PROCEEDINGS OF THE BALKAN SCIENTIFIC CONFERENCE OF BIOLOGY IN PLOVDIV (BULGARIA) FROM 19TH TILL 21ST OF MAY 2005 (EDS B. GRUEV, M. NIKOLOVA AND A. DONEV), 2005 (P. 7–13)

EFFECT OF IRON COMPLEXES WITH MANNICH TYPE LIGANDS ON VIABILITY OF TUMOUR CELL LINES

R. I. Alexandrova[1*], G. Rashkova[1], T. Popova[2], S. Slavov[1], N. Stefanova[1], E.-M. Mosoarca[3], R.Tudose[3], O. Costisor[3]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria
rialexandrova@hotmail.com
[2]Faculty of Veterinary Medicine, Forest Technical University, 10 Kliment Ohridski Blvd, 1756 Sofia, Bulgaria
[3]Institute of Chemistry Timisoara of the Romanian Academy, 24 Mihai Viteazu Blvd, RO-300223, Timisoara, Romania

ABSTRACT. The effect of three complexes of iron with Mannich type ligands were studied *in vitro* on animal and human tumour cells using neutral red uptake cytotoxicity assay. The following cell lines were used in the experiments – LSCC-SF-Mc29 (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29), LSR-SF-SR (transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin), Hep2 (human hepatocellular adenocarcinoma), 8 MG-BA (human glioblastoma). On the basis of their cytotoxic activity the compounds investigated were grader as follows: $Fe_2(TAMEN)Cl_6$ > $Fe_2(BAMP)Cl_4$ > $Fe_2(BAMP)Cl_6$, Tested independently, both ligands were no or very low toxic at the concentrations examined.

KEY WORDS. Mannich bases, pyrazolone, polynuclear complexes, iron, cyto-toxicity, tumour cell lines

INTRODUCTION

In 1912 Mannich and Krösche discovered the property of formaldehyde to bind an amine with a carbon acid *via* a methylene bridge (Mannich, Kather, 1919). The method was utilized to obtain pharmaceutical products by implication of acid components, which were recognized like substances with therapeutic action. Our main point of interest refers to study the Mannich base complexes of some first row metal ions, in order to explain their biological activity as well as to find new

compounds with biological effects. Iron is known to be essential for fundamental cell functions, such as DNA synthesis, transport of oxygen and electrons, and cell respiration (Rosenzweig, Volpe, 1999; Pietrangelo, 2003). The aim of the present study was to evaluate the potential cytotoxic activity of three complexes of iron with ligands containing an antipyrine moiety like the Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) on animal and human tumour cell lines. We decided to evaluate the cytotoxic properties of these compounds because of two main reasons: 1) There are data published about antineoplasic effect of several iron compounds (Kopf-Meier, Klapotke, 1989; Padhye et al., 1992; Hall et al., 2000; Shrivastav et al., 2002); 2) It has been found in our previous investigations that some copper, cobalt and nickel complexes with BAMP and TAMEN significantly reduce the viability of different human and animal tumour cell lines (in press).

MATERIAL AND METHODS

Compounds. The experiments were performed with three complexes of iron with ligands containing an antipyrine moiety like the Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) (Fig.1) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) (Fig.2): $Fe_2(BAMP)Cl_4$, $Fe_2(BAMP)Cl_6$, $Fe_2(TAMEN)Cl_6$. The effect of both ligands on cell viability was also examined.

The compounds were obtained according to the previous work (Costosor et al., 1981, 1994 a, b).

Iron complexes were initially dissolved in dimethylsulfoxide (DMSO, Serva) and then diluted in culture medium. The solutions were stored in dark and were used in the experiments no longer than two weeks after their preparation.

Cell lines and cultivation. The following permanent cell lines were used in the experiments: LSCC-SF(Mc29), established from a transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29 (Alexandrova et al., 2002), LSR-SF(SR), derived from a transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin (SR-RSV) (Alexandrov, 1993), Hep2 (hepatocellular adenocarcinoma) and 8-MG-BA (human glioblastoma) (Perzelova, 1998).

