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STUDY ON THE COMPLEX FORMATION AND ANTICANCER EFFECT OF COMPLEX, ZINC (II)- DACARBAZINE

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ABSTRACT

Dacarbazine, also known as imidazole carboxamide, is an antineoplastic chemotherapic drug, which interfere with cell growth and impede the formation of new tissues. The complexation properties of dacarbazine (dac), being a compound of interest for anticancer research, may be of interest. The physiochemical, microbial and pharmocological studies of Zn-(II)-dacarbazine complex have been done in solid and aqueous phase. According to their polarographic studies, elemental analysis and IR spectral study, the formula of complex has been found to be 1 : 1 Zn (II)-dacarbazine. By using polarographic method at 25 ± 1°C and ionic strength of μ = 10 (KCl), the metal ligand interaction of complex has been found.

The "Raper′s method" is used for microbial study against various pathogenic bacteria The amperometric titration and polarographic studies show that there is a (1 : 1) (M : L) metal ligand interaction. The microbial and pharmacological studies of metal-drug complex revealed the anticancer activity of Zn (II)-dacarbazine as compared to synthetic drug.

Key words: Anticancer, Zinc, Dacarbazine, Complex.

INTRODUCTION

Dacarbazine; [5-(3,3,-dimethyltriazo) imidozole-4-carboximide] is a chemotherapic drug that is used in the treatment of various cancers like malignant melanoma, Hodgkin lymphoma, sarcoma, and islet cell of carcinoma of pancreas[1]. Therefore, the biochemical[2], pharmacological and medicinal studies of metal-drug complex is important. In continuation of the work done on the microbial[3] and pharmacological[4] effect of some metal-drug complexes, the present paper deals with the studies on the, Zn (II)-dacarbazine anticancer drug complex.

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EXPERIMENTAL

Chemicals and reagents

The chemical were used of Anala R (BDH) grade. The drug dacarbazine ($C_6H_{10}N_6O$) was procured from Sigma Chemical Company USA. The solvent used were absolute ethanol and double distilled water.

The pulse polarograph model CL-90-Elico, Hyderabad was used for polarographic measurement and it is coupled with a X-Y polarocard model LR-180. The amperometric titrations[5] were performed on a manually operated set up (a polyflex galvanometer having sensitivity 8.1×10^{-9} amp./div. and an AJCO Vanier potentiometer). The Elico digital pH meter model LI-108 was used to measure the pH of all the test solutions.

Synthesis of solid complex

$ZnCl_2$ and dacarbazine drug solutions were separately prepared in ethyl alcohol (40 : 60 v/v) and were mixed in 1 : 1 molar ratio. The mixture was then refluxed in a round bottom flask for one-two hours. The residue complex was filtered and washed thoroughly to remove any unreacted materials. The complex was dried at low temperatures (40^oC) and stored over P_4O_{10}.

The elemental analysis (C, H, N and O) of the complex was done on Heraeus Varlo Erba elemental analyser model-1108 at CDRI, Lucknow, where as gravimetric method was used for estimation of Zn in the complex.

Biological study of Zn (II)-dacarbazine complex

The microbial screening[6] of Zn (II)-dacarbazine complex against various bacteria *viz. Pseudomonas mangifera, Staphylococus aureus, Salmonella typhi and Vibreo colarae* and *fungi i.e. Trichothesium* and *chrysosporium species.* Sterilized filter paper discs (6 mm) were dipped into complex solution of 0.01 M concentration. Before starting experiment, the bacteria and fungi were separately homogenized with nutrient agar and potatodextrose media at 27-30^oC plated onto the sterilized Petri dishes.

The incubation period is completed after 24 hours and then antimicrobial activities were recorded by measuring the inhibition zone[7] against complex under study. The same experiment was repeated with the control drug, dacarbazine.

