THE POTENTIAL OF THE CRUDE SNAKE VENOM IN TREATMENT OF HUMAN BREAST CANCER WITH AND WITHOUT COMBINATION OF ANTICANCER DRUGS

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Background: Clinical usefulness of the snake venom has been worked out since the last many decades to treat blood pressure, cancer of breast, ovary and etc. we wanted to evaluate the effect of natural compounds of cobra snake venom *in vitro* on nucleic acid in normal and breast cancer tissues in combination with and without anticancer drugs. **Methods:** Surgically removed tissue from cancerous and non cancerous of same human breast were homogenized and extraction of nucleic acids were prepared. All homogenate samples were incubated with and without snake venom and antineoplastic drugs (Cyclophosophamide and Mitomycin-C) for 30 min at 37 ^{0}C and measured at 490 mμ for DNA and at 660 mμ for RNA by spectrophotometer (Sspectron-21®). **Results:** The effect of the snake venom was compared singly as well as in combination with the two chemotherapeutic agents *viz* mitomycin-C (an antibiotic) and Cyclophosophamide (an alkylating agent) on the nucleic acids (RNA and DNA). In human breast cancer tissue shows significant reduction in nucleic acids contains when treated with venom (25 μg/ml) in combination and compared with singly use anticancer drugs. Cyclophosophamide and Mitomycin produced some effect in human breast tissue at 10 μg/ml on the DNA only for short time and eventually destroyed. However snake venom does have potential and is more effective with or without anticancer drugs on both nucleic acids (RNA and DNA) suggests wide range of receptors to act. Both drugs could not produce inhibition or potentiating effect on normal and cancerous tissues *in vitro* by all different doses. **Conclusion:** Anticancer drugs (Cyclophosophamide and Mitomycin-C), specific medicines for solid tumour (Ca. Breast) used in this study showed no significant reduction at the same dose of snake venom (25 μg/ml) *in vitro* in comparison to snake venom. However drugs at 10 μg/ml has shown some effect on the DNA but only for short time and possibly, eventually are destroyed. However significant and promising anticancer effect of snake venom seems to have a better future as an alternative. None toxic dose of snake venom affects on both DNA and RNA.

Keywords: Breast Cancer, Snake venom, Cyclophophamide, Mitomycin, contortrostatin.

INTRODUCTION

In continuation to our previous findings it is becoming clear that snake venom would be best choice to treat the different types of tumours in comparisons to few available anticancer drugs. A complete cure in not expected from present available anticancer drugs. Moreover they cause irreparable side effect that multiply miseries.[1-4] However no doubt the present clinical trials are aimed to prolong life and to restrict proliferation.

Natural toxins especially non-toxic dose of snake venom has attracted the attention hopefully to reduce the solid tumour size and to block the angiogenesis.[5-8]

Failure of some therapeutic regimes as they do more harm than good and the toxic effect (side effect) should also be considered to avoid risk. It is observed that beneficial effect of these drugs seems to be inseparable from their side effects. Hence drug developers should take a different approach.[10]

Number of combination of drugs are being encouraged and dramatic good results are reported, most of these are alkylating and antibiotic together.[4,9-13]

Due to difference in biochemical levels between normal and cancerous cells focus is being given on specific therapy selection of effective drugs and its concentration. This helps to kill tumour cells for longer period and to prevent relapse. The considerable advancement has been made to treat cancer patients with cytotoxic drugs irrespective of the stages. Snake venom is one of natural cytotoxic compound. Hence it is being encouraged to treat different types of tumours especially its positive effect on breast cancer is being reported during last few decades.[6,16-20] In our last few reports we have shown its advantageous effect, is seen *in vitro* studies. However in literature the use of CAP and some other anticancer drugs are discouraged in Invitro.[5, 9, 10 19]In the present study we have tried both drugs (CAP and Mitoc) Separately and in combination with cobra snake venom *in vitro* with the hope that it might have better prospective clinical usefulness.

MATERIAL AND METHODS

All the chemicals/reagents were of "Analar" grade and were supplied by BDH chemicals, Ltd. Poole (England): E. Merck (USA) and Riedel De- Haein AG

Secize-Hannover (Germany). The macromolecules (Nucleic acids: RNA and DNA) were supplied by Fluka (USA) for standardization while chromic acid for cleaning the glassware etc, was prepared in the laboratory. The estimations were made in triplicates.

Sample collection:

Breast (cancerous & unaffected supposed normal) tissues were received from different hospitals of Sindh (Peoples Medical College Hospital, Nawabshah, (LUMHS), Jamshoro, Jinnah Postgraduate Medical Centre Hospital, Karachi) and Ihsan Laboratories, Karachi.

