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In vivo anticancer activity of vanillin semicarbazoneShaikh M Mohsin Ali^{1*}, M Abul Kalam Azad¹, Mele Jesmin¹, Shamim Ahsan¹, M Mijanur Rahman¹, Jahan Ara Khanam², M Nazrul Islam³, Sha M Shahan Shahriar⁴¹Department of Applied Chemistry and Chemical Engineering, University of Rajshahi²Department of Biochemistry and Molecular Biology, University of Rajshahi³Department of Chemistry, University of Chittagong⁴Department of Food Science & Technology, Chittagong Veterinary and Animal Science University

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ABSTRACT

Objective: To evaluate the anticancer activity of vanillin semicarbazone (VSC) against Ehrlich ascites carcinoma (EAC) cells in Swiss albino mice. **Methods:** The compound VSC at three doses (5, 7.5 and 10 mg/kg i.p.) was administered into the intraperitoneal cavity of the EAC inoculated mice to observe its efficiency by studying the cell growth inhibition, reduction of tumour weight, enhancement of survival time as well as the changes in depleted hematological parameters. All such parameters were also studied with a known standard drug bleomycin at the dose of 0.3 mg/kg (i.p.). **Results:** Among the doses studied, 10 mg/kg (i.p.) was found to be quite comparable in potency to that of bleomycin at the dose of 0.3 mg/kg (i.p.). The host toxic effects of VSC was found to be negligible. **Conclusions:** It can be concluded that VSC can therefore be considered as potent anticancer agent.

1. Introduction

Schiff bases are well known for their importance in biological fields^[1–6]. Anticancer activities of these compounds have also been studied to some extent. Schiff bases derived from benzoin, salicylaldehyde, amino phenol with 2,4-dinitrophenyl hydrazine and acetone semicarbazone have shown pronounced anticancer properties. Not only the schiff bases, schiff base complexes with transition metals have also been investigated for their anticancer activities against Ehrlich ascites carcinoma (EAC) cells in Swiss albino mice^[7,8]. A novel schiff base complex with copper with potential anticancer mechanism, DNA binding, cytotoxicity and apoptosis induction activity has appeared very recently^[9]. In the present situation, we have selected vanillin semicarbazone (VSC) as a test compound and studied its anticancer activity against EAC cells *in vivo*. In support of this work, hematological studies

have also been done accordingly.

2. Materials and methods*2.1. Chemicals*

All chemicals and reagents used to carry out the research work were of reagent grade.

2.2. Experimental animals

Swiss albino mice of 5–7 weeks old, weighing 20–26 g were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B), Mohakhali, Dhaka.

2.3. Animal care

Mice were kept in iron cages with saw dust and straw bedding which were changed once a week regularly. Standard mouse diet (recommended and prepared by ICDDR'B) and water were given in adequate.

*Corresponding author: Dr. Shaikh M Mohsin Ali, Professor, Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Bangladesh.

Tel: +88-0721-750180

Fax: +88-0721-750064

E-mail: jammsbhs@yahoo.com

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2.4. Ethical clearance

Protocol used in this study for the use of mice as animal model for cancer research was approved by the University Animal Ethical Committee (27/08/RUBCMB)

2.5. Synthesis of VSC

The procedure for the synthesis of VSC was similar to that described in our recent published paper^[10].

2.6. Characterization

2.6.1. Physical form

The compound in pure form was obtained as white crystalline solid. Its melting point was found to be 229 °C which was in accordance with the literature value^[9]. The compound was found to be soluble in 2% dimethyl sulfoxide (DMSO), partially soluble in ethanol and acetone.

2.6.2. Infrared (IR) spectra

A strong band appeared at 1690 cm⁻¹ confirmed the formation of C=N bond. The other peaks appeared in IR spectra are in accordance with -NH₂, -OH, -OCH₃ groups.

2.7. Structure of VSC

The structure of VSC is given below:

Figure 1. Structure of VSC.