Cells were grown as monolayer cultures in a combination of medium H-199 and Minimum Essential medium (AppliChem, Germany), supplemented with 5-10% fetal bovine serum (Cambrex, Belgium), 100 U/ml penicillin and 100 μg/ml streptomycin. The cultures were maintained at 37°C in a humidified CO_2 incubator. For routine passages adherent cells were detached using a mixture of 0.05% trypsin (Gibco) – 0.02% ethylendiaminotetraacetic acid (EDTA). The experiments were performed during the exponential phase of cell growth.

Cytotoxicity assay. The cells were seeded in 96-well plates (Cellstar) at a concentration of 2 x 10^4 cells/well. At the 24th h the cells from the monolayer were washed and covered with media modified with different concentrations of the compounds tested (each concentration in 6 to 8 repetitions). Samples of cells, grown in a non-modified medium served as a control. After 24 h and 48 h incubation

periods, each plate was examined under inverted microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Effect of the compounds on cell viability was evaluated by neutral red uptake cytotoxicity assay (Borenfreund, Puerner, 1985). Optical density was measured at wave length 540 nm by Organon Teknika Reader 530. Relative cell viability, expressed as a percentage of the untreated control, was calculated for each concentration. All experiments were triplicated.

Statistical analysis. The data are presented as mean ± standard error of the mean. Statistical differences between control and treated groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

RESULTS AND DISCUSSION

Applied at concentrations of 1, 10, 50, 100 and 200 μg/ml BAMP and TAMEN did not significantly reduce the viability of tumour cells investigated. More than 95% of LSR-SF(SR), Hep2 and 8-MG-BA, and > 90% of LSCC-SF(Mc29) cells cultivated in the presence of these compounds were found to be alive after 24 h and 48 h treatment.

Among the metal complexes investigated $Fe_2(TAMEN)Cl_6$ expressed relatively the most pronounced cytotoxic activity, especially on LSCC-SF-Mc29 chicken hepatoma cells (Fig. 3). The relative viability of LSCC-SF-Mc29 cells treated with 50 μg/ml or 100 μg/ml $Fe_2(TAMEN)Cl_6$ was calculated to be 50.83% ± 2.48 ($P < 0.01$) and 30.41% ± 0.62 ($P < 0.01$), respectively. Only 61.63% ± 4.4 ($P < 0.01$) of the LSR-SF-SR sarcoma cells and 78.71% ± 4.3 ($P < 0.05$) of 8 MG BA human glioblastoma cells survived after 48 h treatment with 100 μg/ml $Fe_2(TAMEN)Cl_6$. Administered at a concentration of 100 μg/ml for 48 hours $Fe_2(BAMP)Cl_4$ reduced the number of alive Hep2 cells to 69.56% ± 3.1 ($P < 0.01$). In all the other cases the complexes examined were not found to reduce tumour cell viability by more than 20%.

Cytopathological changes, such as detachment and lysis were observed in LSCC-SF-Mc29 chicken hepatoma cells treated for 48 h with $Fe_2(TAMEN)Cl_6$ at concentrations of 50, 100 and 200 μg/ml.

The potential antineoplastic effects of different metals and metal compounds have been under special interest during the recent years (Galanski et al., 2003; Alexandrova, Nikolova, 2004). In the literature there are data that different iron containing compounds possess antintumour activity *in vitro* and *in vivo*. Thus, complexes containing Fe bound to small carbone ligands are found to be potent cytotoxic agents in murine and human leukemia and lymphoma cultured cells. These complexes inhibit nucleic acid metabolism, specifically DNA and purine *de novo* synthesis and are moderate inhibitors of human DNA topoisomerase II (Hall et al., 2000). Some water-soluble ferricenium complexes have been shown to suppress the growth of human B16 melanoma and colon 38 carcinoma by 35-60% and 50-73%, respectively (Kopf-Meier, Klapotke, 1989). Fe (III) complexes of naphthoqionone

thiosemicarbazones inhibit DNA synthesis in P388 murine leukemia cells (Padhye et al., 1992). Fe(III) complexes of o-hydroxydithiobenzoate has been reported to prolong the survival of Dalton's lymphoma bearing mice by directly killing the tumour cells and reversing tumour-associated immunosuppression (Shrivastav et al., 2002).

