Cytotoxic effects of lectin from elderberry bark on cell line from pupa fat body tissue of Colorado potato beetle, *Leptinotarsa decemlineata*

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Sambucus nigra L-agglutinin-I referred to as SNA-I is a chimeric lectin composed of an A-chain with enzymatic activity and a B-chain with carbohydrate-biding activity. BCIRL-Lepd-SL1 (CPB) cell line established from pupa fat body of the Colorado potato beetle, *Leptinotarsa decemlineata* (Col. Chrysomelidae). Treatment of insect pupal fat body cells with SNA-I caused a loss of cell viability after four days of incubation with different concentrations of SNA-I (0.1 – 30 µg/ml) based on the MTT test. It was clear that SNA-I had a strong growth inhibitory effect on CPB cells. Increased cytotoxicity was observed in SNA-I treated cells in a dose and time dependent manner.

اثرات سمی *Sambucus nigra* L-agglutinin-I روی لاین سلولی بافت شفیره سوسک کلرادوی سیب زمینی *Leptinotarsa decemlineata*

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Sambucus nigra L-agglutinin-I که متعاقبا به اختصار SNA-I نامیده شده است، یک لکتین استخراج شده از گیاه آقطی *Sambucus nigra* است. SNA-I یک chimeric lectin متشکل از یک زنجیره A با فعالیت آنزیمی و یک زنجیرهB با خاصیت ایجاد پیوند با کربوهیدراتها می باشد. تیمارسلولهای BCIRL-Lepd-SL1 (CPB) برگرفته از بافت چربی شفیره سوسک کلرادوی سیب زمینی با غلظتهای مختلف SNA-I (0/1030 µg/ml) باعث کاهش بقای سلولها گردید. نتایج بطور روشنی نشان داد که SNA-I به شدت روی رشد و تکثیر سلولهای CPB اثر بازدارنده دارد. سمیت SNA-I وابسته به دوز و همچنین مدت زمانی بود که سلولها در معرض آن قرار می گرفتند.

کلید واژه *Sambucus nigra* agglutinin, CPB cell, Cytotoxicity, *Leptinotarsa decemlineata*

1- Introduction

In order to achieve demands for food of the increasing world population, there is need of new ways for protecting plant crops against predators and pathogens at the same time keeping away from the use of environmentally aggressive chemicals. Plants have grown a wide array of defensive compounds and complicated defense mechanisms that give resistance against phytophagous predators and infectivity by viruses, bacteria, fungi, nematodes, etc. The best known plant proteins evidently involved in defense mechanisms are lectins. Lectins are a class of proteins of non-immune origin that possess at least one non-catalytic domain that specifically and reversibly binds to mono- or oligosaccharide.

Ribosome-inactivating proteins (RIPs) are a group of proteins known as translation inhibitors. RIPs are capable of killing eukaryotic cells by arresting protein synthesis at the translocation step [1, 2, 3]. There are evidences that RIPs of types 1 and 2, inhibitors of proteolytic enzymes and glycohydrolases have potential entomotoxic effects [4, 5].

Sambucus nigra (elderberry) expresses a complex mixture of so-called ricin B-related lectins with different carbohydrate-binding specificities referred to as *Sambucus nigra* agglutinins (SNA), including several type-2 RIPs as well as hololectins [3, 6, 7]. SNA-I is a type-2 RIP composed of an A-chain with *N*-glycosidase activity that is covalently linked through a disulphide bridge to a B-chain with carbohydrate-binding activity, leading to a unique molecular structure with four [A-s-s-B] pairs. SNA-I exhibits specificity for NeuAc(α-2,6)Gal/GalNAc [8].

Previously we have shown entomotoxic effects of SNA-I and the closely related protein SNA-I' in several important pest insects such as the beet armyworm *Spodoptera exigua* (Lepidoptera) and the aphids *Myzus nicotianae* and *Acyrthosiphon pisum* (Hemiptera) [9, 10]. The rules of a number of RIPs and their functions in program cell death (PCD) have been elucidated mostly in mammalian cells [11]. Nevertheless, the mechanism of action of RIPs in general and SNA-I in particular towards insects is not known. In continuation of our previous investigation, insect cell line from pupal tissue (fat body) of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae) was exposed to SNA-I to investigate the physiological changes caused by this lectin.

Some RIPs are very effective toxins, the best known being ricin, a type-2 RIP from castor beans (*Ricinus communis*), which is extremely cytotoxic. On the other hand, a number of type-2 RIPs were identified, which have structural and enzymatic properties similar to ricin although are much less toxic to cells and animals.

2- Materials and methods

2-1- Cell lines and culture condition

The continuous cell line CPB cell was established from pupal tissue (fat body) of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). Cell number of 1.5×10^5 to 2.0×10^5 cells/ml in 25-cm2 flask were cultured in a total volume of 5 ml per flask Cells at 27°C.