The Breast cancer patients on chemotherapy and radiotherapy at LUMHS and Atomic Energy Medical Centre, Jamshoro took part in the study. The diagnosis of Ca-Breast was made by biopsy and the patients were classified according to staging criteria.

Cobra snakes were supplied by Laghari Snakes Association and from Jogi Colony of Thatta, Thur and Jamshoro. Fresh Snake Venom was collected by compressing the glands of the snakes in the laboratory. The charmers were also requested for Venom from Cobra snakes. The venom thus obtained, was then lyophilized. Cobra Venom was also purchased from India (Sigma loeate). The venom, thus obtained was used for all biochemical quantitative *in vitro* studies.

Fresh venom was placed directly in a fine sterilized glass container fixed in coloured and airtight box. The whole procedure was carried out in the dark room at normal temperature. After two weeks venom got dried and changed into the solid transparent crystals of light yellow colour and was ready for use.

After surgical excisions (mastectomy specimen) the tissues were cut into slices and kept separately as the affected (treated cancer) and non-affected (treated normal) portion of same breast. Again cut into small pieces (1mm thick) and were put into ice-cold normal saline and kept immediately into deep freezer till further experimentation. All tissue homogenates and extraction of nucleic acids were prepared to the method of Ceriotti[4] and Schneider[20].

RESULTS

Quantitative estimation of nucleic acids (RNA and DNA) was carried to compare the effect of the anticancer drugs and snake venom on normal and cancerous breast tissue *in vitro* singly and in combination with snake venom as shown in Table-1. Tissues were treated with different concentrations (10, 25, 50 μg/ml) of both drugs (cylophosphaide and Mitomycin-C) and snake venom. No significant reduction in RNA was observed. However some insignificant reduction in DNA contents was seen by both antineoplastic drugs only at the dose of 10 μg/ml.

The similar *in vitro* combined effect of snake venom and drugs was studied on cellular nucleic acids. A significant reduction has evidently been noted specifically at our proposed non-toxic and survival dose (25 μg/ml) of venom. Although decrease in RNA content was small but remained persistent in presence of snake venom.

It is therefore suggested that constant reduction in contents of nucleic acids by venom at the dose of 25 μg/ml and combined additional potentiating effect seems to have involvement of different receptors.

DISCUSSION

An attempt is made to explore the biological/clinical role of cobra snake venom in treatment of human breast cancer, at molecular level and its comparison to that of available anticancer drugs to understand the feasibility of venom, if any. Since presently available drugs do cause their many side effects[2] one cannot expect complete cure.[1,3] However it is claimed that these available drugs prolong the life and can enhance quality of life.

It has been suggested that the conditional treatment by CAP in combination with 5-DFUR is worth considering for the patients who have metabolic breast cancer that is not life threatening.[4] Some workers treating the patients with metabolic breast cancer in combination of CAP and trastuzumab is minimal toxic in advance cancer patients.[13]

It has been demonstrated that snake venom not only inhibit ovarian cancer dissemination but also prevented the recurrence of blood vessels (angiogenesis)[5,8] to tumour secondary sites. It is reported that snake venom limits breast[6-8] cancer progression. The snake venom is more potent as anticancer agent for the breast and prostate cancer,[14,21] especially in the cancer with metastasis instead of the available chemotherapy and radiotherapy which induce unwanted side effects.[1-3]

The purpose of present study is to determine weather in combination of CAP and venom could target and give better response.

Recently it is reported that snake venom component[2,7,8,22] shows promising anticancer effect may be better than available drugs do. In our previous study it is suggested that reduction in macromolecules (RNA and DNA) by snake venom may be possible basis for reducing the population of cell proliferation in cancer tissue by its cytotoxic action and metabolic disturbance.[17,18]

Table-1: The overall results showing effects of various concentrations of anticancer drugs (cyclophophamide and Mitomycin-C) in single and in combination of venom, on Nucleic acids. Average±SEM Contents of Ca-breast tissues, summarized

