MERCURY(II) CYSTINE COMPLEX AS ANTINEOPLASTIC AGENT

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SUMMARY: Antineoplastic activity of mercury(II) cystine complex was studied against ehrlich ascites carcinoma (EAC) cells in Swiss Albino mice. Cell growth inhibition, increase of life span, haematological parameters, alkaline phosphatase activity of tumour bearing mice inoculated with EAC cells were studied with the test compound. It was found that this compound significantly inhibited the tumour cell growth, enhanced life span of the tumour bearing mice at dose 6 mg/kg i.p. Such treatment also restored the altered haematological parameters and serum alkaline phosphatase activity very closely towards normal. The compound can be considered as a potent antineoplastic agent.

Key Words: Mercury(II) cystine complex, antineoplastic activity, ALK activity, EAC cells.

INTRODUCTION

Chemicals both organic and inorganic now used in biomedicinal purposes possess the property of selectivity in action on various living beings. These chemicals are used in such a dose and condition that they do not show any evident detrimental effect to the host. Consequently a good number of metal complexes are now being used in biochemical researches. Complexes of nickel, cadmium and mercury both with cystine and tyrosine are found to be potent antibacterial and antifungal agents (1,2). The pesticidal activity of such complexes has also been reported recently (3). The applicability of nickel(II) complexes as antineoplastic agents has been studied (4-6). Realizing such biological activities, the present piece of work has been carried out to investigate the effectiveness of mercury(II) cystine complex as antineoplastic agent against *Ehrlich Ascites Carcinoma* (EAC) cells *in vivo*. A comparative study has also been made here with the data obtained in parallel experiments with a standard *drug bleomycin*.

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MATERIALS AND METHODS

Chemicals

All chemicals were purchased from BDH (England) and used without further purification.

Animals

Male Swiss Albino mice (collected from International Center for Diarrhoeal Diseases Research, Bangladesh, ICDDR'B, Dhaka) of 6-8 weeks old, weighing 20-25 grams were used throughout the study. Animals were fed standard mouse pellet obtained from ICDDR'B.

Tumour cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained through the courtesy of Department of Pharmacy, Jahangir Nagar University, Dhaka and maintained every 12 days intraperitoneal (i.p) inoculation of approximately $2x10^6$ cells/mouse.

Synthesis of mercury(II) cystine complex

Mercury(II) cystine complex was prepared by mixing saturated solutions of mercury(II) acetate and cystine in 1:1 molar ratio by the method as described elsewhere (1).

Characterization of the complex

The infrared spectra of the compound as KBr disc were recorded by using Shimadzu FTIR 8101 (Japan). Thermogravimetric analysis for water content was done with a thermoanalyser of Mettler Instrument Corporation (Highstown NJ). Hg(II) content was determined colorimetrically (7). The other physical parameters were determined by usual methods.

LD_{50} determination

LD_{50} was estimated by 'acute toxicity test' (8). The complex was dissolved in distilled water and injected at different doses (i.p) to different groups of mice. LD_{50} was evaluated by recording the mortality up to 24 hours.

Cell growth inhibition

For this study, five groups of mice (six in each) were used. EAC cells ($2x10^6$) were inoculated in all mice on day 0. Treatments were started after 24 hours of EAC cells inoculation and continued for five days. Group 1 served as control. Group 2 received *bleomycin* (0.3 mg/kg). Group 3 to 5 received the test compound at the doses of 2 mg/kg i.p, 4 mg/kg i.p and 6 mg/kg i.p. Mice in each group were sacrificed on the sixth day and the total intraperitoneal tumour cells were harvested by normal saline (0.98%). Viable cells were first identified by using trypen blue and then counted by a haemocytometer. Total number of viable cells in every animal of the treated groups was compared with those of control group (EAC treated only).

Survival time

For this experiment five groups of mice (six in each) were used. For therapeutic evaluation $2x10^6$ EAC cells were inoculated into all mice on day 0. Treatments were started after 24 hours of tumour cell inoculation at the doses of 2 mg/kg i.p per day (Group 1), 4 mg/kg (i.p) per day (Group 2) and 6 mg/kg (i.p) per day (Group 3) respectively. Group 4 was treated with *bleomycin* at the dose of 0.3 mg/kg (i.p) per day. Group 5 was considered as untreated (control). Treatments were continued for 10 days. Body weight of each mouse was measured everyday by a laboratory balance. Tumour weight was obtained by subtracting the weight of tumour bearing mouse from its weight on starting day of inoculation with EAC cells. Mean survival time (MST) of each group of mice (six in each) was noted. Survival time of treated group of mice was compared with those of control group by using the following calculation.

