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In Vivo Antitumor activity of Bis (4-bromobenzaldehyde-4-iminacetophenone) tetraaquochromium (III) Sulphate Complex against Ehrlich Ascites Carcinoma Cells Induced in Mice

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ABSTRACT

Metal complexes are important resource for the generation of chemical diversity in the search for novel therapeutic and diagnostic agents, especially in the area of anticancer drug development. It was of our interest to investigate the in vivo antitumor activity of Bis (4-bromobenzaldehyde-4-iminacetophenone) tetraaquochromium (III) Sulphate (BBIA-Cr) complex against Ehrlich ascites carcinoma cells induced in male albino mice. 125 Male Swiss albino mice were equally divided into five main groups: Normal, EAC, vehicle, BBIA-Cr and treated groups. Results indicated that treatment with BBIA-Cr had prevented the accumulation of ascetic fluid and consequently decreased cell viability, which in turn resulted in increased life span of tumor bearing mice. Intraperitoneal injection of BBIA-Cr to EAC inoculated mice had significantly improved hematological parameters compared to non-treated EAC group. These entire indicate that BBIA-Cr complex exhibited antitumor activity against EAC induced in mice.

Keywords: Chromium complexes; Biological activity; Ehrlich tumors; In vitro cytotoxicity; In vivo cytotoxicity.

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INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world. It is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, local tissue invasion and distant metastases. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans (1). Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells, whereas the rest may damage healthy cells and tissue (2). A need for new drugs stems from the increased body resistance to the available chemotherapeutic agents.

Metal complexes with Schiff bases have numerous application, as cancer treatment (3), antibacterial agent (4) and antivirus agent (5). Metal complexes are important resource for the generation of chemical diversity in the search for novel therapeutic and diagnostic agents especially in the area of anticancer drug development (3). Cis-dichlorodiammine platinum (II); cisplatin; represents one of the most active and clinically useful agents used in the treatment of cancer. In common with many other cytotoxic drugs, cisplatin induces normal tissue toxicity particularly to the kidney (6). Despite the success of cisplatin, however, it lacks selectivity for tumor tissue, which leads to severe side effects. Various tumor cell lines are now growing resistance to cisplatin (7).

A large number of Chromium (III) complexes show promising antimicrobial activities against bacterial strains and fungi (8). Furthermore, several chromium complexes have been reported for their potent cytotoxicity (7; 9). A novel chromium (III) complex was synthesized, Ramadan et al. (10), and was characterized by elemental analysis, mass and IR spectrometry. The complex showed promising antimicrobial activity against gram positive and gram negative bacteria and fungi. The complex, also, exhibited moderate in vitro cytotoxic activity against liver carcinoma cell line (HepG2, IC50 value =12.2µg/ml).

Experimental tumors have great importance for the purposes of modeling, and Ehrlich ascites carcinoma (EAC) is one of the commonest. It appeared firstly as a spontaneous breast cancer in a female mouse and then Ehrlich and Apolant in1905 used it as an experimental tumor by transplanting tumor tissues subcutaneously from mouse to mouse (11). EAC is referred to as an undifferentiated carcinoma, and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and does not have tumor specific transplantation antigen (12). These entire make EAC an efficient model of experimental tumor induced in animals.

Our interest in investigation of some transition metal complexes as antitumor (10; 13) drugs has prompted us to carry out the in vivo antitumor activity of bis (4-bromobenzaldehyde-4-iminacetophenone) tetraaquochromium (III) sulphate complex against EAC tumor induced in mice.

MATERIALS AND METHODS

Materials

Preparation of bis-(4-bromobenzaldehyde-4- iminacetophenone) tetraaquochromium (III) sulphate (BBIA-Cr) complex

The complex under investigation was prepared according to the method described by Ramadan et al. (10). All solvents were of analytical reagent grade and were purified by standard methods. 4-aminoacetophenone, 4-bromobenzaldehyde and chromium sulphate were purchased from Aldrich.

EAC Cells

Cells were obtained from American Type Tissue Culture Collection, Manassas, VA, USA. Cells were maintained in vivo in Swiss albino mice by intraperitoneal (ip.) transplantation of 2 x106 cells/mouse after 8 days (14). EAC cells were used for cytotoxicity study (2 x106 cells/mouse in 0.2 ml).