The percentage inhibition was calculated using the formula -

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100 \quad \ldots(1)$$

Where

A = Diameter of inhibition zone for control drug dacarbazine and

B = Diameter of inhibition zone for sample Zn (II) – dacarbazine complex.

Pharmacological studies

In vitro and *In vivo*[8] study of anticancer activity of drug-metal complex have been done by following procedure:

In vitro – Mouse sarcoma cell line – 180 were obtained from National Centre for Cell Science, Pune, India as a monolyer culture in Roux bottles (Corning plastics, USA).

Cell culture – The cells obtained were cultured in 5 mL, 24 well cultured plate. The cells were seeded in 2 x 10^5 cells per cell in 1.0 mL of Dalbecco Modified Eagles Medium (DMEM) containing 10% (v/v) foetal calf serum. Pencillin (100 µg/mL) and streptomycin (100 µg/mL) was added to each well. The cells were kept in incubator at 37°C for 4 hr in 5% CO_2 atmosphere and 95% humidity. The cell count was made on Neubaurs chamber (Fine Optik, Germany). Three dilutions i.e. 1 µM, 10 µM and 100 µM/mL of pure drug and its Zn complex was made and then the cells were treated as follows :

Column	Free drug	Column	Melal complex
A	1 µM (1 mL)	D	1 µM (1 mL)
B	10 µM (1 mL)	E	10 µM (1 mL)
C	100 µM (1 mL)	F	100 µM (1 mL)

The culture plate was incubated at 37°C for 4 hour after addition of respective solution and then the cells under study were counted and after this, it was compared with the cell cultured in DMEM without treatment.

Cell viability counts

The "Trypan Blue Dye Exclusion Test[9]" was used for cell viability counts. In this test, two drops of trypan blue were added to each cell culture well and kept it for 15 minutes. The number of stained, non-stained and total number of cells were counted by adding a culture to hemocytometer[10]. Therefore, the percentage inhibition was calculated using the equation :

$$\frac{\text{No. of viable cells} - \text{No. of viable cell after treatment}}{\text{No. of viable cells without treatment}} \times 100 \quad \ldots(2)$$

The experiment of each concentration of the drug and the complex was repeated thrice and statistical conclusions were drawn.

In vivo – The comparative efficiency of pure and complex forms of dacarbazine drug were evaluated from the difference in response after treatment with the two forms of drug :

Animal model : Balb/C mice weight 40-50 g.

Tumor model : Sarcoma cell line -180

Drug : Dacarbazine and its Zn (II) complex.

The nutrient medium DMEM was used for cell growing obtained from NCCS, Pune. The trypsinization[10] (0.2% trypsin) is suspended into tumor cell. The cell suspension was centrifuged to prepared concentrated suspension (1-2 × 10^5 cell/mL). Approximately 10^5 cells of tumor were injected on the dorsal surface of the mouse and allowed to grow. The palpable size was reached by 6-8 days.

The antitumor efficacy of pure and complexed in S-180 tumor bearing mice was done by measuring the time which is required for double the tumor volume from 100-200 mm^3 and it is known as volume doubling time[12]. The indicated dose equivalent to 0.2 mg of free drug and drug complex were injected intravenously and tumor growth was monitored. The size of tumor was calculated by the formula ½ LW2. Where L-long diameter and W-short diameter of the tumor in the above *in vivo* experiment was repeated on two other sets of mice groups.

RESULTS AND DISCUSSION

Polarographic behaviour of dacarbazine with Zn (II)

The Zn (II) and its complex with ligand at pH 7.0 ± 0.02 in 1.0 M KCl under study were found to be reversibly reduced involving three electrons, which was evidenced from the plots of log $i/(i_d-i)$. The reduction was found to be diffusion controlled, which was evidenced by the plot.

$$i_d \text{ vs. } \sqrt{h} \text{ Corr.}$$

The half wave potential[11] of Zn (II) metal ion was shifted to more negative value

with the increase of the dacarbazine concentration and the value of diffusion current also decreases; thereby, showing complex formation of Zn (II) with dacarbazine.

