Journal of BUON **15:** 768-773, 2010
© 2010 *Zerbinis Medical Publications. Printed in Greece*

ORIGINAL ARTICLE

The cytotoxic effects of some selected gold(III) complexes on 4T1 cells and their role in the prevention of breast tumor growth in BALB/c mice

V. Volarevic[1], M. Milovanovic[1], A. Djekovic[2], B. Petrovic[2], N. Arsenijevic[1], Z.D. Bugarcic[2]

[1]Department of Microbiology and Immunology, Centre for Molecular Medicine, Faculty of Medicine, University of Kragujevac; [2]Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia

Summary

Purpose: *To investigate the cytotoxic activity of newly synthesized gold(III) complexes $[AuCl_2(en)]^+$, $[AuCl_2(SMC)]^+$, $[AuCl_2(DMSO)_2]^+$ (en: ethylenediamine, SMC: S-methyl-L-cysteine and DMSO: for dimethylsulfoxide) in 4T1 mouse breast cancer cell line in vitro and in vivo and to compare their antitumor characteristics with cisplatin complex $[PtCl_2(NH_3)_2]$.*

Methods: *The in vitro, effects of the tested complexes on 4T1 cell viability were determined using MTT colorimetric technique. In vivo, progression of mouse breast tumor growth in BALB/c mice was measured by using external caliper.*

Results: *Among the tested gold(III) complexes, $[AuCl_2(en)]^+$ showed best cytotoxic effects in vitro. The cytotoxic effects of $[AuCl_2(en)]^+$ and $[PtCl_2(NH_3)_2]$ were similar at all concentrations. The data from the in vivo experiment showed that among the tested gold(III) complexes only $[AuCl_2(en)]^+$ can prevent the primary breast tumor growth. $[AuCl_2(en)]^+$ was tolerated well and much better than $[AuCl_2(DMSO)_2]^+$, $[AuCl_2(SMC)]^+$ and $[PtCl_2(NH_3)_2]$ complex which was confirmed by weight gain in mice that received $[AuCl_2(en)]^+$. In addition, mice that received $[AuCl_2(en)]^+$ showed better survival time in comparison with mice that received $[PtCl_2(NH_3)_2]$ complex.*

Conclusion: *$[AuCl_2(en)]^+$ complex seems to be good candidate for future pharmacological evaluation in breast cancer research.*

Key words: cytotoxic effects *in vitro/in vivo*, gold(III) complexes, mouse breast cancer, prevention

Introduction

In preclinical studies it is necessary to test the cytotoxic effects of newly synthesized anticancer drugs *in vitro* and *in vivo* [1]. The use of orthotopic systems gives the most precise information about the capacities of the tested, newly synthesized anticancer drugs to prevent and/or to stop progression of primary tumor growth [2]. In addition, it is of crucial importance to test the newly synthesized therapeutic agents on murine cell lines that will metastasize in a similar manner and to similar locations as the same tumor type will in human.

The human breast cancer is the most prevalent malignancy among women, representing the second cause of cancer-related deaths [3,4]. The 4T1 mammary carcinoma cell line, originally isolated by Fred Miller and coworkers at the Karmanos Cancer Institute [5,6], when introduced orthotopically is useful model for evaluation of primary breast cancer tumor growth progression [7]. In addition, the 4T1 tumor cell line has the capacity to metastasize to all organs affected in human breast cancer, including lungs, liver, brain and bone [8-14].

Although novel molecular pathways relevant to breast cancer biology and breast cancer therapy are explored continuously [15,16], it is expected that a whole array of new agents should be tested in combination or in sequence to standard chemotherapy with the aim to improve the outcome of high-risk breast cancer patients.

The big successes of cisplatin in cancer chemotherapy on one side, and the toxic side effects related to cisplatin treatment on the other side, stimulated the search for new and more selective metal-based anticancer drugs [17].

During the last 20 years, much interest has fo-

Correspondence to: Vladislav Volarevic, MD. Faculty of Medicine, University of Kragujevac, 69 Svetozara Markovica Street, 34000 Kragujevac, Serbia. Tel/fax: +381 34 306800, E-mail: drvolarevic@yahoo.com

Received 05-02-2010; Accepted 30-03-2010

cused on gold(III) complexes and several reports that described their anticancer characteristics suggested gold(III) compounds as very good candidates for further anticancer investigations [18,19]. Although there is some evidence about their *in vivo* antitumor effects [20,21], gold(III) complexes are not stable under physiological conditions [19], so during the past few years much interest has focused on gold(III) complexes in a number of new synthesized complexes [22,23]. The acceptable solution stability of these newly synthesized gold(III) complexes facilitated extensive pharmacological investigation, both *in vitro* and *in vivo* [24-28].