2-2- Cell viability and cell proliferation determination following incubation with SNA-I

To verify the relative potencies of the type-2 RIP SNA-I on cell proliferation, 2×10^5 cells/ml were treated for four days with different concentrations of SNA-I (0.01− 30 µg/ml)

2-3- Time course exposure of CPB cells with SNA-I

Time course experiments, using a final concentration of 30 µg/ml SNA-I were also carried out for 0, 24, 48, 72, 96 and 120 h. After loading the required wells of a 96-well microtiter plate with 100 µl of the cell solution, 1 µl of the SNA-I with adjusted concentration was added and the plates

were incubated for four days at 27°C. In the controls, cells were treated with the same volume of phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM Na 2HPO4, 1.47 mM KH2PO4, pH 7.4). For every concentration four replicates were prepared and each experiment was repeated two or three times. After incubation, the cell numbers were counted using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma, Bornem, Belgium) as a substrate according to [12]. The MTT assay is based on the enzymatic conversion of a yellow tetrazolium salt to an insoluble formazan product by the mitochondria of viable cells [13]. Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) values on cell proliferation were calculated with Prism v4® (GraphPad Software Inc., San Diego, CA). Absorbance of the produced formazan was measured at 560 nm in a microtiter plate reader (PowerWave X340, Bio-Tek Instruments Inc., Winooski, VT).

3- Results

3-1- Cytotoxicity of SNA-I on CPB cell line with different doses

After four days incubation of cells with different doses of SNA-I (0.1 - 30 µg/ml) percentages of cell proliferation inhibition were calculated (0.5%-130%) (Figure 1).When CPB cells were exposed to the lower concentrations of SNA-I (0.1 and 1 µg/ml) no significant reductions in cell proliferation were observed. Absorbance of the produced formazan was measured at 560 nm in a microtiter plate reader (Power wave x 340, Bio-Tek instruments Inc, Winooski, VT). Median lethal concentration to kill 50% of cells (LC_{50}) for SNA-I when exposed to CPB cells was 3.7 µg/ml.

3-2- Cytotoxicity of SNA-I on CPB cell in different time points

In the present experiment to calculate the percentage of cell viability, CPB cells were incubated for 0, 24, 48, 72, 96 and 120h with the 30 µg/ml of SNA-I.

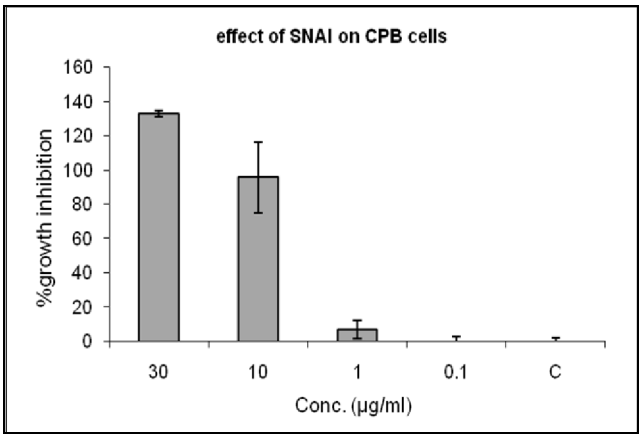

Figure 1. Inhibition of cell proliferation of CPB insect cell line after treatment with different concentration of SNA-I.

The time needed for cell growth inhibition of 50% of the cells (LT_{50}) exposed to 30 and 10 µg/ml of SNA-I was 40h and 84h respectively. For the concentrations lower than 1µg/ml no significant cell growth inhibition was observed (Figure 2).

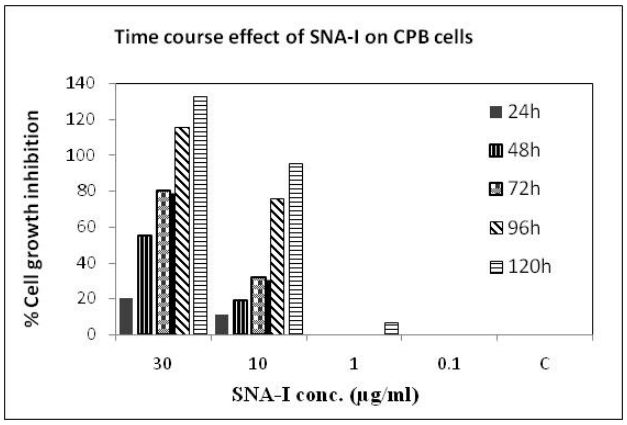

Figure 2. Inhibition of cell proliferation of CPB insect cell line after treatment with different concentration of SNA-I at different time point.

4- Discussion

Over the years different research groups have shown that type-2 RIPs show (strong) cytotoxicity to several cell lines and animal models. However, it

should be noted that the toxicity of type 2 RIPs varies considerably between different RIPs [7]. In this work, SNA-I was examined against pupal tissue (fat body) of the Colorado potato beetle, *Leptinotarsa decemlineata.* The viability assay used based on MTT to determine the quantitative data on cells surviving. Data showed that type 2 RIP SNA-I has a strong growth inhibitory effect on CPB cell line. Microscopic analysis of the cells revealed the conventional apoptotic morphological characteristics such as cell shrinkage, cell membrane blebbing, and formation of apoptotic bodies. In our previous research investigating the mode of action of SNA-I against a midgut cell line (CF-203) from the spruce budworm, *Choristoneura fumiferana* it was revealed that SNA-I causes identical apoptotic symptoms and effects on cells. Cytotoxicity effects and morphological changes observed in the cells treated with SNA-I can lead our hypothesis to the fact that RIPs can induce cell death through mechanism which is separate from their function as inhibitors of protein-synthesis and present results suggest that type 2 RIP, SNA-I may induce apoptosis in insect fat body cell line in a pathway independent of its A-moiety with enzymatic activity. But, further experiments are needed to clarify the function of type 2 RIPs SNA-I during the apoptosis process. Increased cytotoxicity was observed in SNA-I treated cells in a dose and time dependent manner

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