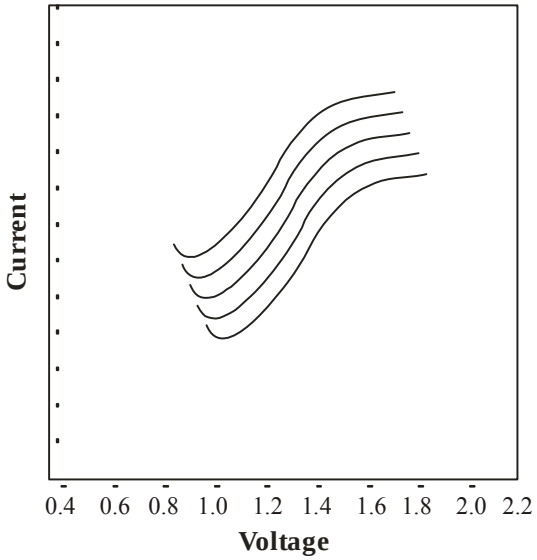


Fig. 1: Polarograms of Zn (II) (1 mM) in 1 M KCl supporting electrolyte at pH 7.0 ± 0.02 and A – without dacarbazine; B – 1 mM dacarbazine; C, D and E – 3, 5 and 6 mM dacarbazine

The plots of $\Delta E_{1/2}$ (shift in the $E_{1/2}$) i.e. $\Delta E_{1/2} = (E_{1/2})_S$ against log C_x (logarithm of the concentration of ligand) were drawn to study the composition and formation constant of complex. Lingane treatment[12] of observed polarographic data reveals 1 : 1 metal : dacarbazine complex formation with log $\beta = 6.2$

Amperometric determination of dacarbazine with Zn (II)

Zn (II) gives a well defined polarographic wave in 1.0 M KCl at pH 7.0 ± 0.02. The diffusion current was found proportional to concenteration of Zn (II). The dacarbazine drug does not produced any wave under the said experimental conditions. The plateau potential for the polarographic curve of Zn (II) at -1.2 V vs Hg was applied for carrying out titration.

The amperometric titration[13] of drug solution with standard solution of Zn (II) gives the shaped curve by plotting the current volume plots and the end point, which is located by graphical method. It reveals metal to drug ratio 1 : 1, which is agreement with the authors observations on the metal ligand equilibria using polarographic method.

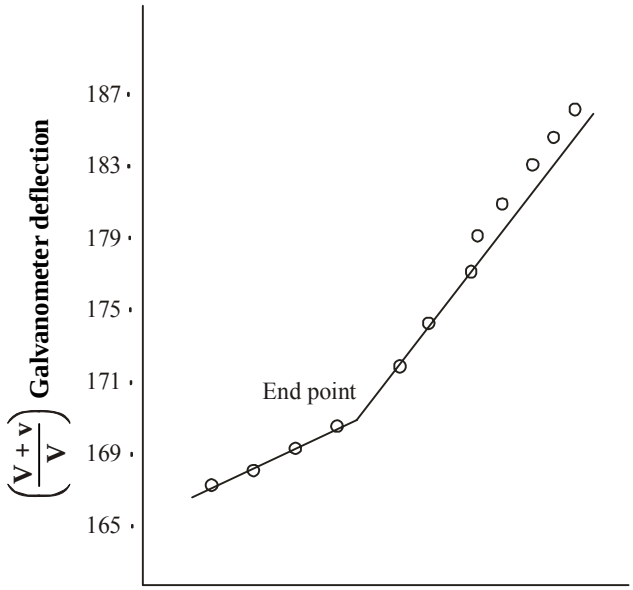


Fig. 2: Amperometric titration of 2 mM/10 mL Dacarbazine; 1 mM/10 mL Zn (II) solution

Characterization of Zn (II)-dacarbazine complex

Elemental analysis

By study of the elemental analysis[14] (Table 1) of drug and its complex with Zn (II) revealed (1 : 1) metal drug : ratio in this complex, which supports the data of polarographic and amperometric method.