However, compared to the corresponding platinum(II) complexes, ligand substitution reactions of gold(III) complexes have not been extensively studied [29].

Because gold(III) is isoelectronic (d^8) with platinum(II) and tetracoordinate gold(III) complexes have the same square-planar geometries as cisplatin [30] we decided to investigate the potential cytotoxic activity of some gold(III) complexes: $[AuCl_2(en)]^+$, $[AuCl_2(SMC)]^+$, $[AuCl_2(DMSO)_2]^+$ in 4T1 mouse breast cancer cell line *in vitro* and *in vivo* and to compare their cytotoxic effects with the cytotoxic effects of cisplatin $[PtCl_2(NH_3)_2]$ (Figure 1).

It was envisaged that this study could throw more light on the anti-cancer properties of gold(III) complexes and to suggest the newly synthesized gold(III) complex that showed best anti-tumor characteristics for future pharmacological investigation.

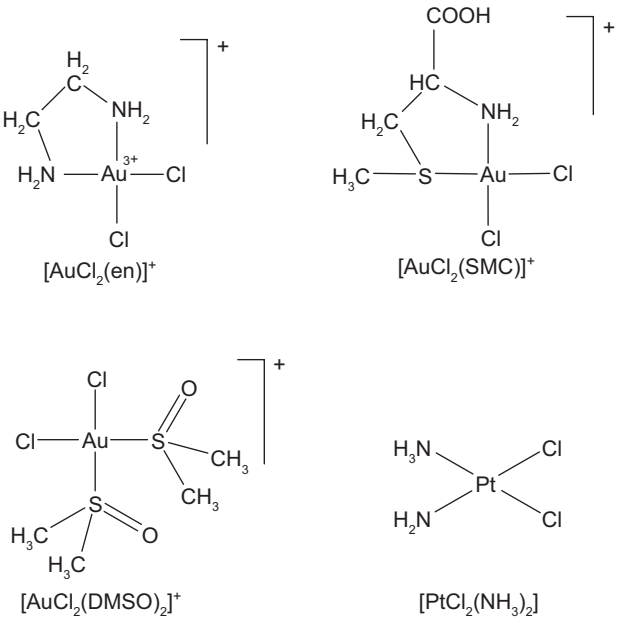

Figure 1. Structures of the investigated complexes.

Methods

Chemicals and ligands

The ligands: ethylenediamine (en), S-methyl-L-cysteine (SMC) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (Munich, Germany). Starting potassium tetrachloridoaurate(III) complex, $K[AuCl_4]$, was purchased from ABCR GmbH & Co. (Karlsruhe, Germany), while cisplatin *(cis*-diamminedichloroplatinum(II), *cis*-$[Pt(NH_3)_2Cl_2]$), was purchased from Sigma-Aldrich. All chemicals were of the highest purity commercially available and were used without further purification. Ultra pure water was used in all experiments.

For the cytotoxicity determination further chemicals were used: fetal bovine serum (FBS), growth medium RPMI 1640, penicillin G, streptomycin, (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide (MTT), phosphate buffered saline (PBS). dimethylsulfoxide (DMSO), trypan blue staining (all from Sigma Chemicals, Germany); 96-well plates (Sarstedt, Germany) and Haemaccel (Theraselect Gmbh, Germany).

Synthesis of complexes

The $[AuCl_2(en)]Cl{\cdot}2H_2O$ complex was prepared according to the published procedure [31]. Dichloridobi(dimethylsulphoxide)gold(III)-chloride, $[AuCl_2(DMSO)_2]Cl$, was synthesized by dissolving $K[AuCl_4]$ (0.2 g, 0.53 mM) in 5 cm^3 0.05 M HCl in the dark. Under continuous stirring, to the solution were dropped, first, 75 μL of DMSO (1.06 μM) and later 0.1M NaOH, to adjust pH about 4.5. The mixture was stirred for 5 h at room temperature and the obtained yellow solution was left in the darkness to evaporate. After few days, the formed dark yellow crystals were filtered and dried. Found: H 1.87; C 7.21; S 7.18. Calc. for $AuC_4S_2O_2H_{12}Cl_3$: H 1.84; C 7.32; S 7.76%.