2.8. Tumour cells

Transplantable tumour (EAC) cells were used in this experiment. The initial inoculum of EAC cells was kindly provided by the Indian Institute of Chemical Biology (ICB), Kolkata, India. The EAC cells were thereafter propagated in our laboratory biweekly through intraperitoneal (i.p.) injections of 136×10^4 cells per mouse.

2.9. Determination of median lethal dose (LD₅₀)

The test compound was dissolved in 2% DMSO and injected intraperitoneally to six groups of mice (each group containing six animals) with different doses of (*viz.* 30, 50, 60, 70, 90, 105, 120, 130 mg/kg i.p.). The LD₅₀ value was estimated from the plot of number of mortality versus dose curve.

2.10. Study of anticancer activities

The procedures followed for the anticancer activities of the compound were similar to those described in our recent published papers^[7,10,11].

2.10.1. Cell growth inhibition

Five groups of Swiss albino mice (6 in each group) weighing 20–25 g were used for the experiment. In every mouse 136×10^4 EAC cells were inoculated into each group on day 0. Treatments were started after 24 h of tumour inoculation and continued for five days. Group one to three received the test compound at the doses of 5, 7.5, 10 mg/kg (i.p.), respectively. Group four received bleomycin at the dose of 0.3 mg/kg (i.p.) and group five was used as control.

Mice in each group were sacrificed on day six and the total intraperitoneal tumour cells were harvested by normal saline (0.98%). Viable cells were first identified by using trypan blue and then counted by a haemocytometer. Total number of viable cells in every animal of the treated groups was compared with that of control (EAC treated only) group.

2.10.2. Average tumour weight and mean survival time (MST)

Five groups of Swiss albino mice (6 in each group) were used for the experiment. EAC cells (136×10^4) were inoculated in each mouse on day 0. Treatment was started after 24 h of tumour cell inoculation and continued for 10 days. The weight changes of each mouse were recorded daily and the increase in tumour weight was monitored. The host survival was recorded and expressed as mean of survival time in days. The percent increase of life span (ILS) was calculated by using the following formulae:

$$\text{MST} = \frac{\sum \text{Survival time (days) of each mouse in a group}}{\text{Total number of mice}}$$

$$\text{ILS\%} = \left(\frac{\text{MST of treated group}}{\text{MST of control group}} - 1 \right) \times 100$$

2.10.3. Hematological parameters in normal and tumour bearing mice

The effect of the test compound on haematological parameters was studied in both normal and tumour bearing mice. For tumour bearing mice, treatment was started after 24 h of EAC cell transplantation and continued for 10 days. For normal mice, the same procedure was followed. Obviously the normal mice were free from EAC cells. Blood was drawn out (from tail vein) from each group of mice on day 5, 10, 15, and 25 for such studies.

2.10.4. Effect of the test compound on normal peritoneal cells

Effects of the test compound on normal peritoneal cells were measured by counting total peritoneal cells and number of macrophages. Four groups of normal mice (4 in each group) were treated with the test compounds (i.p.) at all the doses (group 1–3 with 5, 7.5 and 10 mg/kg, respectively), each for three consecutive days. The untreated group (group 4) was used as control. On the fourth day the animals were sacrificed after injecting 5 mL of normal saline (0.98%) into

the peritoneal cavity of each mouse. Intraperitoneal exuded cells and number of macrophages were counted with 1% neutral red by haemocytometer.

2.11. Statistical analysis

All values were expressed as mean $\pm$ standard error of mean (SEM). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's 't' test using SPSS statistical software of 14 version. $P < 0.05$ was considered to be statistically significant when compared with control.

3. Results

The results of the experiments as described in the previous sections were presented here. In most cases average values of repeated experiments were taken.

Lethal dose of VSC was found to be 120 mg/kg for intraperitoneal treatment in male Swiss albino mice. For anticancer activities, three doses *viz.* 5, 7.5 and 10 mg/kg (i.p.) were selected throughout the work.

The effects of the test compound and bleomycin on EAC cell growth on day six after tumor transplantation were shown in Table 1. Treatment with VSC resulted in cell growth inhibition at the doses of 5, 7.5 and 10 mg/kg (i.p.) to be 67.05%, 78.10% and 84.42%, respectively. The maximum result

was shown by the drug, bleomycin at the dose of 0.3 mg/kg (i.p.) which showed 88.20% cell growth inhibition.