Table 1: Analytical data of dacarbazine and its complex with Zn (II)

S. No.	Element	Dacarbazine Calculated (found)	Zn (II)-Dacarbazine complex Calculated (found)
1.	Zn (II)	-	24.62 (23.60)
2	C	38.71 (39.70)	30.18 (30.23)
3	H	4.40 (5.38)	4.12 (4.02)

Cont…

S. No.	Element	Dacarbazine Calculated (found)	Zn (II)-Dacarbazine complex Calculated (found)
4	N	43.02 (46.15)	32.42 (36.35)
5	O	7.97 (8.78)	6.47 (6.08)

IR Spectra

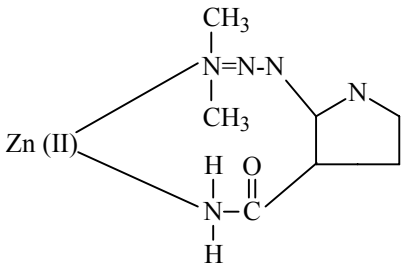
The IR spectra[15] of dacarbazine and its complex with Zn (II) metal gives different frequencies of IR bands from which one can determine their structure. Frequencies obtained are tabulated in Table 2.

Table 2: Principal IR frequencies (cm^{-1}) and their assignment for dacarbazine and its complex

	Ligand (cm^{-1})	Assignment	Zn (II)-dacarbazine (cm^{-1})
1	620, 650 (s)	Imidazole vibrations	620, 650 (s)
2	880 (s)	$CONH_2$ stretching vibrations	850 (s)
3	1280, 1325, 1340 (w)	$-N_2$ stretching	1280, 1325, 1340 (w)
4	1430 (s)	-N = N stretching vibrations	1430 (3)
5	1601 (br)	C-N aliphatic vibrations	1565 (br)

s – Sharp, w-weak, br-Broad

The IR spectral data of dacarbazine drug gives IR bands at frequency 880 cm^{-1} and 1601 cm^{-1}, where as the IR bands in drug complex are shifted to 850 cm^{-1} and 1565 cm^{-1}, respectively due to the involvement of the two nitrogen[17], one each of primary amide and triazo (attached to dimethyl group) groups of the drug in complex[16]. The tentative structure of Zn (II)-dacarbazine complex may be given as under :



Zn (II) – Dacarbazine complex

Microbial study

The result of antimicrobial activities[3] of the Zn (II)-dacarbazine complex are shown in Table 3. A perusal of the data in table clearly shows that zinc-dacabazine complex is found to be more toxic as compared to the control drug against some bacteria and fungi.

Table 3: Antimicrobial study of Zn (II)-dacarbazine complex

Organism	Inhibition zone (mm) conc. of complex (per 10 mL)		Control Zn (II) metal (A) 1.0 M/10 mL	Percentage change over control metal (A-B/A) × 100	Control drug (Y) 1.0 M/10 mL	Percentage change over control drug (Y-B/Y) × 100
	5 mM	**1.0 mM (B)**				
1. Bacteria						
a. *Pseudomonas mangifera*	4.3	11.3	56.3	79.92	10.7	-5.6
b. *Staphylococus aureus*	4.9	12.5	39.7	69.01	13.5	8.88
c. *Salmonella typhi*	6.9	13.8	51.6	73.25	20.5	32.68
d. *Vibreo colarae*	-	12.5	51.5	8.0	9.5	-31.5
2. Fungi						
a. *Trichothesium*	6.5	13.5	38.7	65.11	-	-
b. *Chryosporium species*	5.5	15.6	37.5	58.4	-	-