Percent increase of life span,

$$ILS\ \% = \frac{(MST\ of\ treated\ group)}{(MST\ of\ control\ group)} \times 100$$

$$MST = \frac{\Sigma\ Survival\ time\ in\ days\ of\ each\ mouse\ in\ a\ group}{Total\ number\ of\ mice}$$

Bioassay of EAC cells

The procedure was a modification of the method as described by Fernandes and Klubes (9). Two groups of mice (n=6) were inoculated with $2.0x10^6$ EAC cells. Group 1 was treated with the test compound at the dose of 6 mg/kg i.p for three consecutive days and Group 2 received the vehicle only (served as control). On day 4, tumour cells from the mice were harvested in cold (0.9%) saline, pooled, centrifuged and reinoculated ($2x10^6$ cells/mouse) in to two fresh groups of mice (n=6) as before. No further treatment was done on these mice. On day 5, the mice were sacrificed and viable tumour cell count/mouse was performed.

Haematological studies

Haematological parameters were studied with the test compound at the doses 2 mg/kg i.p, 4 mg/kg i.p and 6 mg/kg i.p both in normal and tumour bearing mice following usual method (10).

Alkaline phosphatase activity (ALP)

ALP activity of the serum of normal and tumour bearing mice was measured according to the method as described in the literature (11) using para-nitrophenyl phosphate (PNPP) as substrate, in glycine-sodium hydroxide buffer (pH 10). Absorbance was measured at 410 nm using a spectrophotometer. One unit of the enzyme is the amount that hydrolyzes one ?mol of PNPP/minute. ALP activity of the serum of normal and tumour bearing mice treated with the complex (2 mg/kg i.p, 4 mg/kg i.p and 6 mg/kg i.p) was assessed on day 12.

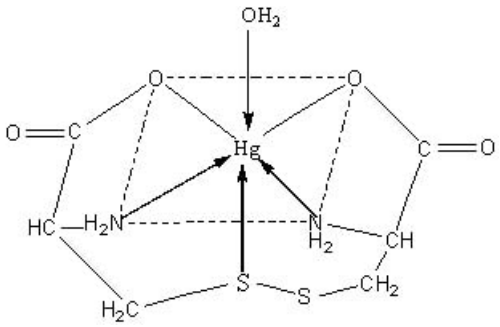
Statistical analysis

The student t-test were used for the statistical analysis of the results.

RESULTS AND DISCUSSIONS

The compound was found to be partially soluble in water and alcohol. The density was found to be 3.50 g/cm^3 The structure of the complex is supposed to be as

Figure 1: Structure of the test compound.



follows with one molecule of coordinated water (Figure 1). IR spectra showed a broad peak at 3600-3300 cm^{-1} for coordinated water and also for NH_2 groups. The shifting of peak from 473 cm^{-1} (for S-S- bond) to 415 cm^{-1} indicated the formation of M-S bond. About 3.77% weight loss at 120-140°C found (due to evaporation of water molecules) from TG analysis, was also in accordance with the proposed structure (1).

Lethal dose (LD_{50}) of Hg(II) cystine complex was found to be 50 mg/kg, which indicated the toxicity of the compound to the host. The compound also showed cytotoxic effect in brine shrimp lethality bioassay with LC_{50} value of 8.0 μg/ml (1). All such data are presented in Table 1.

The effect of Hg(II) cystine complex on EAC cell growth inhibition (*in vivo*) has been shown in Table 2. Treatments with the complex at the doses studied, showed significant tumour growth inhibition. The dose 6 mg/kg (i.p.) enhanced their life span to the same extent as did *bleomycin* at dose 0.3 mg/kg i.p. (Figure 2). Such treatments also decreased the tumour weights remarkably as shown in Figure 3. Evidently the doses above 4 mg/kg i.p were quite comparable with that obtained for *bleomycin*. The effect of the test compound on transplantibility of tumour cells was observed by 58% reduction of intraperitoneal burden in mice. All the haematological parameters in tumour bearing mice (on

Table 2: Effect of Hg(II) cystine complex on EAC cell growth inhibition of tumour bearing mice.

