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Synthesis, characterization and biological evaluation of a novel vanadium complex as a possible anticancer agent

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ABSTRACT

Chemotherapeutics are the sole treatment modality for cancer patients at advanced condition. In the pursuit of getting an efficient, selective and non toxic anticancer agent that has not been discovered yet, we rationally synthesized a vanadium complex with a Schiff base ligand *N*-(-2-hydroxyacetophenone) glycinate. Characterization of the synthesized complex $VO(NG)_2$, was carried out with the help of various spectroscopic means. The complex proved to be a broad spectrum anticancer agent with significantly less toxicity towards normal cells as evident from cell viability assay. Furthermore, $VO(NG)_2$ treatment increased the life-span of ascitic and solid tumor bearing Swiss albino mice and did not show any significant symptomatic sub acute toxicity on normal Swiss albino mice.

Introduction

Success of cancer chemotherapy relies on selective induction of cytotoxicity to transformed self cells, but such specific drug is elusive till date. Application of a transition metal complex, e.g., cisplatin as anticancer drug (ACD) showed us a new direction to fight against cancer [1,2]. Our laboratory has also participated in the crusade against cancer by introducing various transition metal complexes having the organic moiety *N*-(2-hydroxyacetophenone) glycinate (NG) as their ligand [3]. These metal chelates viz., CuNG, FeNG, ZnNG, MnNG imparts oxidative stress in the malignant cells by depleting cellular glutathione and thus induce apoptosis to the same [4–8]. Herein, we tried to explore the anticancer potential of a vanadium complex formed with the same ligand, NG. Vanadium; a trace element for various mammalian species has therapeutic applications to abrogate diabetes and AIDS [9,10]. Growing body of evidences discloses that vanadium complexes are potent anticancer agent but proves to be toxic at higher doses [11].

Deregulated cell cycle checkpoints is one of the pivotal hallmarks of cancer [12], and vanadium exerts its intracellular effect by inhibiting or degrading the protein tyrosine phosphatases (PTP's) which triggers apoptosis in neoplastic cells via cell cycle arrest [13,14]. Vanadium compounds also trigger apoptosis in malignant cells either by inducing proapototic genes or by inhibiting anti-apoptotic gene expression [15]. Contemporary studies reveal that transformed cells maintain an abnormal redox homeostasis that helps them to acquire optimal genetic instability, which is instrumental for cancer progression [16]. Vanadium being redox-active can generate reactive oxygen species (ROS). This heightened ROS disrupts the redox homeostasis in neoplastic cells and imparts redox stress leading to apoptosis either by cleaving DNA or by facilitating mitochondrial membrane permeabilization [17].

Under this scenario, antiproliferative effect of the synthesized complex $VO(NG)_2$ on a number of human and murine cancer cell lines were evaluated. The anticancer potential was further established on ascites and solid tumor bearing mice models. The subacute toxicity of the complex was also evaluated on normal mice.

Materials and methods

Reagents

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), glycine, *N*-(2-Hydroxy) acetophenone, vanadyl sulphate were purchased from Sigma Chemical Company (St. Louis, USA). All

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other chemicals used were of highest purity and purchased from commercial sources.

Synthesis of the ligand, potassium N-(2-hydroxy acetophenone) glycinate (NG)

The ligand, potassium *N*-(2-hydroxy acetophenone) glycinate (NG) was prepared following the method of Majumder et al. as reported earlier [18]. In brief, KOH (18.39 mM) solution was added drop wise to glycine (18.4 mM) solution. The mixture was kept in an ice bath and stirred continuously at 15–20 °C. An ethanolic solution (10%) of 2 hydroxyacetophenone was added slowly to the mixture. Stirring was continued for 1 h following addition and the mixture was kept at room temperature for 5 h. The solvent was removed from the mixture (deep yellow) by a rotary evaporator. The yellow mass so obtained was washed with pet-ether and precipitated with methanol-diethyl ether mixture (1:1). The crude product was recrystallised from methanol. The pure crystallized NG deposited, yield 75%, m.p. 258–260 °C.

Synthesis of the vanadium complex of NG

Vanadium *N*-(2-hydroxy acetophenone) glycinate [$VO(NG)_2$] was synthesized from the ligand, potassium *N*-(2-hydroxy acetophenone) glycinate by its reaction with vanadyl sulphate. The aqueous solution of vanadyl sulphate (1 mM) was added to the aqueous solution of NG (1.1 mM) drop-wise. The solution was stirred magnetically at room temperature for 2 h and kept at 4 °C for 1 week. Brown crystalline precipitate was deposited from the solution and was filtered off and dried. Yield 67%, m.p. 191–193 °C. Anal. Calc. $C_{20}H_{20}O_7N_2V$: C, 53.21, H, 4.42, N, 6.2; Found: C, 52.46; H, 4.04; N, 5.9.

