Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 19
Sofia • 2012

Influence of Metal Compounds on Viability and Proliferation of Rat Insulinoma Cells

T. Zhivkova, L. Dyakova, B. Andonova-Lilova, R. Kalfin*, A. Tolekova**, E.- M. Mosoarca***, R. Tudose***, O. Costisor***, R. Alexandrova*

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria;
**Institute of Neurobilogy, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria;*
***Deaprtment of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria;*
****Institute of Chemistry Timisoara of the Romanian Academy, 24 Mihai Viteazu Blvd, RO-300223, Timisoara, Romania*

In the present study the influence of ammonium vanadate (0.01-20 μg/ml) and Cu(I,II)complexes with Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (1–200 μg/ml) on viability and proliferation of RIN-38 rat insulinoma cells was investigated. The experiments were carried out by thiazolyl blue tetrazolium bromide (MTT) test after 72 h of treatment. Some of the Cu(II) complexes – $Cu_2(BAMP)I_3$ (TS-2) and $Cu(TAMEN)(NO_3)_2$ (TS-17), did not express cytotoxic activity. Ammonium vanadate decreased in a time- and concentration- dependent manner the viability and proliferation of RIN-38 cells.

Key words: RIN-38 rat insulinoma cells, ammonium vanadate, copper(I,II) complexes, cytotoxicity, diabetes mellitus

Introduction

The lifestyle changes characteristic to the second half of the 20 century, have evoked diabetes mellitus (DM) epidemic which drastically impairs the quality of life and is the underling cause of many demises. To treat DM, which has many severe complications, several types of insulin preparations and synthetic drugs for Type 1 diabetes and Type 2 diabetes, respectively, have been developed and are in clinical use. However, there are several problems concerning the insulin preparations and synthetic drugs, such as physical and mental pain due to daily insulin injections and defects involving side effects, respectively. Consequently, a new class of therapeutic agents is anticipated [2, 3]. The idea of using metal ions for the treatment of diabetes originated in the late 19th century. A wide class of vanadium, copper and zinc complexes was demonstrated to be effective for treating diabetes in experimental animals [2, 3, 4, 6]. As a first step in our search for new metal compounds with antidiabetic properties in this study we present

data about the influence of ammonium vanadate and three newly synthesized Cu(I,II) complexes with Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) on viability and proliferation of RIN-38 rat insulinoma cells.

Materials and Methods

Chemicals and other materials. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco-Invitrogen (UK). Dimethyl sulfoxide (DMSO), and trypsin were obtained from AppliChem (Germany); thiazolyl blue tetrazolium bromide (MTT) was from Sigma-Aldrich Chemie GmbH (Germany). All other chemicals of the highest purity commercially available were purchased from local agents and distributors. All sterile plastic and syringe filters were from Orange Scientific (Belgium).

Compounds. The experiments were performed with amonium vanadate (NH_4VO_3, Valerus) and three newly synthesized complexes of Cu(I, II) with Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) – $Cu_2(BAMP)(NCS)_4$ (TS-1), $Cu_2(BAMP)I_3$ (TS-2), $Cu(TAMEN)(NO_3)_2$ (TS-17). The compounds were obtained according to the previous work [5]. Ammonium vanadate (Valerus) was dissolved initially in bidistilled water, sterilized by filtration (diameter of pores 0.2 μm) and then diluted in culture medium. The concentration of the compound in the stock solution was 1 mg/ml. Copper complexes were initially dissolved in dimethylsulfoxide (DMSO, Serva) and then diluted in culture medium. The concentration of DMSO in the stock solution (where the concentration of the copper complex was 1 mg/ml) was 2 %.