All anticancerous drugs show a significant effect on survival time of EAC cell bearing mice. The effects of test compound at different doses were summarized in Table 2. It was observed that life span was increased significantly in tumor induced mice treated with the test compound at different doses. Treatment with VSC at the doses of 5, 7.5 and 10 mg/kg (i.p.) increased life span of EAC cell bearing mice by 44.43%, 63.48% and 88.90%, respectively, when compared to that of control mice. Evidently the survival time was found to be increased with increased dose. Bleomycin at the dose of 0.3 mg/kg (i.p.) increased life span by 90.47% when compared to control.

Figure 2. Effect of VSC on tumour weight.

Treatment time: 10 days; Number of mice in each group: 6.

Table 1

Effect of test compound on EAC cell growth inhibition (*in vivo*) (mean $\pm$ SEM) (n=6).

Treatments	Nature of the drug	Dose in mg/kg (i.p.)	No. of EAC cells in mouse on day 6 after tumour cell inoculation	Cell growth inhibition (%)
Control (EAC cell bearing mice)	—	—	$(4.00 \pm 0.05) \times 10^7$	—
EAC + bleomycin	Antibiotic	0.3	$(0.47 \pm 0.01) \times 10^7$ *	88.20
EAC + VSC	Synthetic	5	$(1.32 \pm 0.11) \times 10^7$	67.05
		7.5	$(0.88 \pm 0.10) \times 10^7$	78.10
		10	$(0.62 \pm 0.04) \times 10^7$ *	84.42

Treatment days=5; * $P < 0.05$ between control and treated group.

Table 2

Effect of VSC on survival time of EAC cells bearing mice (mean $\pm$ SEM) (n=6).

Treatments	Nature of the drug	Dose in mg/kg (i.p.)	MST (days)	% ILS
Control (EAC bearing mice)	—	—	21.00 $\pm$ 1.41	—
EAC + bleomycin	Antibiotic	0.3	40.00 $\pm$ 0.86*	90.47
EAC + VSC	Synthetic	5	30.33 $\pm$ 1.82*	44.43
		7.5	34.33 $\pm$ 0.55*	63.48
		10	39.67 $\pm$ 0.82*	88.90

Treatment days=10; * $P < 0.05$ between control and treated group.

Table 3

Effect of VSC on the enhancement of normal peritoneal cells in mice (mean $\pm$ SEM) (n=6).

Experiment type	Dose (mg/kg)	Macrophages	Total peritoneal cells
Control (normal)	—	$(0.26 \pm 0.05) \times 10^6$	$(3.87 \pm 0.04) \times 10^6$
Normal + VSC	5	$(0.30 \pm 0.08) \times 10^6$ *	$(4.10 \pm 0.02) \times 10^6$ *
	7.5	$(0.36 \pm 0.04) \times 10^6$ *	$(4.40 \pm 0.05) \times 10^6$
	10	$(0.40 \pm 0.06) \times 10^6$	$(4.75 \pm 0.25) \times 10^6$ *

Treatment days=3; * $P < 0.05$ between control and treated group.

Effects of the test compound at different doses and the standard antitumor drug bleomycin (0.3 mg/kg) on the tumor weight due to tumourgenesis were shown graphically in Figure 2. Treatment of the animals with test compounds, previously inoculated with EAC cells, resulted in the inhibition of tumor growth pronouncedly.

Hematological parameters were found to be altered from normal values along with the growth of tumor. Hemoglobin content, red blood cell (RBC) counts were found to be decreased and white blood cell (WBC) counts were found to be increased after inoculation of EAC cells in Swiss albino mice. After treatment with the test compound at different doses, it was observed that the parameters restored moderately only at high doses of VSC (10 mg/kg). The effects of test compound on hematological parameters of both tumor bearing mice and normal mice were shown in Figure 3–8.

Figure 3. Effect of VSC on RBC of tumour bearing mice on day 5, 10, 15 and 25.

Treatment time: 10 days; Number of mice in each group: 6.