Animals Management and groups

125 Male Swiss albino mice (8-10 weeks of age, 27-33 g body weight) were obtained from King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were kept for one week acclimatization period under controlled conditions of temperature (23-25 Cº), humidity (50-55%) and light, dark cycle (12 h L / 12h D). Animals had free access to water and standard diet throughout the experiment. Mice were equally divided into five main groups as follows: Normal group (mice were i.p. injected with 0.2 ml saline, 3 times/week for 2 weeks), EAC group (mice were ip. inoculated with 2x106 Ehrlich ascites carcinoma cells/mouse and were monitored for 14 days), Vehicle group (healthy mice were injected with 0.2 ml of DMSO: H2O, 4:1, 3 times/week for 2 weeks. BBIA-Cr group (animals in this group were ip. injected with LD10 of BBIA-Cr complex; 70 mg of BBIA-Cr/Kg body weight freshly dissolved in vehicle; 3 times/week for 2 weeks and treated group (animals were i.p. inoculated with EAC cells; 2x106 cells/mouse; followed after 24 h, by ip. injection of LD10 of BBIA-Cr dissolved in vehicle, 3 times/week for 2 weeks.

Blood Samples and Ascetic Fluid Collection

Animals were monitored regularly for alterations in body weight, for the development of any signs of toxicity and mortality. Body weight was registered at day 0,3,6,9 and day15 (day of animal sacrifice). After the last dose, five mice from each group were left for survival study, while the rest of animals were fasted overnight, blood was withdrawn for determination of hematological parameters.

Methods

Food Intake

The weekly intake of food by mouse was calculated using the formula described by Anand et al. (15):

Feed consumed by 5 animals/cage/week =

Total quantity of feed offered during that week (gm) – Feed left over on last day of week (gm)

Feed consumed by individual animal/week =

Feed consumed by 5 animals/cage/ week/5 (number of animals/cage)

Change in Body Weight

Body weight was registered for each mouse at begging of the experiment and every 3 days till day 15 (day of animal sacrifice). The percent change in body weight was calculated using the formula described by Kuttan et al. (16):

$$\text{Percentage change in weight} = (W2 - W1) / W1 \times 100$$

Where W1 is the body weight of each mouse at the start of the experiment and W2 is the body weight of animal at the end of observation.

Measurement of relative organs weight

The relative organs weight of the vital organs for each animal was calculated using the formula described by Sahgal et al. (17).

$$\text{Relative organ weight} = (\text{Absolute organ weight} / \text{body weight of mice on the day of sacrifice}) \times 100$$

Mean Survival time and Percent in Life Span

Mean survival time (MST) and lifespan (% LS) were calculated for each group using the equations described by Mazumder et al. (18) and Gupta et al. (19).

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MST = (Day of 1st death + Day of last death) / 2
% LS = {(MST of treated group / MST of control)-1} × 100

Measurement of Ascetic Fluid Volume & EAC Cells Viability

The developed ascetic fluid was obtained from each mouse in EAC-bearing animals and BBIA-Cr treated groups under aseptic conditions and was measured. The fluid was centrifuged to collect EAC cells. The cells were tested for viability by trypan blue using hemocytometer.

Hematological Parameters

Blood obtained from retro-orbital was used for determination of hemoglobin (Hb) contents, red blood cells (RBCs) and white blood cells (WBCs) count.

Statistical analysis

Statistical analysis was performed using SPSS 24.0 for windows (SPSS Inc, USA). Descriptive statistics were shown as ± standard error of mean & using mean rank to describe the ordinal data. Kruskal-Wallis test was performed for comparing more than two groups of ordinal, non-parametric data to determine if they are statistically different. Independent samples' Mann-Whitney (U) test was used as a post hoc test to compare the medians of two groups of ordinal, non-parametric data to determine if they are statistically different. Correlation coefficient (r) was used to estimate the effect of size that describe the proportion of total variability attributable to a variable & used Cohen's effect size estimates to interpret the meaning of the (r) score. P value smaller than 0.05, was considered statistically significant.

