REVIEW ARTICLE

The Effects of ICD-85 (Venom Derived Peptides) In Vivo and In Vitro in Treatment of Cancer

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Abstract

Background: Breast cancer is now the most important type of cancer in women around the globe and accounts for 25% of all types of cancer. Prevention and treatment of cancer are essential.
Methods: The main methods for treating cancer include chemotherapy, surgery, radiotherapy, gene therapy, and hormone therapy. Chemopreventive test programmes began in 1987, when over 1,000 agents and agent combinations were selected and evaluated in preclinical studies of chemopreventive activity against various types of cancers.
Results: An important feature of anticancer drugs is a cytotoxic effect on cancer cells; these drugs have some cytotoxic agents found in animal venom. The ICD-85(venom derived peptides) is a combination of three peptides, ranging from 10,000 to 30,000 Da, and derived from the venom of the Iranian brown snake (Gloydius halys) and the yellow scorpion (Hemiscorpius lepturus).
Conclusion: ICD-85 has an anti-proliferative effect and anti-angiogenesis activity on cancer cells. The side effects of chemotherapy are multiple drug resistance and effects on natural tissues, among others. Therefore, cytotoxic anticancer drugs are useful in treating cancer. The present work investigates the effects of ICD-85 on in vivo and in vitro studies.

Keywords: Breast Cancer; Gloydius Halys; Hemiscorpius Lepturus; ICD-85

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INTRODUCTION

Cancer is now the second leading cause of death in developing countries. Breast cancer is the most important type of cancer in women and accounts for 25% of all cancers around the globe (1–3). Although the level of disease and the age of a person vary in cases of breast cancer, but the prevalence of breast cancer in women is 100 times higher than that of men (4). The survival rate in developed countries is high, while it is lower in developing countries (5). As much as 80%–90% of people in England and America are at least five years old (6, 7). Factors that increase the risk of breast cancer include obesity, physical inactivity, alcohol consumption, etc. (8). Efforts have been made to treat breast cancer, some of which include chemotherapy, radiation therapy, immunotherapy, gene therapy, endocrine treatment, and surgical combination (breast surgery or mastectomy) (9, 10). In Iran, about 8,000 people have breast cancer every year, with an estimated 30–35 cases per 100,000 in Iranian women (12). Researchers and physicians now look for drugs with less complications and higher efficacy to increase the specificity of the drug to targeted tumour cells with targeted therapies (11, 13–15). Finding new therapies and new medicines from natural sources is a concern for many researchers (11, 16). Over the past three decades, numerous studies have been published on the anticancer properties of toxins. Toxins contain complex compounds, such as proteins and peptides, that have specific biological and chemical functions (14, 17, 18). The application of scorpion and snake venoms works as a promising route for the treatment of some incurable diseases in the development of anticancer agents due to the presence of enzymes, such as hyaluronidases, phospholipases, sphingomyelinases, alkaline phosphatases, choline esterase, metalloproteinases, and disintegrins, with specific biological and chemical activity, as well as the presence of biological resources (19, 20).

The active agent ICD-85 is a combination of three peptides (two enzymes of the scorpion and one enzyme of the snake). The composition is within the range of 10,000 to 30,000 Da; it is derived from *Gloydius halys* and *Hemiscorpius lepturus* (21).

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Effects of ICD-85 (Venom Derived Peptides)

MDA-MB-231 and MRC-5 CELL LINEs

Zare Mirkabadi et al have examined the effects of this substance on MDA-MB-231 cells in case of breast cancer (due to the highly invasive behaviour of these cells) to understand the apoptotic and cytotoxic effects of ICD-85 (22) in a better way. In MDA-MB-231 cells affected by ICD-85, initially cytosolic activity of lactate dehydrogenase (LDH) enzyme was measured and transferred to the culture medium after membrane injury. The cytotoxicity test that measures LDH activity can be used to distinguish between apoptosis and necrosis. The morphological changes were observed between the control group cells and the cells receiving the cytotoxic agent (ICD-85). The results showed that in the test group, some of the cells that were incubated with 16 µg/mL ICD-85 lost their membrane adherence and cytoplasm (Figure 1). At higher concentrations in the membrane surface, ICD-85 allowed the ions to pass through the concentration gradient leading to osmotic changes and membrane lysis, followed by several unknown mechanisms that ultimately caused cell death. In this regard, apoptosis and other forms of cell death can be created based on various stimuli such as chemical toxicity, radiation, or genotoxicity (23). At lower concentrations, ICD-85 can prevent cell growth by another mechanism—one of the causes of apoptosis in the cell. The effect of ICD-85 on normal lung cells (MRC-5) at low concentrations (5, 10, and 15 µg/mL) after 24 hours of incubation showed no significant cellular events. However, the ICD-85 cytotoxic effect was clear when the concentration increased to more than 20 µg/mL. The results of this study showed that the cytotoxic effects are dose-dependent, and ICD-85 cells are killed by various mechanisms that directly damage the cells. Membrane damage is more toxic in the early stages, while other types of cell damage, including swelling, rupture, or immobilization, occur in the next stages (22).

