Original Article

Cobra Snake Venom reduces significantly tissue nucleic acid levels in Human Breast Cancer

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Abstract

Objective: To look into the feasibility of using cobra snake venom in suppressing breast cancer tissue through inhibition of nucleic acid synthesis .

Methods: Samples of breast cancerous tissue, obtained from Atomic Energy Medical Center, LMCH, Jamshoro, incubated with varying concentrations of venom and without venom (as controls) were assayed for macro-molecular (RNA and DNA) levels

Results: No. change in the control samples while a prominent and significant fall in nucleic acid contents has been noted in venom treated tissues. Also, maximum effect was observed with 25ug/ml dose.

Conclusion: Snake venom strongly inhibited the formation of nucleic acids in the breast cancerous tissues. It may bring a fall in cell proliferation, thus there is hope that venom could be used as an anti-cancerous drug in the future (JPMA 55:71;2005).

Introduction

Cancer is the second most common cause of death in Europe and United States accounting for about 25% of deaths in all ages.[1] Among all cancers, breast cancer is a very common malignancy in women aging between 35-55 years. It is not only a health problem of the industrialized world but is now affecting the developing countries also.[2]

In Pakistan, breast cancer is a major health problem of women and according to Pakistan Medical Research Council, it is very common and its incidence is steadily rising.[3] It constitutes 25% of all malignant tumors and has increased by over 100% during the last five years, affecting age group of 30-35 years both urban and rural areas, rich and poor families with multiple pregnancies.[4,5]

Special attention has been paid recently to the studies on the influence of toxic substances in malignant tissues. The first chemical agents are sodium cacodylate, caryo-dastic poisons, colchicine and then various plant and animal toxins, especially snake venoms. Some of the drugs are found to be poisonous to cancer cells as well as normal ones. Some researchers believed on the fact that cancer cells have enormous appetites against poisonous drugs as compared to that of normal cells. It may be said that the use of venom which is a poison has shown to possess cytotoxic or lytic invitro effects on tumour cells[6,7], could prove a weapon to combat the cancer disease, especially the solid tumours. If desired results are had in the positive direction, it will open a door for the designing and developing of a new anti-neoplastic drug from the venom, in the future.

In this study, the invitro effect of crude cobra snake venom (ccsv) on different cellular macromolecules (Nucleic acids RNA, DNA) was quantitatively evaluated in normal and cancerous breast tissues of human. Thus, an attempt has been made to study and compare breast cancer tissues response towards the venom with that of normal breast tissue.

Materials and Methods

All chemicals were supplied by Fluka (USA), ARC (New York), BDH, Ltd, Poole (England), E. Merck (USA) and Riedel-de Haein AG. Seclze - Hannover (Germany). Venom was collected from living cobra snake and different dosage forms 10ug, 25ug and 50ug per ml were prepared by the method.[8]

Human breast tissues (cancerous and normal) were collected from different hospitals of Sindh (PMC Hospital, Nawabshah; Liaquat Medical College Hospital, Jamshoro; Jinnah Post-graduate Medical Centre, Karachi) and Ihsan laboratories, Karachi. The breast cancer patients on chemotherapy and radiotherapy at LMC and Atomic Energy Medical Center, Jamshoro were selected. The diagnosis of breast cancer was made by an oncologist through biopsy and patients were classified according to the staging criteria.

After surgical excisions (mastectomy specimen), the tissues were cut into slices and kept separately as the affected (treated as cancerous) and non-affected (treated as normal) portions of the same breast. These were then cut into small pieces (1mm thick) and were put into ice-cold normal saline and kept immediately into the deep freezer till further processing. The methodology used for homogenate (KIW-Ultra-Turrax) preparations were incubations, (with and without venom) and extractions, estimations.[9]

All spectrophotometer estimations were made as mentioned previously.[9] The level of significance was calculated by the method of students 't' test. The RNA, DNA - content is expressed as µg/50mg tissues homogenate per 30 minutes at 37^0C.

Results

No significant change was found to occur in nucleic acid levels when normal tissues were treated with varying concentrations of the venom (Table). However, cancerous breast tissues responded well and DNA and RNA content reduced to a maximum with 25µg/ml venom.