RESULT

Changes in food intake:

Significant elevation in food intake in EAC bearing mice compared to normal mice at first and second weeks was observed (Fig.1a). Same observation was noted in treated mice group (Fig. 1b). No significant alteration in food intake was observed in healthy mice injected with BBIA-Cr.

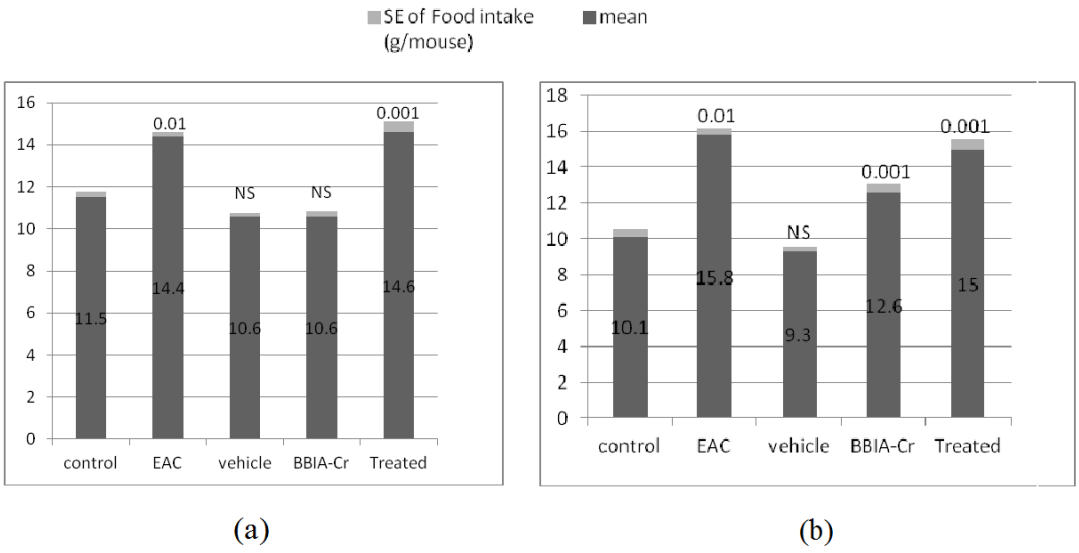

Fig. 1: Food intake during first (a) and second (b) weeks. Numbers above the columns indicate p value for each group versus normal group.

Changes in body weight:

At the beginning of the experiment (zero days), the mean values of body weight for both EAC bearing mice and treated groups were matched with normal group mean value. Significant increase in body weight was noted in EAC group after 9 and 15 days compared to normal group. BBIA-Cr treated mice bearing tumor

showed significantly decreased body weight compared to normal mice group after 3, 6, 9 and 15 days of treatment, although the body weight was matched with those for normal mice group at the beginning of the experiment. The changes in body weight of the studied groups during 15 days of experiment are shown in Table 1.

The percent changes in body weight, in all studied groups, between day zero and day 15 are shown in Fig. 2. Mice inoculated with EAC tumor cells showed a maximum gain in body weight, at the end of experiment, that amount to 33.4 %, which was significantly higher than the percent increase in normal mice ($p < 0.001$). Treatment by BBIA-Cr complex after tumor inoculation resulted in a net decrease in body weight by 5.4% at the end of experimental period compared to zero days, which was significantly lower compared to normal mice. Results indicated no significant difference in the percent changes of body weight between day zero and day 15 for vehicle compared to normal mice.

Table 1: Body weight (B.Wt) during experimental period in all studied groups

groups Parameters	Normal	EAC	Vehicle	BBIA-Cr	Treated
B.Wt at 0 day *p **p	30.6±0.7 - -	30.7±0.5 NS -	31.6±0.5 NS NS	29.2±0.61 NS 0.05	30.7±0.49 NS NS
B.Wt after 3 days *p **p	34.9±0.92 - -	33.6±0.52 NS -	34.1±0.75 NS NS	30.1±0.66 0.01 0.001	30.8±0.62 0.02 0.001
B.Wt after 6 days *p **p	35.1±1.09 - -	35.2±0.86 NS -	35.2±0.58 NS NS	32±0.51 0.02 0.001	30.7±0.62 0.01 0.001
B.Wt after 9 days *p **p	34.4±0.94 - -	37.9±1.45 NS -	34.8±076 NS 0.02	31.6±0.64 0.04 0.001	30.8±0.73 0.01 0.001
B.Wt after 15 days *p **p	35.1±1.09 - -	40.9±1.73 0.01 -	35.8±0.58 NS 0.001	31.8±0.82 0.03 0.001	32.4±0.91 NS 0.001

*p: values vs. normal group mean value; **p: values vs. EAC group mean value; NS: non-significant; p value > 0.05 is significant.