Mouse Models of Breast Cancer

Given that breast cancer is one of the most prevalent diseases with a high level of management, the survival rate of people is not more than 20%–25% which has raised concern among the communities (24). Koohi et al have examined the effect of ICD-85 on preventing breast cancer in in vivo condition (25). In vivo studies showed that ICD-85 also affects the longevity of cancerous mice (Figure 2). Additionally, ICD-85 is known as angiography at an injectable dose. Since all animals receive ICD-85 as a treatment, bleeding in the place of the tumour was stopped within 21 days of the treatment (Figures 2 and 3). Anti-angiogenesis is a promising alternative treatment for cancer treatment; it is also useful for preventing metastasis or recurrence (25).

HUVEC CELLs

The formation of new capillaries from the arteries is called angiogenesis. In various diseases, including cancer, preventing the formation of new blood vessels leads to reduced tumour size and metastasis (26). Therefore, the basis for the transformation of tumours into malignant ones is through this process. Mombeinipour et al have examined the anti-angiogenic effects of ICD-85 in vitro with CAM and in vivo from human umbilical endothelial cells (HUVECs). Anti-proliferative ICD-85 activity was also determined by the MTT assay in HUVEC (27). The ICD-85 used in this study was a combination of three peptides with a molecular weight of about 10–30 kDa and derived from *Gloydius halys* and *Hemiscorpius lepturus*. The results of this study showed anti-proliferative ICD-85 activity on the HUVEC cell line with 12 µg/mL inhibitory concentration (IC50). The CAM in vivo test showed that when the chick embryo is exposed to 0.15 µg/mL disc of ICD-85, it prevents the formation of new vessels (Figure 4). Based on the results obtained in this study, ICD-85 has anti-angiogenic activity, which has been shown to prevent capillary tube formation and CAM test. The results

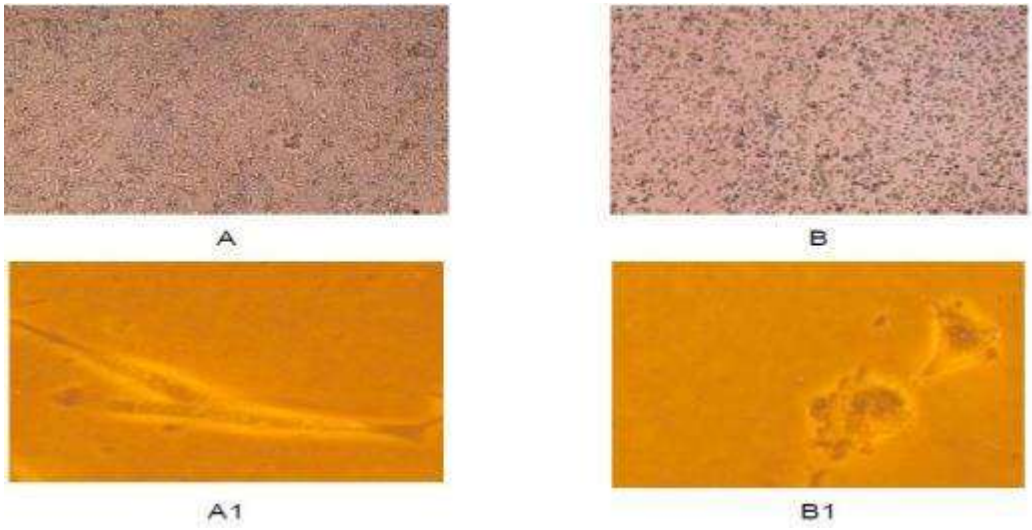

Figure 1. ICD-85 treatment in MDA-MB-231 cells (before and after) .

A and A1 are control cells. Figures B and B1 have been treated with ICD-85 (16 µg/mL). The cells have reduced the cytoplasmic branch (22).