$[AuCl_2(SMC)]Cl{\cdot}H_2O$ complex was synthesized starting from acid $H[AuCl_4]$. An acid (0.2 g, 0.8 mmol) was dissolved in a little amount of water on magnetic stirrer. S-methyl-L-cysteine (0.105 g, 0.8 mmol) was added to the solution. After mixing, the pH was adjusted between 4-5 with 0.1 M NaOH. The mixture was stirred a few hours and the clear solution was left to evaporate in the dark at room temperature. A brown powder was formed after few days. Found: H 3.08; C 10.73; N 2.47; S 7.01. Calc. for $AuC_4SO_3H_{11}NCl_3$: H 3.07; C 10.52; S 7.02%.

Cell culture

The mouse breast tumor cell line 4T1, syngeneic

in BALB/c mice, was purchased from American Type Culture Collection (ATCC).

Animals

The cytotoxic effects of the tested complexes were evaluated on BALB/c female mice. Animals were housed in cages under constant temperature and humidity conditions, exposed to light and dark for 12 h respectively, with food and water available *ad libitum*.

BALB/c female mice (n=30), 7-9 weeks old, were divided into one control and 4 experimental groups (n=6 in each group).

Tumor implantation and drug injection

4T1 mouse breast tumor cells (5×10^4), previously resuspended in 60 µl of RPMI 1640, were inoculated into the 4th mammary fat pad of BALB/c mice. Ten min after tumor inoculation (1st day) and at 4th, 8th, 12th, 16th and 20th day of experiment, mice from the control group received saline (100 µl) in the tail vein, while mice from the experimental groups received $[AuCl_2(DMSO)_2]Cl$, $[AuCl_2(SMC)]Cl$, $[AuCl_2(en)]Cl$ and cisplatin $[PtCl_2(NH_3)_2]$ (100 µl in the tail vein in a concentration of 62.5 µM, previously diluted in DMSO).

Cytotoxicity assay (MTT)

Gold(III) complexes are biologically active substances of special interest as potential anticancer agents. Effects of $[AuCl_2(en)]^+$, $[AuCl_2(SMC)]^+$, $[AuCl_2(DMSO)_2]^+$ and cisplatin, $[PtCl_2(NH_3)_2]$, on cell viability were determined using MTT colorimetric technique [32]. 4T1 cells were diluted with medium to 1×10^6 cells/ml and aliquots (5×10^3 cells/100 µl) were placed in individual wells in 96-multiplates. About 24 h later, after the cell adherence, each well received 100 µl of either $[AuCl_2(DMSO)_2]Cl$, $[AuCl_2(SMC)]Cl$, $[AuCl_2(en)]Cl$ or $[PtCl_2(NH_3)_2]$, which had been serially diluted 2-fold in medium to concentrations ranging from 2000 µM to 0.98 µM. Cells were incubated at 37° C in a 5% CO_2 incubator for 24 h. After incubation, multiplates were centrifuged, the supernatant was removed, fresh medium and MTT solution (5 mg/ml in PBS) 20 µl were added to each well and the plates were incubated for an additional 4 h. The multiplates were centrifuged, cell-free supernatants were suctioned off, and DMSO (150 µl) and glycine buffer were added to dissolve the crystals. The plates were shaken for 10 min. The optical density of each well was determined at 595 nm using microplate multimode detector Zenyth 3100.

The percentage of cytotoxicity was calculated using the formula: cytotoxicity % = 100-[(TS−BG0)−E/(TS−BG0) × 100], where BG0 is for background of medium alone, TS is for total viability/spontaneous death of untreated target cells, and E is for experimental well.

Measurement of primary tumor growth and tumor volume

External caliper is currently the standard method for determination of tumor volume due to the low cost and high throughput of this simple method [33,34]. During 21 days, the growth of primary tumors was measured every day by external caliper. In order to determine tumor volume by external caliper, experimental animals were sacrificed on the 21st day and tumors were removed. The greatest longitudinal diameter (length) and the greatest transverse diameter (width) of tumors were determined. Tumor volume based on caliper measurements was calculated by the modified ellipsoidal formula: $Tumor\ volume = 1/2 \times (length \times width^2)$ [33,34].

Results

All 4 complexes showed cytotoxic effect *in vitro* on 4T1 cells (Figure 2 and 3).