Chemical characterization and instrumentation

Elemental (C, H, N) analysis was performed with the help of Perkin–Elmer 2400 Series II CHN analyzer. UV–Vis spectra for the complex and the ligand were recorded in Varian Cary 100 Scan (range 800–200 nm) in dimethyl sulfoxide (DMSO). IR spectra were recorded in Perkin–Elmer RX 1 FT spectrophotometer in KBR discs, range 4000–400 cm^{-1}. Mass spectrum was recorded in Waters Micromass Quattro micro API mass spectrometer, in DMSO. ^1H NMR and ^{13}C NMR spectra of the complex were recorded at room temperature in a Bruker Avance 300 MHz spectrophotometer using 5 mm BBO probe in DMSO-d_6 solution. Thermogravimetric analysis was performed on a TA instrument (SDT Q600) thermal analyzer in a dynamic atmosphere of ultra high pure dinitrogen (flow rate: 100 cc min^{-1}). The sample was heated in platinum crucible at a rate of 10 °C min^{-1} from 25 °C to 900 °C. Cyclic voltametric study was done in BASi Epsilone EC instrument (USA). Electrodes used were Ag/AgCl (reference electrode), platinum auxilary electrode and platinum working electrode in DMSO using tetrabutylammonium hexafluorophosphate, [*N*-(*n*-Bu)4] PF_6; 0.1 M as supporting electrolyte at a scan rate of 100 mv/s (25 °C), reference against Ferrocinium/Ferrocene (Fc+/Fc). The complex solution was purged with N_2 for 30 min before each experiment.

Cell culture

The human T-cell acute lymphoblastic leukemia cell line CCRF-CEM (gifted by Prof T. Efferth, University of Mainz, Germany) and human colorectal carcinoma HCT-116 (ATCC) were maintained in RPMI 1640 medium (GIBCO Invitrogen Corp, Carlsbad, California, USA) supplemented with 10% foetal bovine serum (FBS) (GIBCO), glutamine (0.15%), HEPES (25 mM) and gentamycin (50 mg/ml). Furthermore the human breast cancer cell line MCF-7 and human astrocytoma (glioblastoma) cell line U373MG (NCCS, Pune) and mouse normal fibroblast cell line NIH 3T3 (provided by Dr. M. M. Gottesman, NIH, USA) were maintained in DMEM medium (GIBCO), supplemented with 10% FBS, additional glutamine (0.15%), HEPES (25 mM) and gentamycin (50 mg/ml). Cells were grown in plastic tissue culture flasks (Greiner Bio-One, Frickenhausen, Germany) in a 5% CO_2 atmosphere at 37 °C. Cells were passaged twice weekly. Cells from exponentially growing cultures of 15th passage were used for all experiments.

EAC (Ehrlich ascites carcinoma) and S180 (Sarcoma 180) cells were cultured *in vitro* by isolating the ascitic fluid from EAC or S180 bearing mice within 7–10 days of inoculation with the cancer cells. Cells were collected and washed thrice in PBS and then seeded accordingly in each well of flat bottomed 96-well plate for MTT assay with RPMI 1640 (for EAC) or DMEM (for S180) medium (GIBCO), containing HEPES (Sigma), penicillin–streptomycin and 10% FBS.

Isolation of PBMC

Heparinized human blood (peripheral) was collected and diluted with RPMI 1640 (1:1, v/v). The Lymphocyte-enriched mononuclear cell layer [Peripheral Blood Mononuclear Cells (PBMC)] were then isolated with the aid of Histopaque 1077 (Sigma). The procured cells were washed and finally resuspended in cold RPMI 1640 with above-mentioned supplements.

Treatment

For in vitro assay a solution of $VO(NG)_2$ (1 mg/ml) was prepared just before experiments by dissolving the lyophilized compounds in DMSO (0.01%) following serial dilution in FBS containing medium to reduce the concentration of DMSO to nontoxic level i.e. less than 0.01%. For MTT assay, $VO(NG)_2$ treatments were performed with a concentration range from 2 to 28 μg/ml. For *in vivo* experiments a solution of $VO(NG)_2$ (1 g/ml) was prepared just before experiments by dissolving the lyophilized compounds in DMSO (0.01%) following serial dilution in PBS containing medium to reduce the concentration of DMSO to nontoxic level i.e. less than 0.01%).

In vitro viability assay (MTT assay)

Cell viability was determined by the method as described previously [19]. In brief, cells were seeded in 96-well plates at a density 4×10^4 of cells per well. For single-agent studies, cells were seeded and allowed to acclimatize for 24 h before treatment with increasing concentrations of $VO(NG)_2$ and was incubated for further 48 h or 72 h with 5% CO2 at 37 °C. Following completion of incubation cells were further incubated with 5 mg/ml of MTT dye for 4 h at 37 °C. The cells were suspended in DMSO (0.1 ml) and the absorbance was read at 540 nm in an ELISA reader (Tecan 200) and the control value corresponding to drug untreated (solvent-treated) cells was taken as 100%. The viability of treated samples was expressed as percent of control. The IC_{50} values were determined as the concentration of $VO(NG)_2$ that reduced cell viability by 50% compared to control cells.

Animals

Swiss albino mice (originally obtained from National Institute of Nutrition, Hyderabad, India and reared in the institute animal facilities) were used for all the experiments with prior approval (Approval ID: IAEC-1.2/SKC-7/2007/4 dated 22.01.2008) of the institutional animal ethics committee (IAEC). The experimental protocols described herein were in accordance with the IAEC (Registration No.: 175/99/CPCSEA, dated 28.01.2000) guidelines laid down by the committee for the purpose of control and