In this study we report for the first time the data about cytotoxic activity of iron complexes with ligands containing an antipyrine moiety like the Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN). The results presented here indicate that: 1) On the basis of their relative cytotoxic activity metal complexes investigated follow the order: $Fe_2(TAMEN)Cl_6 > Fe_2(BAMP)Cl_4 > Fe_2(BAMP)Cl_6$; 2) Among the cell lines used in the experiments chicken hepatoma cells LSCC-SF(Mc29) are the most sensitive to the cytotoxic effects of the compounds; 3) Applied at doses examined both ligands – N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) do not significantly reduce the viability of tumour cell lines tested. More investigations will be required to clarify the potential cytotoxic activity of the complexes examines as well as their mechanism of action.

Acknowledgements. This study was supported by Grant CC 1402/04, National Scientific Council, Bulgarian Ministry of Education and Science.

REFERENCES

ALEXADROVA, R. I., V. OGNEVA, P. JORDANOVA. 2002. Tumor heterogeneity investigations in virus-induced transplantable tumor. – Int. J. Cancer, Suppl., **13**, P. 563.

ALEXANDROV, I. 1993.Immunobiological characterization of transplantable sarcoma in rats. – Compt. Rend. Acad. Bulg. Sci., **46**: 97-100.

ALEXANDROVA, R., E. NIKOLOVA. 2004. Metals as potential anticancer agents. – J. Bulg. Acad. Sci., **1**, 19-23.

BORENFREUND, E., J. PUERNER. 1985. Toxicity determination in vitro by morphological alterations and neural red absorption. – Toxicol. Lett., **24**: 119-124.

COSTISOR, O., A. MAURER, S. POLICEC. 1981. New pyrazolone, complex compounds. II. Homobinuclear complex compounds of some first row transition.elements with N, N' – bis(antipyril-methyl)-piperazine. – St. Tehn. Inst. Pol. Timisoara, Ser. Chim. **26**: 87-92.

COSTISOR, O., C. STANESCU, R. TUDOSE, I. EREMIA, S. POLICEC. 1994 a. New Pyrazolone Complex Compounds VII. *N,N*'-tetra(antipyryl-Methyl)-1,2-Ethanediamine A New Dinucleating Ligand.- Bul. St. Teh. Inst. Politehnic "Traian Vuia" Timisoara, Ser. Chim., **39**: 79-84

COSTISOR, O., W. LINERT, S. DEUSCH, C. STANESCU. 1994 b. Novel Complexes of Antipyrine Ligands – Dinuclrear Copper(II), Cobalt(II) and Nickel(II) Complexes of *N,N'*-tetra(antipyrylmethyl)-1,2-diaminoethane. – J. Coord. Chem., **33**: 229-234

GALANSKI, M., V.B. ARION, M.A. JAKUPER, B.K. KEPPLER. 2003. Recent developments in the field of tumor-inhibiting metal complexes. – Curr. Pharm. Des., **9**: 2078-2089.

HALL, I. H., C. B. LACKEY, T. D. KISTLER, R. W. DURHAM J R, J.M. RUSSEL, R. N. GRIMES. 2000. Antitumor activity of mono-and dimetallic transition metal carbone complexes of Ta, Fe, Co, Mo, or W. – Anticancer Res., **20**: 2345-2354.

KOPF – MAIER, P., T. KLAPOTKE. 1989. Tumor inhibition by ferricenium complexes. Activity against some solid experimental tumors. – Arzneimittelforschung, **39**: 369-371.

MANNICH, C., B. KATHER. 1919. Ueber Kondensationsprodukte aus Aminsaltzen, Formaldehyd und Antipyrin. – Arch. Pharm., **257**: 18-33

PADHYE, S., R. CHIKATE, A. KUMBHAR, J.M. SHALLOM, M.P. CHITNIS. 1992. Novel, quinone-thiosemicarbazone hybrid (QTSCHY) non-platinum antutumor agents: inhibition of DNA biosynthesis in P388 lymphocytic cells by coordinativelly unsaturated copper (II) and iron (III) complexes of naphthoquinone thiosemicarbasones. – Biometals, **5**: 67-71.