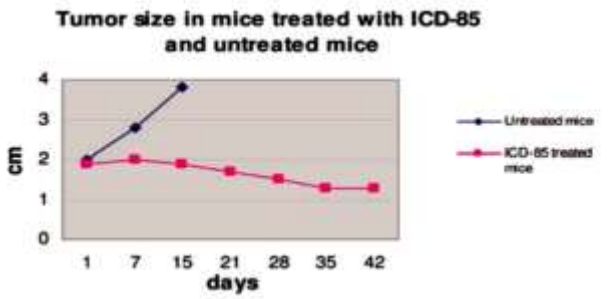

Figure 2. Comparison of tumour size among treated and untreated mice with ICD-85.
All mice that had not been treated died within 15 days (25).

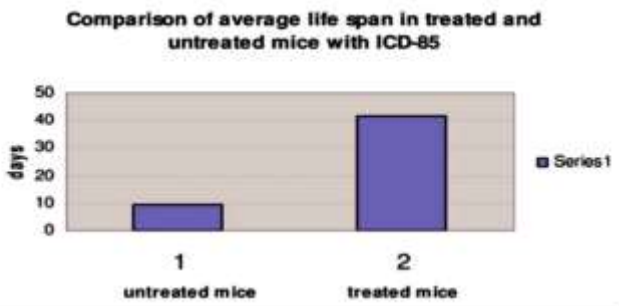

Figure 3. The mean lifetime of both groups of mice (treated and untreated) (25)

of this study showed that ICD-85 severely inhibits the growth and proliferation of cells, decreases HUVE survival, and promotes the ICD-85 inhibition of endothelial cells in a concentration-dependent manner (Figure 5). However, the maximum ICD-85 concentration, which indicated the compatibility and proliferation of the results by inhibiting 72% of HUVEC cells, was 24 µg/mL. Therefore, agents that inhibit angiogenesis can be effective in controlling the growth and development of tumours as well as secondary metastasis tumours. It implies that anti-angiogenic treatment is suggested as a promising way to control cancer (27).

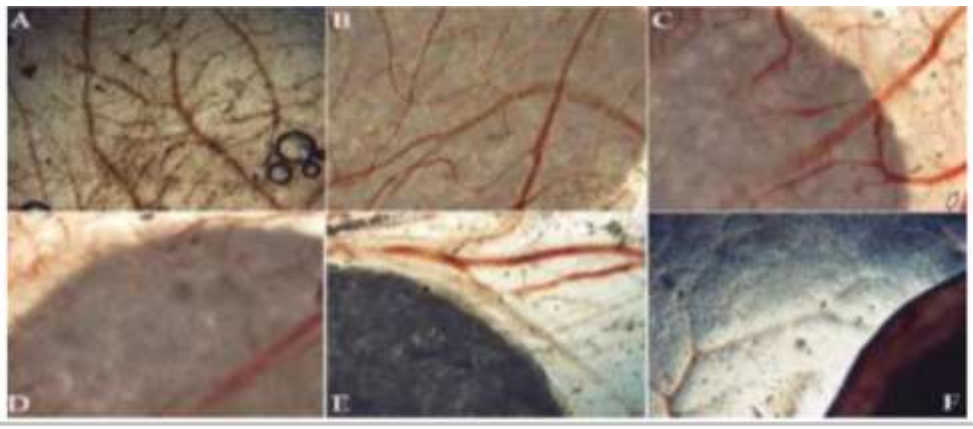

Figure 4. Comparison of tumour size among treated and untreated mice with ICD-85.
All mice that had not been treated died within 15 days (27).

