Summary: A Schiff base acetone semicarbazone has been synthesized and characterized. Its anticancer activities have been studied against Ehrlich Ascites Carcinoma (EAC) cells in Swiss albino mice by monitoring the parameters like tumour cell growth inhibition, tumour weight, survival time, peritoneal cells and haematological parameters. It was found that this compound significantly reduces tumour cell growth rate, decreases tumour weight and increases life span in comparison to those of EAC bearing mice. The test compound restored the depleted haematological parameters of EAC bearing mice towards normal. The compound also enhanced number of macrophages in normal mice. The results obtained were compared with those obtained with the standard drug bleomycin.

Key words: Anticancer activity, EAC cell, Schiff base, Acetone thiosemicarbazone.

Introduction

Biochemical and pharmacological properties of Schiff bases have been extensively investigated owing to their potential applications such as antibacterial (1-2), antiviral (3-4), anti-inflammatory (5-6), anti-tubercular (7-8), antileprosy (9), antileukemia (10) activities. Because these bases appear to have such wide applications, many investigators are now focusing attention on the possibility of developing chemotherapeutic agents from Schiff bases (11-13). Schiff bases of thiosemicarbazone and their derivatives also possess potential anticancer activities. 1, 2-naphthaquinone-2-thiosemicarbazone and its metal complexes have pronounced anticancer activities against MCF 7 human breast cancer cells (14). Copper (II) complex of thiosemicarbazone derivatives showed potent anticancer activities against human leukemia cell lines K562 and U937 (15). Thiosemicarbazones, semicarbazones and acetyl hydrazones of phthalic, o-benzo-sulfinide, naphthalimide and diphenimide demonstrated...
potent cytotoxicities against different cancer cell lines (16). The anticancer activities of pyridine-2-carboxaldehyde thiosemicarbazones and a series of di-2-pyridyl ketone thiosemicarbazone ligands have also been reported in literature (17). In the present paper we have studied the anticancer activities of acetone thiosemicarbazone (ATSC) against Ehrlich Ascites Carcinoma (EAC) cells in Swiss albino mice.

MATERIALS AND METHODS

Synthesis of ATSC

The procedure for the synthesis of ATSC was similar to that described in the literature (18). Acetone and thiosemicarbazide were mixed together in 1:1 molar ratio and refluxed for a period of 3-4 hours and then distilled to half of the total volume. The solution was then allowed to stand overnight till a white crystalline product was obtained. The crystals were recrystallized, filtered, washed several times with ethanol and dried at 60°C in an oven. It was then cooled to room temperature and stored in a desiccator.

Characterization of the compound (ATSC)

The formation and purity of the compound was confirmed by taking melting points and infrared spectrum. A strong band appeared at 1646.68 cm⁻¹ confirmed the formation of C=N bond. The other peaks appeared in IR spectra are in accordance with – NH₂, -NH- etc at 3178-3000 cm⁻¹, for C = S at 1164 cm⁻¹ etc. The melting of the compound is very much similar in accordance with the literature value (172-175°C).

Structure of ATSC

The structure of ATSC given below:

\[
\begin{align*}
\text{S} & \quad \text{NH}_2 - C - \text{NH} - N = C(CH_3)_2 \\
\text{NH}_2 - C - \text{NH} - N = C(CH_3)_2
\end{align*}
\]

(Acetone thiosemicarbazone)

N – (1-methylethylidene)-thiosemicarbazone.

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

Tumour cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained by the courtesy of Indian Institute for Chemical Biology (IICB), Kolkata, India. The EAC cells were thereafter propagated in our laboratory biweekly through intraperitoneal injections.

Determination of median lethal dose (LD₅₀)

The LD₅₀ value was determined following conventional methods (19). The test compound was dissolved in 3% DMSO and was injected intraperitoneally to six groups of mice (each group containing six animals) with different doses of [10, 20, 25, 30, 35 and 40 mg/kg (i.p.)]. LD₅₀ was evaluated by recorded mortality after 24 hrs. The dose, at which the 50% mice survived, was considered as LD₅₀ value of the compound.

Preparation of the solution of standard drug

Solution of the standard drug, bleomycin was prepared by dissolving bleomycin in distilled water. Bleomycin was introduced through intraperitoneal injection into mice at the dose of 0.3 mg/kg to determine its antineoplastic activities.