The changes in the nucleic acids in the normal and cancerous breast tissues, brought about by the venom are mentioned in Table. From the data, RNA, DNA could be observed in the normal breast samples whereas, cancerous tissue samples which have larger concentrations of nucleic acids at basal level, showed a significant ($p<0.001$) fall in the RNA and DNA contents.

Figure 1. The amount of RNA in venom treated breast tissue of normal and cancer (human).

Figure 2. Amount of DNA in venom treated breast tissues of normal and cancer (human).

The corresponding values regarding the reduction of RNA / DNA levels indicate that cancerous tissues could be more sensitive towards the venom. The dose response curve (Figures 1 and 2) shows that a concentration of 25µg/ml produced maximum inhibition of both the nucleic acids conversely, further increased dose (50µg/ml) failed to produce any significant change. Various concentrations of the venom caused gradual reduction in the RNA/DNA content of cancerous tissues; when compared with contents (without venom) of same cancerous tissue. From these observations, it can be assumed that venom might have maximum effect at 25ug/ml in cancerous tissues.

Discussion

The study was designed to investigate whether venom does have any effect to alter cellular nucleic acids and if any; then to compare its influence in the normal as well as cancerous breast tissues within the range of the dose regime being studied throughout.

Table. Effect of various concentrations of snake venom on the RNA and DNA levels of human breast cancer and normal breast tissue.

		Various concentrations in sname venom			
Nucleic acids (RNA, DNA)	Control (normal and cancerous)	I 10 µg/ml	II 25 µg/ml	III 50 µg/ml	Wheher inhibition significant or not
RNA (N)	0.3250±0.0524	0.3267±.0378	0.3267±0.0427	0.3150±0.0339	NS
RNA (Ca)	0.3525±0.0322	0.3383±.214	0.2617±0.0454	0.3267±0.0266	SS
DNA (N)	0.0278±0.0111	0.0228±0.0040	0.0243±0.0068	0.0252±0.0071	NS
DNA (Ca)	0.0437±0.0068	0.0293±0.0078	0.0210±0.0051	0.0302±0.0032	SS

The data obtained so far showed that cancerous tissues (human-breast) possessed considerably larger amounts of nucleic acids than did the normal tissues. This agrees with the observation[10] for both RNA, DNA that rapidly growing tissues always contain larger amount of non-sedimentable DNA than differentiated tissues.[11] An increased RNA content in cancer tissue controls is likely to be due to increased DNA-dependent - RNA polymerase activity. There are two major causes (first a change in membrane permeability and second, change at nuclear level)of cell proliferation resulting in metabolic disturbances[12] and excessive lactate production[13] and produce changes in nucleoproteins that could affect the process of protein synthesis.[14]

Snake venoms are supposed to be the most complex of animal secretions containing a vast number of compounds with different pharmacological and biochemical activities. Literature shows that cobra venom components especially DNAase and RNAase and other enzymes inhibit cancer growth.[15] Cytotoxin of cobra venom was found to have a more cytotoxic effect on tumour cells than normal cells upon invitro incubation.[16] In our results also no significant change was found to occur in nucleic acids levels when normal tissues were treated with varying doses of the venom whereas, the cancerous tissues responded well. The RNA, DNA content, were deeply reduced with 25µg/ml venom.

Thus, it may be assumed that venom must first penetrate the nucleus then bind to specific receptor proteins and initiate an activation of genetic components leading to a number of events. In other words; cancer tissues seemed to be more sensitive towards venom as has been reported.[17]

Results conclusively show that venom in small doses can cause inactivation of stimulating enzymes and/or activating the inhibitory enzymes at that site and this might be one aspect through which venom interferes with the nuclear functions by inducing alterations in the nucleus to restrict the RNA / DNA synthesis through active DNA synthesis which enhance or initiate rapid cell division.

Because of these alterations, DNA reduction occurs due to reduced ability of cells to synthesize DNA from altered templates.[18] This adds weight to the hypothesis concerning venoms mode of action at nuclear as well as cellular level and it may be demonstrated that appropriate concentration of the venom to impair abnormal cell growth could be 25µg/ml. As far as Ca-breast tissues are concerned, more profound reduction was observed with 25ug/ml venom. It could be proposed that the said dose could be more practical and needs further exploration to confirm the findings.

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