Cell cultures and cultivation. The permanent rat insulinoma cell line RIN 38 (Cell Culture Collection, IEMPAM – BAS) was used as experimental model in our study. The cells were grown as monolayer cultures in DMEM medium, supplemented with 5-10% FBS, 100 U/mL penicillin and 100 g/mL streptomycin. The cultures were maintained at 37 °C in a humidified CO_2 incubator (Thermo scientific, Hepa class 100). For routine passages adherent cells were detached using a mixture of 0.05% trypsin and 0.02% EDTA. The experiments were performed during the exponential phase of cell growth.

Cytotoxicity assay. The cells were seeded in 96-well flat-botommed microplates at a concentration of 1×10^4 cells/well. After the cells were grown for 24 h to a subconfluent state (~ 60-70%), the cells from monolayers were washed with phosphate buffered saline (PBS, pH 7.2) and covered with media modified with solutions, containing different concentrations of the compounds tested: 0.01-20 μg/ml (for NH_4VO_3) and 1 – 200 μg/ml (for Cu complexes). Each solution was applied into 4 to 6 wells. Samples of cells grown in non-modified medium served as controls. After 72 h of incubation, the effect of the compounds on cell viability and proliferation was examined by thiazolyl blue tetrazolium bromide (MTT) test as described by Mossman [1]. The method consisted of three hours incubation with MTT solution (5 mg MTT in 10 mL D-MEM) at 37 °C under 5% carbon dioxide and 95% air, followed by extraction with a mixture of absolute ethanol and DMSO (1:1, vol/vol). Optical density was measured at 540/620 nm using an automatic microplate reader (TECAN, Sunrise™, Austria). Relative cell viability, expressed as a percentage of the untreated control (100% viability), was calculated for each concentration. Concentration–response curves were prepared. All data points represent an average of three independent assays.

Statistical analysis. The data are presented as mean ± standard error of the mean. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test (GraphPad 5.00 program – La Jolla, CA, USA).

Results and Discussion

In our investigations the compounds tested were applied for 72 h at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20 μg/mL (for NH_4VO_3) and 1, 10, 20, 50, 100, 200 μg/ml (for Cu complexes with Mannich bases). The obtained data about viability/proliferation of the treated RIN 38 rat insulinoma cells are summarized in Figures 1 and 2.

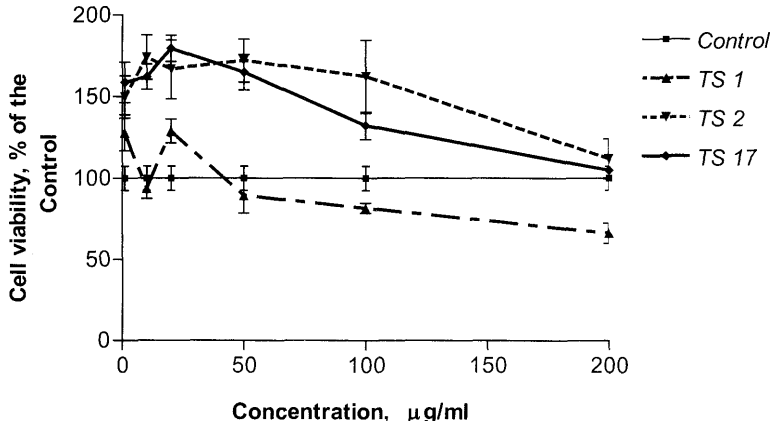

Fig. 1. Effect of Cu(I, II) complexes with Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TA-MEN) on viability and proliferation of RIN-38 rat insulinoma cells. The compounds are applied at concentrations of 1, 10, 20, 50, 100, 200 for 72 h. The investigation was performed by MTT test. TS1 = $Cu_2(BAMP)(NCS)_4$; TS2 = $Cu_2(BAMP)I_3$; TS3 = $Cu(TAMEN)(NO_3)_2$ (TS-17).