Cell growth inhibition

In vivo tumour cell growth inhibition was carried out by the method as described in the literature (20). For this study five groups of mice (6 in each group) were used. For therapeutic evaluation 13.6x10⁵ cells/mouse were inoculated into each group of mice on the first day. The mice were sacrificed on the 6th day after transplantation and tumour cells were harvested by normal saline. Viable tumour cells per mouse of the treated group were counted and compared with those of control.

Average tumour weight and survival time

These parameters were measured under similar experimental condition as stated in the previous experiment. Tumour growth was monitored daily by measuring weight change. The host survival time was recorded and expressed as mean of survival time in days. The percent increase of life span was calculated as follows:

\[
\text{Percent increase of life span (ILS)} \% = \left( \frac{\text{MST of treated group}}{\text{MST of control group}} - 1 \right) \times 100
\]

Mean survival time

\[
\text{Mean survival time} = \frac{\sum \text{Survival time (days) of each mouse in a group}}{\text{Total number of mice}}
\]

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

The haematological parameters viz. white blood cell (WBC), red blood cell (RBC) and haemoglobin content were determined by standard method using cell dilution fluids and haemocytometer. For this purpose blood was collected from mouse by tail puncture. Four groups (with n = 6) were taken for this purpose. Group one to three received the test compound at the doses 1.0, 1.5 and 2.0 mg/kg (i.p.) respectively. Group four was served as untreated control. Treatment was started after 24 hours of tumour inoculation and continued for 10 consecutive days.

Determination of the effects of ATSC on Peritoneal Cells

Effects of the test compound on normal peritoneal cells were determined by counting total peritoneal cells and the number of macrophages. Three groups of normal mice were treated with the test compounds (i.p.) at the dose of 1.0 mg/kg, 1.5 mg/kg and 2.0 mg/kg for three consecutive days and the fourth group (untreated) was served as control. After 24 hours of last treatment animals were sacrificed after injecting 5 ml of normal saline (0.98%) into peritoneal cavity of each mouse. Intraperitoneal exuded cells and number of macrophages were counted with 1% neutral red by haemocytometer.

Statistical analysis

All values were expressed as mean ± 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.

RESULTS

Lethal dose of ATSC was found to be 20 mg/kg for intraperitoneal treatment in swiss albino mice. The effects of the test compound and bleomycin on EAC cell growth on day six after tumour transplantation were shown in Table 1. Treatment with ATSC resulted in cell growth inhibition at the doses of 1.0, 1.5 and 2.0 mg/kg (i.p.) by 69.22%, 78.47% and 83.18% 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 the test compound at different doses were summarized in Table 2. Treatment with ATSC resulted in cell growth inhibition at the doses of 1.0, 1.5 and 2.0 mg/kg (i.p.) by 69.22%, 78.47% and 83.18% 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.

Treatments of the animals inoculated with EAC cells previously with the test compound resulted in the inhibition of tumour growth. Tumour weight reduction ability of the compound was shown in Figure 1.
Haematological parameters were found to be altered from normal values along with the growth of tumour. Haemoglobin content and RBC counts were found to be decreased but WBC counts were found to be increased after inoculation of EAC cells in Swiss albino mice. After treatment with the test compound at doses 1.0, 1.5 and 2.0 mg/kg (i.p.), it was observed that the parameters restored more or less towards normal. During treatment of normal mice with ATSC, haemoglobin content and RBC counts were found to be decreased but WBC counts were found to be increased as shown here (Figures 5-7) for the first 10 days. After treatment period, it was observed that the parameters restored almost towards normal (Figures 5-7).

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.

Table 2: Effect of ATSC on survival time of EAC cell bearing mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nature of the drug</th>
<th>Dose in mg/kg (i.p.)</th>
<th>Mean survival time (Days) Mean ± SEM</th>
<th>% increase of life span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (EAC cell bearing mice)</td>
<td>-</td>
<td>-</td>
<td>22.0 ± 2.5</td>
<td>-</td>
</tr>
<tr>
<td>EAC + Bleomycin</td>
<td>Antibiotic</td>
<td>0.3</td>
<td>40.0 ± 0.86*</td>
<td>90.47</td>
</tr>
<tr>
<td>EAC + ATSC Synthetic</td>
<td>1.0</td>
<td>37.50 ± 1.6*</td>
<td>69.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>39.67 ± 0.82*</td>
<td>80.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>41.0 ± 2.44</td>
<td>86.63</td>
<td></td>
</tr>
</tbody>
</table>

The number of EAC cell 136 ± 10^4 per ml was inoculated into five groups of mice (six in each group) on day 0. Three groups were treated with ATSC at the doses of 1.0 mg/kg (i.p.), 1.5 mg/kg (i.p.) and 2.0 mg/kg (i.p.) respectively after 24 hours of EAC cell inoculation in mice and continued for 10 days. Group four was treated bleomycin at the doses of 0.3 mg/kg (i.p.). Fifth group was considered as untreated control. Mean survival period was measured by monitoring life span of each mouse. Results were shown in mean ± SEM and compared with normal mice. * P<0.05 between control and treated group.