Drug used with EAC treated mice	Dose mg/kg (i.p)	Number of EAC cells/mouse on day 5 after tumour cell inoculation	% Cell growth inhibition
Control (EAC) without drug	-	(6.98 ± 0.51) x 10^7	-
Hg(II) cystine complex	2.0	(0.79 ± 0.32) x 10^7 *	88.68
	4.0	(0.43 ± 0.27) x 10^7 *	93.83
	6.0	(0.39 ± 0.26) x 10^7 *	94.30
Bleomycin	0.3	(0.43 ± 0.10) x 10^7 *	93.83

Results are shown as mean ± SEM ;
Where significant value is * $P < 0.05$ when compared with control.

Table 1: Physical constants and IR spectra of the test compound [Hg(II) cystine complex].

LD_{50}	Yield	Physical form	Melting point	Solubility	Density	Elemental anal. data (%), found (calculated)	Water content, found (calculated)	Characteristic IR spectra cm^{-1}
50 mg/kg	56%w	White crystalline	180°C (Khanam et.al., 2006)	Partially soluble in both water and alcohol	3.5 g/cm^3	C: 15.41(15.77) H: 2.38 (2.63) N: 6.02 (6.13) Hg: 43.45(43.93)	3.77 % (3.94 %)	3600-3300 w (NH_2 and H_2O) 2830 m (CH_2-) 1460 sh (-CH_2-) 415 s (-S-S-)

w - wide; m - medium; sh - shoulder and s - strong

Table 3: Effect of Hg(II) cystine complex on haematological parameters of normal and tumour bearing mice on day 12 of tumour inoculation.

Mice+dose of test compound	RBC cells/mL	WBC cells/mL	Hb %	Lymphocytes %	Neutrophill %	Monocytes %
Normal mice (without test compound)	$(8.01 \pm 1.78) \times 10^9$	$(6.8 \pm 0.21) \times 10^6$	13.15 ± 0.77	70 ± 0.25	20.5 ± 0.5	9 ± 0.75
Control (EAC treated mice)	$(2.87 \pm 0.48) \times 10^9$	$(27.0 \pm 0.15) \times 10^6$	8.45 ± 0.51	44 ± 0.65	40.0 ± 1.4	13 ± 0.18
EAC + 2 mg/kg	$(4.10 \pm 1.02) \times 10^9$	$(22.2 \pm 0.62) \times 10^6$	9.70 ± 0.15	48 ± 0.25	42.0 ± 0.70	10 ± 0.05
EAC + 4 mg/kg	$(6.30 \pm 0.98) \times 10^9$	$(18.0 \pm 0.58) \times 10^6$	10.8 ± 0.98	58 ± 0.18	35.40 ± 0.37	9 ± 0.36
EAC + 6 mg/kg	$(7.70 \pm 0.62) \times 10^9$	$(12.0 \pm 0.71) \times 10^6$	12.0 ± 0.25	60 ± 0.68	28.0 ± 0.82	8 ± 0.13
N + 2 mg/kg	$(6.90 \pm 0.48) \times 10^9$	$(9.0 \pm 0.25) \times 10^6$	10.1 ± 0.24	79 ± 0.50	24.0 ± 0.48	8 ± 0.63
N + 4 mg/kg	$(6.40 \pm 0.62) \times 10^9$	$(8.0 \pm 0.35) \times 10^6$	11.0 ± 0.15	72 ± 0.52	23.0 ± 0.32	7 ± 0.58
N + 6 mg/kg	$(7.20 \pm 0.82) \times 10^9$	$(10.0 \pm 0.58) \times 10^6$	12.0 ± 0.15	80 ± 0.98	22.0 ± 0.52	10 ± 1.20

Number of mice was 6 per group. Results are shown in mean ± SD. N- normal mice (without EAC cell); EAC- EAC cell bearing mice.

day 12) were found to be significantly changed from normal values (Table 3). The total WBC count was found to be increased with a reduction of haemoglobin content of RBC. The total number of RBC showed a significant change. In differential count, % of neutrophill increased while the lymphocytes count decreased. During the same time interval treatment with Hg(II) cystine complex (at doses of 2 mg/kg i.p. and 4 mg/kg i.p.) shifted these altered parameters towards normal values. At higher dose (above 4 mg/kg i.p), these values were found to be restored more effectively. Since interference with the cell division is responsible for effectiveness of most of the anticancer agents, the toxicity associated with their use is usually encountered in various parts of the body (such as gastrointestinal tract and bone marrow), where rapid cell proliferation takes place (12). The treatment with Hg(II) cystine complex inhibited tumour cell growth, enhanced survival time of treated mice and restored the haematological parameters.

Figure 2: Effect of Hg(II) cystine complex on increase of life span of tumour bearing mice. Number of mice in each group was 6.