Anti cancer drugs/ drugs + venoms	Nucleic acids (RNA, DNA)	Control (Normal & cancerous)	Various concentrations of anticancer drugs and S. Venom			Whether Significant or not
			I 10 µg/ml	II 25 µg/ml	III 50 µg/ml	
Cyclophosphamide	RNA (N)	0.1032 ±0.0115	0.1015 ±0.0135	0.1012 ±0.0082	0.1083 ±0.0111	N.S
	RNA (Ca)	0.1183 ±0.0092	0.1162 ±0.0244	0.1093 ±0.0127	0.1037 ±0.0127	N.S
	DNA (N)	0.0207 ±0.0050	0.0223 ±0.0061	0.102 ±0.0082	0.0188 ±0.0016	N.S
	DNA (Ca)	0.0390 ±0.0137	0.0237 ±0.0081	0.1093 ±0.0127	0.0207 ±0.0060	N.S
Mitomycin–C	RNA (N)	0.1025 ±0.0221	0.0955 ±0.0189	0.0265 ±0.0091	0.0857 ±0.0146	N.S
	RNA (Ca)	0.1113 ±0.0252	0.0970 ±0.0139	0.0267 ±0.0174	0.0855 ±0.0142	N.S
	DNA (N)	0.0305 ±0.0114	0.0210 ±0.0083	0.0963 ±0.0143	0.0219 ±0.0091	N.S
	DNA (Ca)	0.0316 ±0.0351	0.0174 ±0.0134	0.0965 ±0.0150	0.0125 ±0.0110	N.S
Cyclophosphamide +S. Venom	RNA (N)	0.1092 ±0.0140	0.1013 ±0.0130	0.0187 ±0.0089	0.108 ±0.0120	S
	RNA (Ca)	0.1237 ±0.0227	0.1088 ±0.0195	0.0163 ±0.0130	0.1020 ±0.0129	S
	DNA (N)	0.0350 ±0.0127	0.0258 ±0.0029	0.1123 ±0.0139	0.0255 ±0.0040	S
	DNA (Ca)	0.1022 ±0.0505	0.0672 ±0.0253	0.1017 ±0.0144	0.0562 ±0.0191	S
Mitomycin C +S. Venom	RNA (N)	0.1230 ±0.0165	0.1197 ±0.0098	0.1177 ±0.0104	0.1147 ±0.0176	S
	RNA (Ca)	0.1333 ±0.0080	0.1297 ±0.0124	0.1148 ±0.0096	0.1207 ±0.0097	S
	DNA (N)	0.0315 ±0.0099	0.0320 ±0.0083	0.0283 ±0.0068	0.0302 ±0.0070	S
	DNA (Ca)	0.0545 ±0.0078	0.0452 ±0.0076	0.0400 ±0.0043	0.0467 ±0.0022	S

N=Normal breast tissue sample, Ca=Breast cancerous tissue sample, Control=Without snake venom and drugs

The two drugs though presently are being discouraged due to their poisonous effect on normal cells and because of unwanted side effects are still in use to treat breast cancer. Cyclophospmide (an anticancer alkylating agent), it is the nitrogen mustered group of alkylating antineoplastic, chemotherapeutic agent and is more frequently used and mitomycin-C (an anticancer antibolic agent).[23,24] Despite the differences in their chemical structure and general pharmacological/biological actions were compared and evaluated for reducing cell proliferation in the breast cancer.

Since these drugs have reflected similar effect on both cellular contents RNA (indirectly) and DNA (directly) of cancerous cells[25] hence there cytotoxic (cell killing) effect may be considered through a common routes and centralized to react with nucleic acids. In the present study, the venom, when tested has been found to interact and produce a fall in these molecules.

Sladek[26] has reported that CAP detrimental to tumour cells but also affect on the proliferation of normal cells. The use of CAP and some other drugs *in vitro* is discouraged. However snake venom has been reported that it can be used safely *in vivo* and *in vitro*.[16,27,28]

The effect of snake venom on transport mechanism of cell membrane and mitotic division[1,18] is well documented. Other worker has also reported protease inhibitor from snake venom.[29] CAP act by alklyting DNA polymerase and Mitomycin-C forms covalent cross links between DNA strands.[23,24,30]

Where as snake venom act on both effective and favourable sites of cellular parameters *viz* cell membrane permeability and nucleolus due to poly functional cytotoxic ability to cross link strands of DNA. Our previous results suggest that venom is more effective which seems due to its neucleophilic site of action.[17-18] In human glioma cells the contortrostatin (CN), a component of snake venom,

has the properties to act as anti invasive and anti metastasis without affecting on cell vialbility and extra cellular matrix (ECM) degradation. CN interact with different antigens.[28]

The present findings provide an evidence of specific therapeutic action of venom on neoplastic tissues and this action specifically existed for all studies carried out singly or in combination of the drugs and is consistent to the observation.[12,14,21] More over the above reported results provide an evidence for usefulness of venom (at 25 µg/ml) hence could be used very effectively in the treatment of breast cancer. However, more comparative studies are being carried with new effective drugs to further strengthen the present findings.

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