Figure 1: Effect of ATSC on the rate of tumour growth inhibition in mice.

Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.
DISCUSSION

The potency of ATSC as anticancer agent has been judged by measuring the inhibition of cell growth, reduction in tumour weight and enhancement of mean survival time of the EAC cell bearing mice. The result presented above showed that the compound ATSC at dose 2.0 mg/kg (i.p.) can inhibit cell growth of tumour bearing mice sufficiently, reduce tumour growth rate markedly and increase life span dramatically. These are considered as very important and promising aspects in justifying the potency of a compound in cancer chemotherapy (21). The major problems usually in cancer chemotherapy which are being encountered are myelosuppression and anemia (22-23) due to reduction of RBC and haemoglobin contents. This is probably due to the deficiency of iron in hemolytic or myelopathic condition (24). After treatment with the compound under investigation, all the depleted haematological parameters restore towards normal. The host toxic effect of the compound is not very high. In addition, the treatment in normal mice increases the macrophages and peritoneal cells which have also been considered as a very vital event in acquiring self destroying activity of the living being towards cancer cells (25). Enhancement of macrophages might produce some cytokines such as tumour necrosis factor and interleukins inside the peritoneal cavity, which in turn may be responsible for killing of tumour cells (26). Since the LD50 value of ATSC is found to be 20 mg/kg (i.p.), ATSC is expected to be an effective anticancer agent with low toxicity (at doses up to 2 mg/kg). However, the information obtained from the present investigation is insufficient.

Table 3: Effect ATSC on the enhancement of normal peritoneal cells in mice.

<table>
<thead>
<tr>
<th>Experiment type</th>
<th>Dose in mg/kg</th>
<th>Macrophages Mean ± SEM</th>
<th>Total peritoneal cells Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal)</td>
<td>-</td>
<td>(1.62 ± 0.034) x 10^6</td>
<td>(7.93 ± 0.04) x 10^6</td>
</tr>
<tr>
<td>Normal + ATSC</td>
<td>1.0</td>
<td>(2.24 ± 0.016) x 10^6*</td>
<td>(7.25 ± 0.40) x 10^6*</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>(2.58 ± 0.24) x 10^6*</td>
<td>(8.25 ± 0.61) x 10^6*</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>(3.14 ± 0.13) x 10^6*</td>
<td>(9.65 ± 0.31) x 10^6*</td>
</tr>
</tbody>
</table>

Three groups were treated with ATSC at the doses of 1.0 mg/kg (i.p.), 1.5 mg/kg (i.p.) and 2.0 mg/kg (i.p.) respectively after 24 hours of EAC cell inoculation in mice and continued for 10 days. Fourth group was considered as untreated control. Macrophages and total peritoneal cells were counted by haemocytometer with 1% neutral red dye. Results were shown in mean ± SEM and compared with normal mice. * P < 0.05 between control and treated group.

Figure 2: Effect of ATSC on RBC of EAC bearing mice on day 5, 10, 15 and 25.

Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.
for ATSC to be used as a novel anticancer agent in clinical practice. Many more investigations have to be carried out with this compound and also with its derivatives using various other cancer cell lines and higher animal models in order to confirm it as a potent anticancer agent.

ACKNOWLEDGMENTS

The authors are grateful to IICB (Kolkata, India) authority for providing the EAC cells and ICDDR’B (Dhaka, Bangladesh) for kindly providing mice and the standard mouse-pellet. The authors are also thankful to university grant commission, Bangladesh for monetary support.

Figure 3: Effect of ATSC on WBC of EAC bearing mice on day 5, 10, 15 and 25.

Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.

Figure 4: Effect of ATSC on haemoglobin content of EAC bearing mice on day 5, 10, 15 and 25.

Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.
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**Figure 5**: Effect of ATSC on RBC of normal mice on day 5, 10, 15 and 25. Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.

**Figure 6**: Effect of ATSC on WBC of normal mice on day 5, 10, 15 and 25. Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.

**Figure 7**: Effect of ATSC on haemoglobin content of normal mice on day 5, 10, 15 and 25. Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.
REFERENCES


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