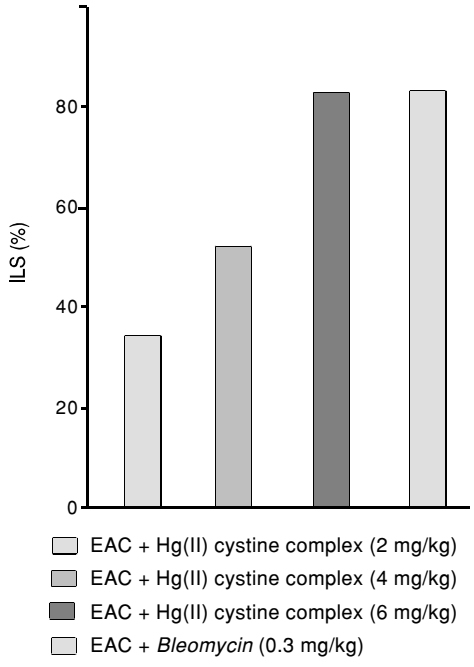
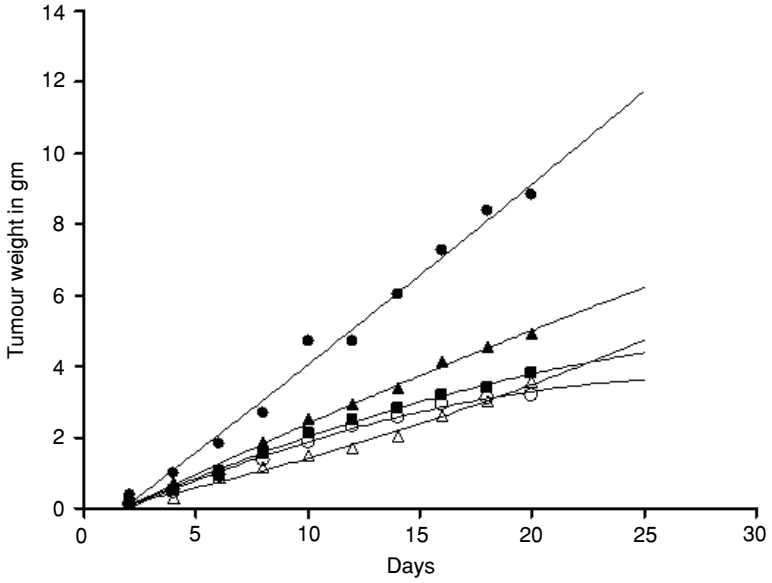


Table 4: Effect of Hg(II) cystine complex on serum alkaline phosphatase activity of normal and tumour bearing mice.

Mice + test compound/*bleomycin*	Enzyme activity (μmol of PNPP hydrolyzed min^{-1} mL^{-1} serum)
Normal	0.135 ± 0.08
Control (only EAC cell bearing mice)	0.050 ± 0.03
EAC + *Bleomycin*	0.118 ± 0.15
EAC + Hg(II) complex (2 mg/kg)	0.060 ± 0.06
EAC + Hg(II) complex (4 mg/kg)	0.090 ± 0.11
EAC + Hg(II) complex (6 mg/kg)	0.119 ± 0.21
Normal + Hg(II) complex (2 mg/kg)	0.120 ± 0.20
Normal + Hg(II) complex (4 mg/kg)	0.125 ± 0.26
Normal + Hg(II) complex (6 mg/kg)	0.130 ± 0.18

Number of mice was 6 per group. Results are shown in mean ± SD.
EAC- EAC cell bearing mice ; Normal- mice without EAC cell.

Figure 3: Effect of test compound on body weight due to tumour growth in mice. ●-Untreated EAC cell bearing mice, ▲-2 mg/kg, ■-4 mg/kg, o-6 mg/kg, of the test compound and △-0.3 mg/kg of *bleomycin*.



Growth of EAC cells in mice showed a depletion of ALP activity in serum (Table 4). It was observed that ALP activity of human cancer cell could be increased by treatment with compounds like sodium butyrate and bromo deoxyuridine (13). Significant recovery of the enzyme activity in the serum with the reduction of tumour growth after treatment with Hg(II) cystine complex was found at dose 6 mg/kg (i.p.).

CONCLUSION

The compound showed strong cytotoxic effect in brine shrimp lethality bioassay. Since LD_{50} value is much higher than the doses used and since the results obtained are promising regarding cancer treatment, the test compound can be taken as an effective antineoplastic agent. Further experiments should be carried out in advanced level with other cell lines to get more effective results.

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