Figure 4. Effect of VSC on WBC of EAC bearing mice on day 5, 10, 15 and 25.

Treatment time: 10 days; Number of mice in each group: 6.

Figure 5. Effect of VSC on haemoglobin content of tumor bearing mice on day 5, 10, 15 and 25.

Treatment time: 10 days; Number of mice in each group: 6.

Figure 6. Effect of VSC on RBC of normal mice on day 5, 10, 15 and 25.

Treatment time: 10 days; Number of mice in each group: 6.

Figure 7. Effect of VSC on WBC of normal mice on day 5, 10, 15 and 25.

Treatment time: 10 days; Number of mice in each group: 6.

Figure 8. Effect of VSC on haemoglobin content of normal mice on day 5, 10, 15 and 25.

Treatment time: 10 days; Number of mice in each group: 6.

Effects of the test compounds on enhancement of total peritoneal cells in normal mice and mice treated with the test compounds at different doses were shown in Table 3. Treatment with the test compound at increasing doses resulted in increase of normal peritoneal cells as well as macrophages.

4. Discussion

The potency of VSC as anticancer agent has been judged by measuring the inhibition of cell growth, reduction in tumor weight and enhancement of MST of the EAC cell bearing mice. The efficiency of the test compound has been compared with the data obtained by running parallel experiments with a known effective anticancer drug, bleomycin at the dose of 0.3 mg/kg (i.p.)

For EAC bearing mice, the tumor weight has been found to increase rapidly. The treatment of such mice with test

compound reduced the growth rate. Similar trend has been found when examined for the cell growth inhibition ability of VSC. The results showed that treatment with the compound at high dose inhibited cell growth efficiently. VSC at the dose of 10 mg/kg (i.p.) inhibited cell growth by 84.42%. The potency is quite comparable to that of bleomycin at the dose of 0.3 mg/kg (i.p.), when 88.20% inhibition of cell growth was observed.

The life span of the EAC cell bearing mice increased remarkably when treated with the test compound. VSC at the dose of 10 mg/kg (i.p.) has increased the life span to more than 88.90%, when bleomycin at the dose of 0.3 mg/kg (i.p.) has increased the same to about 90.47%. The prolongation of the life span of cancer bearing mice is a very important and reliable criterion^[12] for judging the potency of any drug as anticancer agent. The positive effect of the compound against EAC cell bearing mice has further been verified by monitoring the change in hematological and biological parameters. The RBC and hemoglobin contents of EAC bearing mice decrease gradually with time when compared to those of normal mice. The reduction of RBC levels and haemoglobin percentage is the major problem in cancer bearing animal^[13–15]. This is probable owing to the deficiency of iron in hemolytic or myelopathic condition^[16]. Treatment with the compound reverses back RBC and hemoglobin contents towards normal. With the growth of tumor, WBC level increases with time. The rise of WBC count of the EAC bearing treated mice follows much slower rate in comparison with that of EAC bearing untreated mice. Parallel hematological experiments have been done with normal mice to evaluate the host toxic effect of the compound. A very slight deterioration of such parameters has been observed during the treatment period (25 consecutive days). Similar toxic effect has been observed with bleomycin at the dose of 0.3 mg/kg (i.p.).

The immunological effect of the test compound in fresh healthy mice has been performed by counting peritoneal macrophages which has provided further support for the potency of the compound as anticancer agent. The compound has notably enhanced the number of macrophages. This enhancement might have produced some cytokinetic^[17] products, such as tumor necrosis factor, interleukins, interferons, etc. which in turn may be responsible in destroying tumor cells.

In conclusion, the compound, VSC showed pronounced efficiency against EAC cells in Swiss albino mice. Important parameters viz. cell growth inhibition, tumour weight reduction and the enhancement of survival time are quite comparable to those of bleomycin. As the LD₅₀ value is 120 mg/kg (i.p.), treatment with doses more than 10 mg/kg i.p. can be safely used if necessary to get still better results. These data may, therefore, be helpful for advanced cancer researches in future.

Conflict of interest statement

We declare that we have no conflict of interest.

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