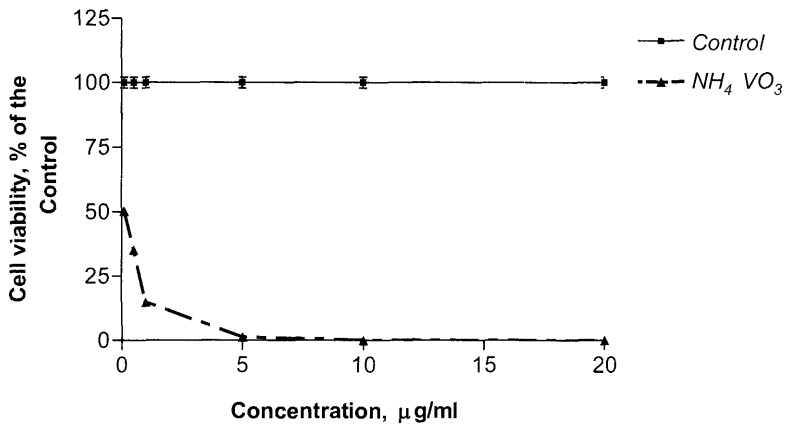

Fig. 2. Effect of ammonium vanadate on viability on viability and proliferation of RIN-38 rat insulinoma cells. NH_4VO_3 was applied at concentrations of 0.1 0.5, 1, 5, 10, 20 μg/mL for 72 h. The investigation was carried out by MTT test.

NH_4VO_3 was found to decrease in a time- and concentration- dependent manner the viability and proliferation of RIN-38 cells. Relatively higher survival rate was observed at concentrations ≤ 0.05 μg/ml ($\geq 75\%$ viable cells, data not presented in Fig. 1). At the same time some of the Cu(II) complexes – $Cu_2(BAMP)I_3$ (TS-2) and $Cu(TAMEN)(NO_3)_2$ (TS-17), did not reduce the viability of the treated cells even when administered at concentrations of 100 and 200 μg/ml.

The permanent rat insulinoma cell line RIN-38, induced by high-dose X-ray irradiation, is one of the most widely used insulin-secreting cell lines and is appropriate for the development of new anti-diabetic strategies. Additional experiments are underway to examine the influence of the compounds tested (especially low toxic copper complexes) on insulin-secreting activity of these cells.

Acknowledgements: This study was supported by Grant DDVU-02-24/2010 from the National Science Fund, Sofia, Bulgaria; European Social Fund and Republic of Bulgaria, Operational Programme "Development of Human Resources" 2007-2013, Grant № BG051PO001-3.3.06-0048 from 04.10.2012.

References

1. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. – J. Immunol. Meth., **65**(1-2), 1983, 55-63.
2. Sakurai, H., A. Katoh, T. Kiss, T. Jakusch, M. Hattori. Metallo-allixinate complexes with anti-diabetic and anti-metabolic syndrome activities. – Metallomics., **2**, 2010, 670-82.
3. Sakurai, H., H. Yasui, Y. Adachi. The therapeutic potential of insulin-mimetic vanadium complexes. – Expert. Opin. Investig. Drugs. **12**, 2003, 1189-203.
4. Vanco, J., J. Marek, Z. Trávníček, E. Racanská, J. Muselík, O. Svajlenová. Synthesis, structural characterization, antiradical and antidiabetic activities of copper(II) and zinc(II) Schiff base complexes derived from salicylaldehyde and beta-alanine. – J. Inorg. Biochem., **102**, 2008, 595-605.
5. Weinberger P., O. Costisor, R. Tudose, O. Baumgartner, W. Linert. A novel mixed valence copper(II)-Copper(I)-bis(antipyryl-methyl)-piperazine complex: synthesis, molecular structure and spectroscopic characterization. – J. Mol. Structure, **519**, 2000, 21-31.
6. Yasumatsu, N., Y. Yoshikawa, Y. Adachi, H. Sakurai. Antidiabetic copper(II)-picolinate: impact of the first transition metal in the metallopicolinate complexes. – Bioorg. Med. Chem., **15**, 2007, 4917-22.