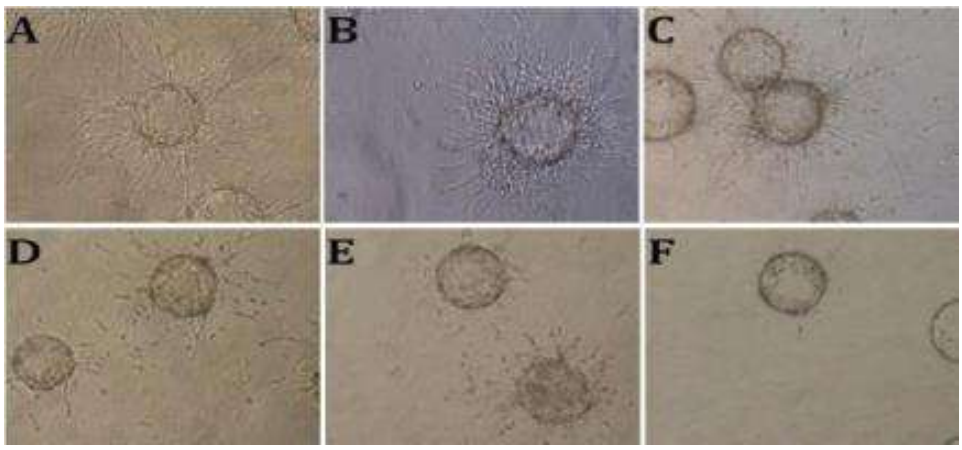

Figure 5. Effect of ICD-85 on in vitro angiogenesis. Suspension was added to the control containing growth supplements (A, B). Angiogenesis of human umbilical endothelial cells were treated by ICD-85 at 9 µg/mL (C). ICD-85 was observed at 18 µg/mL in 100% inhibition culture (D, E). ICD-85 was detected at 27 µg/mL of cellular degradation (F) (27).

HeLa CELLs

Moradhaseli et al have examined the cytotoxic effects of ICD-85 NPs on HeLa cells of the human cervical cancer using the Caspase-8 method. Hela cells have appropriate growth characteristics, including short-term division and low growth factors (29). Cell toxicity was evaluated by MTT and LDH as well as the effect of free ICD-85 and apoptosis ICD-85 nanoparticles (NPs) on HeLa cells using the colorimetric caspase-8 test. Alginate was used to prepare NPs for ICD-85. The MTT test showed that NP-ICD-85 could increase cytotoxicity in in vitro compared to HeLa cells compared to ICD-85. However, the LDH test showed that ICD-85 has dose-dependent cytokines in HeLa cells, while ICD-85 NP has less toxicity in similar cells (Figure 6). The results also showed that ICD-85 apoptosis is associated with the activation of caspase-8 on HeLa cells. There are two main ways to activate caspases: death receptor and committed mitochondrial mechanisms. In addition, the analysis of the caspase-8 test showed that ICD-85 NPs increase apoptotic levels in HeLa cells than the ICD-85s released. In other studies, it was found that Hela cells had a lower sensitivity to ICD-85 than human leukaemia (HL-60).The treatment of HeLa cells with ICD-85 causes morphological changes and increases apoptosis in the cell population. In addition, in vivo studies showed that anti-tumour agents increase polymer-mediated drug resistance in tumours, thereby reducing tumour growth and increasing the survival of animals containing the tumour. These results indicate that ICD-85 NPs may be a promising approach to cancer treatment (29).

MCF-7 and HDF CELL LINEs

Kheirandish Zarandi et al have investigated the effect of ICD-85 in different concentrations on the MCF-7 breast cancer cell line and the normal human dermal fibroblasts (HDF) cell line (21).

This study addressed the inhibitory effect of ICD-85 with MTT and neutralized colorimetric tests, the ICD-85 necrotic effect on the cell lines by the LDH test, cell morphology changes in conjunction with ICD-85, and the study of induction of ICD-85 apoptosis into cancer cells. The breast cancer cells were identified by Kit Caspase 9. MCF-7 cells treated with ICD-85 exhibited apoptosis characteristics such as granulation and rounding of cells and producing apoptotic bodies. These morphological changes were recorded by the invert microscope (Figure 7). This difference was not observed in normal cells. The MTT method was used to study cytotoxic effects. This method is simpler than other methods of cell proliferation; it is available with most facilities in most laboratories. In this technique, the response of different cells to external factors can be evaluated. In ICD-85, the induction of concentration-dependent cytotoxic effects on MCF-7 cells is confirmed by neutral red test. The results of this study showed that the inhibition concentration of 50% of this compound on MCF-7 cells is 36.45±0.38 µg/mL for 24 hours. Increasing the concentration of this compound also increases its inhibitory effect. The highest inhibitory effect of this compound on breast cancer cells was at a concentration of 80 µg/mL, which was able to reduce the survival of 60% of the cells. However, when HDF cells were adjacent to ICD-85, there was no significant increase in lactate dehydrogenase release at concentrations below 20 µg/mL. The results showed a lower toxicity of this compound on normal HDF cells. It can be noted that this compound is somewhat selective on the cancer cells. It can also be noted that the combination of ICD-85 at low concentrations causes cell death of apoptosis, and, at high concentrations, causes cell death of the necrosis type. Also, the induction of ICD-85 apoptosis into MCF-7 cells was achieved by activating Caspase 9, which was thirteenfold that of negative control cells. The present study showed that ICD-85 induces apoptosis in MCF-7 cells by activating caspase and can therefore be considered in further research as a potential treatment for breast cancer (21).

