1 showed the effect of melittin on p53 and caspase 3 expression in HeLa cell line after treated with melittin and doxorubicin 24 hour. The result express as number of positive cell express protein in field. The dose-dependent response to express protein marker (p53 and caspase 3) after treated. Increases express of p53 and caspase 3 indicated the melittin cause death cell through apoptosis. One of the main apoptotic marker cell are p53 and caspase 3 [18-20].

CONCLUSION

We reasoned that p53 and caspase 3 might be able to induce apoptotic cell in HeLa cell line.

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