Pharmacological studies

In vitro

The Table 4 show the results of *in vitro* experiments of pure drug and its complex. On the basis of the *in vitro* experiment[8] results, it was found that Zn (II)-dacarbazine complex is more effective than pure drug. The complex under study showed an increased inhibition against the S-180 tumor cells at all the test concentration i.e. 1, 10, 100 µM/mL. The increased inhibition activity of the complex was 49.1 ± 1.0%, 66.8 ± 1.0% and 90.4 ± 0.9% as against 33.4 ± 1.0%, 51.7 ± 0.6% and 75.6 ± 0.8%, shown by drug respectively. The statistical treatment of the observed inhibition data i.e. standard deviation and coefficient of variance, which never exceeded 0.9 and 1.8%, respectively speaks about the reliability of observed inhibition data.

Table 4: *In vitro* cytotoxity of dacarbazine and Zn (II)-dacarbazine complex against S-180 tumor cells

Compound	Concentration (µM/mL)	% Inhibition after 4 hr.
Dacarbazine	1.0	33.4 ± 1.0 (a)
	10.0	51.7 ± 0.6%
	100.0	75.6 ± 0.8%
Zn (II)-Dacarbazine complex	1.0	49.1 ± 1.0
	10.0	96.8 ± 1.0
	100.0	91.4 ± 0.9

Composite results of three experiments
(a) Mean ± standard error at mean

In vivo

The *in vivo* study of dacarbazine drug and its complex was carried out on mice tumor. The results of dacarbazine drug and its complex are shown in Fig. 3. The results show that the tumor cell injected mice without administering drug or complex after 20 days, percentage was reduced to 0.029 cm^2 on tumor injected mice, who were administered the dacarbazine drug whereas in case of Zn (II)-dacarbazine administered mice (tumor cell injected) shows noted decrease in the tumor volume (0.013 cm^2) was observed. Thus, indicating the *in vivo* tumor inhibition power of the complex over the drug under study, over the experimental time period i.e. 20 days.

The same experiments are repeated for other mice group. However, the statistical treatment of observed inhibition data i.e. standard deviation and coefficient of variance exceeded 1.0 and 2.3%, respectively.

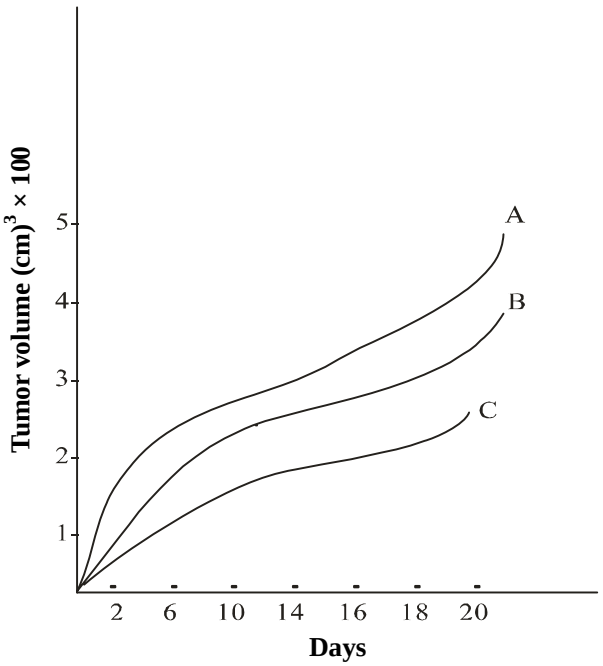


Fig. 3: A – Without drugs; B – With Dacarbazine; C – With Zn (II)-Dacarbazine complex

CONCLUSION

Hence, the *in vitro* and *in vivo* studies of dacarbazine complex with Zn show that it is more effective to control the multiplication of cell by complex than dacarbazine drug. Thus, Zn (II)-dacarbazine complex may be recommended to the therapeutic experts as a more potent anticancer drug in lieu of the drug taken for the present study.

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