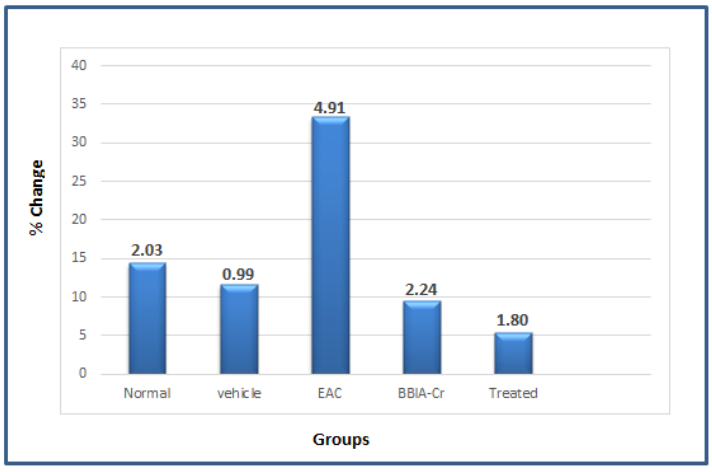

Fig. 2. The means and standard errors of percent changes in body weight.
p value vs. control group: EAC (0.001); vehicle (NS); BBIA-Cr (NS) and treated (0.001).

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Mean survival time (MST) and life span (LS):

MST and LS were calculated for both BBIA-Cr and treated groups using normal mice group as a control for BBIA-Cr while EAC group was a control for treated group. Treatment with 70 mg BBIA-Cr/kg after EAC inoculation improved the mean survival time of mice which in turn resulted in prolonged life span (54.7 %). Normal mice injected with a dose equivalent to 70mg/kg body weight of BBIA-Cr showed reduction in their MST resulting in decreased life span by 56% (Table 1).

Table 2: Mean survival time (MST) and life span (LF) in all groups.

Groups parameters	Normal	EAC	Vehicle	BBIA-Cr	Treated
MST(days)	120	26.5	120	53.2	41
LS (%)	-	-	-	-56	54.7

Tumor volume and EAC cell viability in all groups: Ascetic fluid, viable cell count and percent of cell viability were significantly higher in tumor bearing mice group. Injection of BBIA-Cr after, 24 hours of EAC cells inoculation to mice had significantly increased non-viable cells and consequently decreased cells viability with reduction of ascetic fluid accumulation.

Table 3: Ascetic fluid volume & EAC cell viability in tumor bearing and treated groups.

Groups Parameters	EAC	Treated
Ascetic fluid (ml)	12.8±6.14	1.53±1.47*
Viable cell count (x108cells/mouse)	8.6±5.6	1.48±0.92*
Non-viable cell count (x108cells/mouse)	1.09±0.94	1.62±0.33*
Viable cells (%)	88.82±3.5	64.5±10.1*
Non-viable cells (%)	10.98±3.6	35.3±10.2+*

*p value ≤ 0.001.

Hematological profile:

Hematological parameters of EAC inoculated mice were significantly altered compared to healthy normal animals (RBCs and Hb contents were decreased while WBCs were increased,). Intraperitoneal injection of BBIA-Cr to EAC inoculated mice had significantly improved hematological parameters, compared to non-treated EAC group. It is worth to indicate that administration of Cr-complex to healthy mice (BBIA-Cr group) had significantly affected hematological parameters; resulting in pronounced anemia; compared to healthy mice.

Table 4: Hematological parameters in all studied groups (□ ± SE).