CONCLUSION

According to the International Agency for Research on Cancer (IARC), there are more than 10 million cancer cases around the globe. WHO estimates that the disease will reach more than 13 million by 2030. One of the cancer treatment routes is the use of cytotoxic compounds. Over the past three decades, numerous studies have been published on the

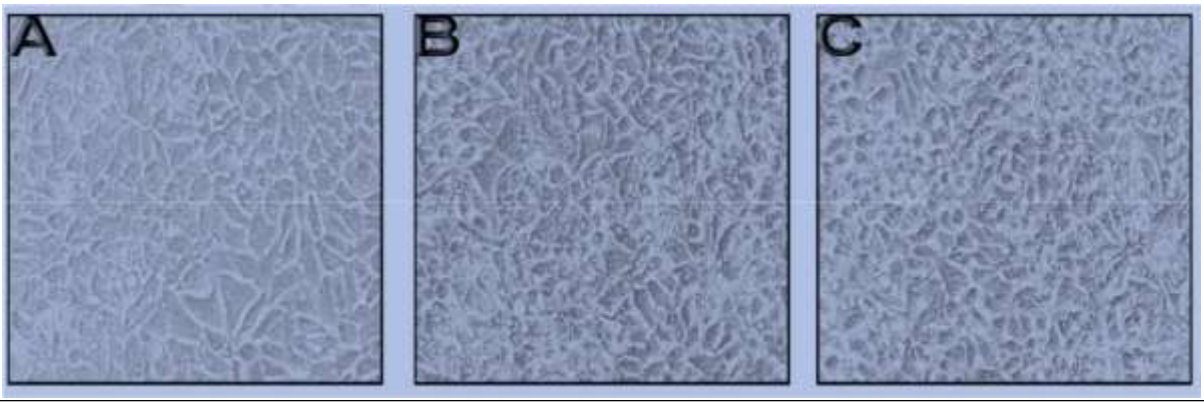

Figure 6. Morphological changes of HeLa cells exposed to ICD-85 and ICD-85 NP. Micrograph of control cells (A). HeLa-treated cells with 28 µg/mL in ICD-85 and ICD-85 NP (B and C, respectively) (29).

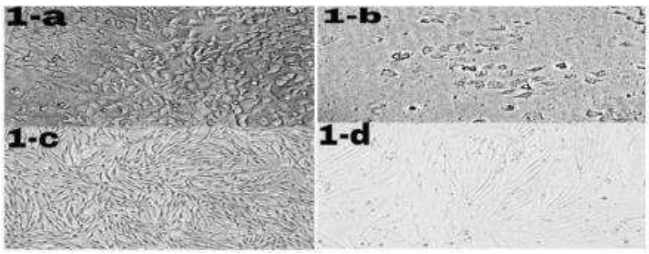

Figure 7. Morphological changes of ICD-85 on MCF-7 and HDF cells. Cell rounding in MCF-7 cells treated with ICD-85 1-b (20X). Untreated MCF-7 cells with ICD-85 (1-a, 20X). There are no significant morphological changes in HDF normal cells treated with ICD-85 (1-d, 20X). Untreated HDF cells with ICD-85 (1-c, 20X) (21).

anticancer properties of toxins. Toxins contain complex compounds, including proteins and peptides, that have specific biological and chemical functions. The application of scorpion and snake venoms works as a promising route for the treatment of some incurable diseases in the development of anticancer agents due to the presence of enzymes such as hyaluronidases, phospholipases, sphingomyelinases, alkaline phosphatases, choline esterase, metalloproteinases, and disintegrins, with specific biological and chemical activity, as well as the presence of biological resources. An anticancer drug is an ideal drug that can kill cancer cells specifically. But unfortunately, most antimicrobial drugs, along with antimicrobials that have fewer toxic effects on healthy cells, also kill growing cells in the body. With the specific features of ICD-85 (preventing cancer cell growth, anti-angiogenesis, and apoptotic and cytotoxic effects), the future of the ICD-85 peptide combination can be used to treat various cancers and many incurable diseases.

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Conflict of interest: None to be declared.

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