PERZELOVA, A., I. MACCOVA, P. MRAZ, I. BZIK, J. STENO. 1998. Characterization of two new permanent glioma cell lines 8-MG-BA and 42-MG-BA. Neoplasma, **42**: 25-29.

PIETRANGELO, A. 2003. Haemochromatosis. – Gut, **52:** Suppl., 1123-1130.

ROSENZWEIG, P. H., S. L. VOLPE. 1999. Iron, termoregulation, and metabolic rate. – Crit. Rev. Sci. Nutr., **39**: 131-148.

SHRIVASTAV, A., N.K. SINGH, G. SRIVASTAVA. 2002. Synthesis, characterization and antitumor stadies of transition metal complexes of 0-hydroxydithiobenzoate. – Bioorg. Med. Chem., **10**: 2693-2704.

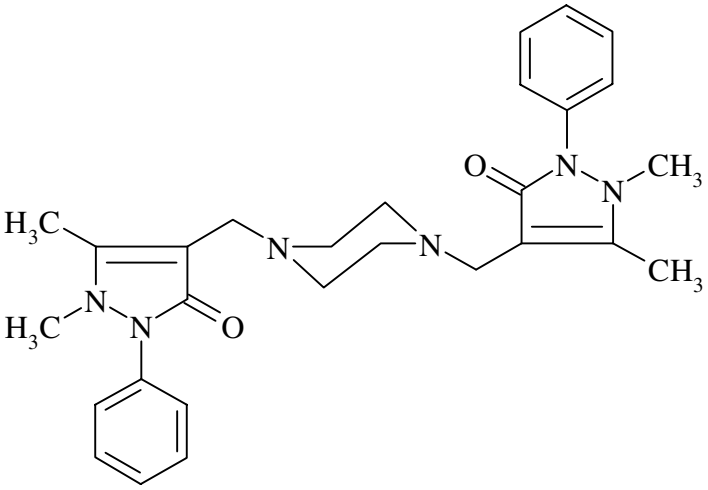

Figure 1. *N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP)*

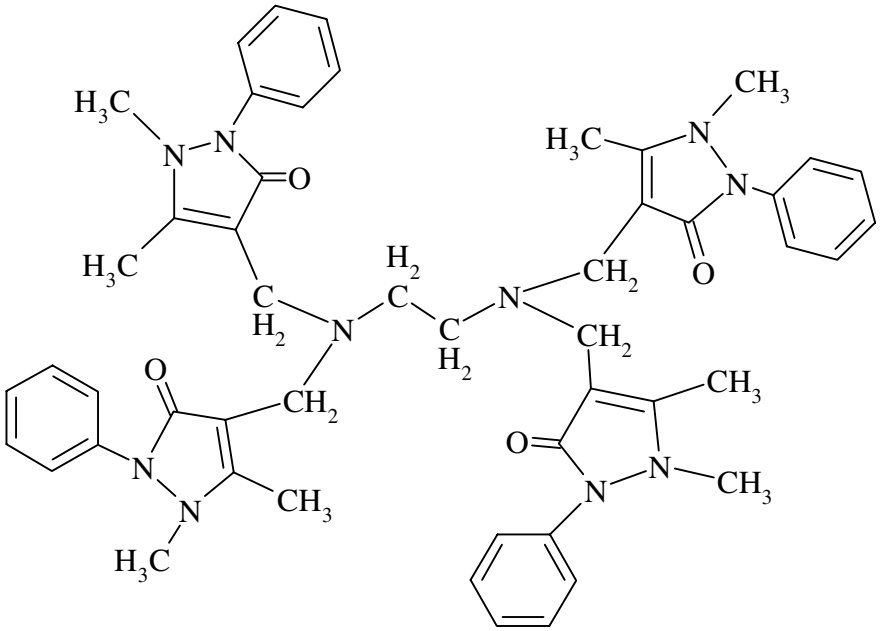

Figure 2. *N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN)*

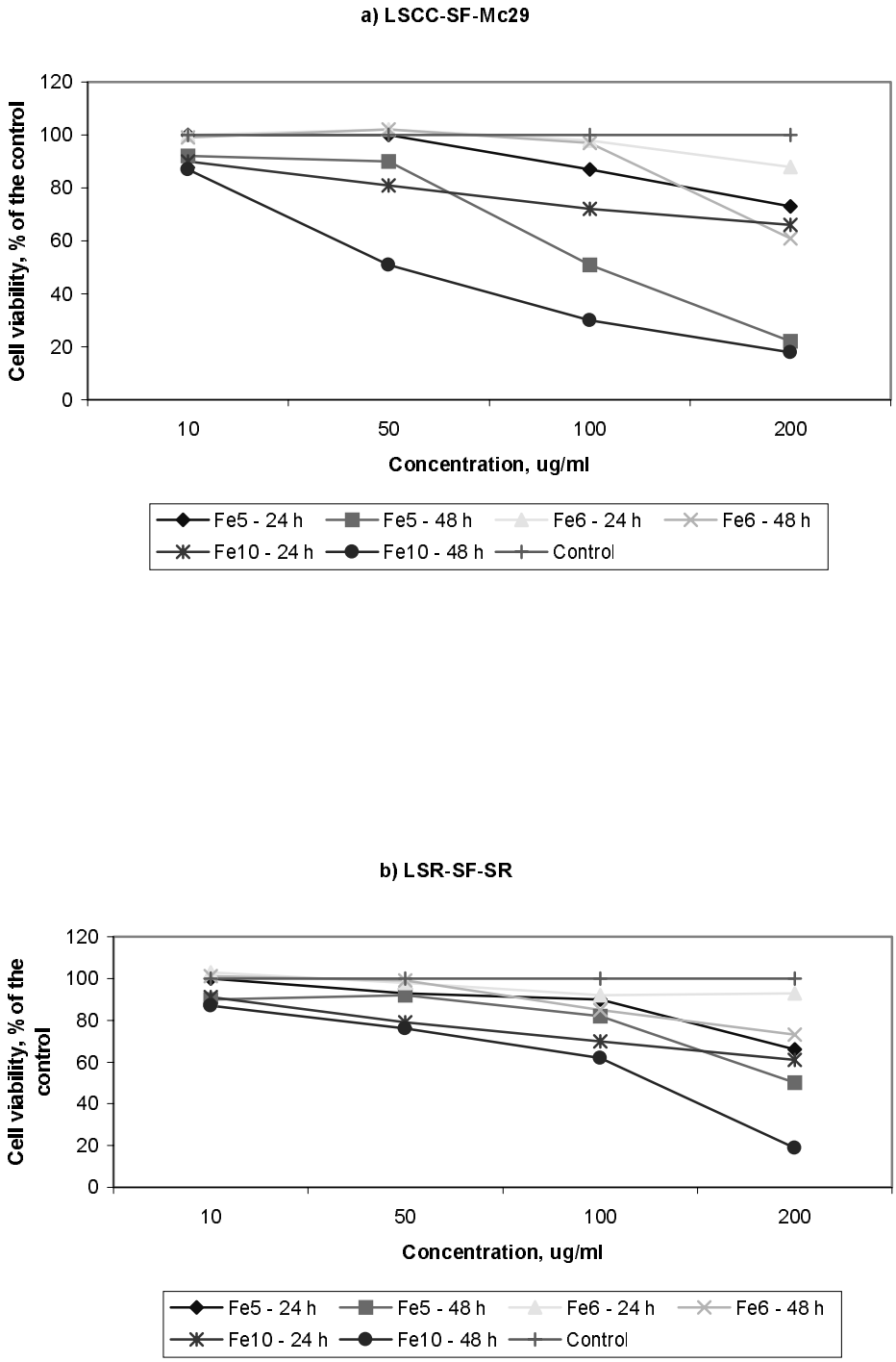

Fig. 3. *Cytotoxic effect of iron complexes with Mannich type ligands on viability of tumour cell lines*