Groups Parameters	Normal	EAC	Vehicle	BBIA-Cr	Treated
Hb (g/dl)	14.5±0.22	11.9±0.19	13.2±0.76	7.7±0.19	11.58±0.14
*P	-	0.001	NS	0.001	0.001
**P	-	-	0.01	0.001	NS
RBCs (x106 cells /mm3)	8.5±0.09	6.7±0.13	7.8±0.47	6.8±0.52	8.1±0.97
*P	-	0.001	NS	0.01	NS
**P	-	-	0.01	NS	0.01
WBCs (x103 cells/mm3)	7±0.40	23.8±3.63	5.4±0.53	12.6±0.28	13.3±0.21
*P	-	0.001	0.02	0.001	0.001
**P	-	-	0.001	0.001	0.001

Hb: hemoglobin; RBCs: red blood cells; WBCs: white blood cells. *p value vs. control group; **P value vs. EAC group. NS: non-significant; P value < 0.05 is significant.

DISCUSSION

The present study is considered a preliminary study. It aimed to evaluate the antitumor activity of Bis (4-bromobenzaldehyde-4-iminacetophenone)tetraaquochromium(III) sulphat (BBIA-Cr) against Ehrlich ascites carcinoma (EAC) induced in mice. The t study indicated significant increase in body weight following inoculation of EAC cells to healthy animals, reaching 33.4 % increase after 15 days of EAC inoculation. The increased body weight in mice bearing EAC tumor was mainly due to accumulation of ascetic fluid. Earlier study (20) indicated that Ehrlich ascetic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascetic fluid formation and accumulation. Anticancer effect is quantified by attenuation of EAC-induced weight gain, decreased ascites fluid volume and increased non-viable cell count (20).

In the current study, Injection of 70 mg of BBIA-Cr complex had significantly attenuated EAC-induced weight gain by decreasing ascetic fluid accumulation. The ascetic fluid which is formed constitutes a direct nutritional source for tumor cells (21). Moreover, treating EAC inoculated mice with BBIA-Cr complex resulted in decreased viable EAC count and increased mean survival time of tumor bearing mice (41 days) compared to non-treated EAC control group (26.5 days), which in turn resulted in prolonged life span by 54.7%. This result might point out to the effectiveness of the complex as an anticancer agent, since one of the reliable criterions for judging the value of anticancer drug is the prolongation of animal's life span by more than 25% (22; 23).

In our work, increased food intake by mice bearing EAC tumor during the studied experimental period, probably may enhance the growth of transplanted tumor cells, by enhancing glycolysis rate of the tumor, Warburg's effect (24; 25; 26). It is well known that dietary energy restriction (DR) often referred to "as under nutrition without malnutrition" is a potent inhibitor of the process of carcinogenesis. DR can dramatically decreases tumor development in multiple models (27). Protection against tumor development by DR is a very general phenomenon, because spontaneously arising (28) as well as chemically induced cancers (27) is suppressed by DR. Many physiological changes occur in response to DR as changes in pituitary derived hormones and decreases in growth hormone, insulin and IGF-1 (29). IGF-1 levels were reduced in DR-treated rats concomitant with a decrease in cancer incidence (30). IGF-1 inhibits apoptosis and stimulates proliferation (31). Furthermore, it has been hypothesized that DR primarily targets neoplastic cells by reducing net energy status and particularly glucose metabolism, therefore lower the metabolism of transformed cells, since more than 75% of tumor types relay on aerobic glycolysis (24). This finding was recently supported by Singh et al. (32) who reported that dietary supplementation of the glycolytic inhibitor, 2-diphosphoglycerate, to mice effectively inhibits tumorigenesis same as DER effects.

In this study, the hematological parameters of untreated tumor-bearing mice were significantly changed from those of the normal group. There were significant decreases in the levels of hemoglobin and RBCs count and an increase in WBCs in EAC-bearing mice. The effect of EAC inoculation on hematological profile of the animals bearing tumor was reported by many authors (33; 34). Myelosuppression and anemia (reduced haemoglobin) have been frequently observed in ascites carcinoma (35), which could be mainly due to iron deficiency, either by haemolytic or myelopathic conditions leading to reduced RBC number (35; 36). Treatment by the complex under investigation had brought back the hemoglobin content, RBCs and WBCs count more or less to normal levels.

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