Decrease of concentration was followed by markedly decrease of apoptotic cells percentage.

Concentrations from 2000 µM to 500 µM of $[AuCl_2(en)]Cl$, $[AuCl_2(DMSO)_2]Cl$ and $[PtCl_2(NH_3)_2]$ complexes showed higher cytotoxic effect in comparison with the same concentrations of $[AuCl_2(SMC)]Cl$ complex.

Concentrations from 500 µM to 62.5 µM of $[AuCl_2(en)]Cl$ and $[PtCl_2(NH_3)_2]$ complexes showed higher cytotoxic effect in comparison with the same concentrations of $[AuCl_2(SMC)]Cl$ and $[AuCl_2(DMSO)_2]Cl$.

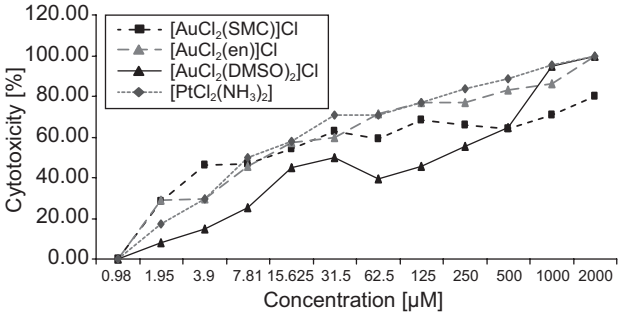

Figure 2. Toxicity of $[AuCl_2(DMSO)_2]Cl$, $[AuCl_2(SMC)]Cl$, $[AuCl_2(en)]Cl$ and cisplatin $[PtCl_2(NH_3)_2]$ complex, using 4T1 cells as target cells. 4T1 cells were cultured with different doses of the tested complexes ranging from 0.98 to 2000 µM. Cell viability was determined based on MTT assay. Each point represents a mean value and standard deviation of 3 experiments with 3 replicates per dose.

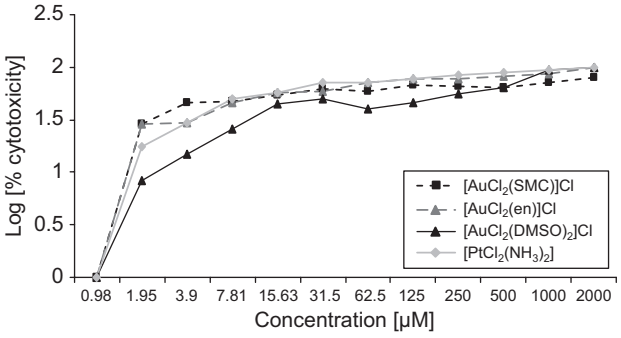

Figure 3. Semi-logarithmic plot of cytotoxic effects of [$AuCl_2$ $(DMSO)_2$]Cl, [$AuCl_2$(SMC)]Cl, [$AuCl_2$(en)]Cl and cisplatin on 4T1 cells. Each point represents a mean value of 3 experiments with 3 replicates per dose.

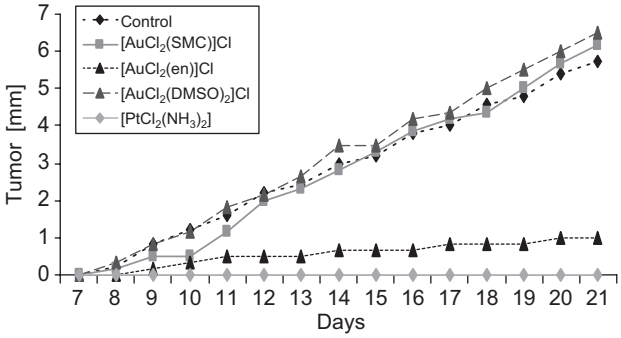

Figure 4. The breast tumor growth in BALB/c mice after injection of 5×10^4 4T1 cells. Each point represents a mean value of tumor size measured daily in mice from each group.

At concentrations 125 μM and 62.5 μM, [$AuCl_2$ (en)]Cl complex showed higher cytotoxic effect than [$PtCl_2(NH_3)_2$], [$AuCl_2(DMSO)_2$]Cl and [$AuCl_2$(SMC)] Cl.

Nevertheless, [$PtCl_2(NH_3)_2$] complex showed higher cytotoxic effect in comparison with the same concentrations of all 3 gold(III) complexes at the concentration of 31.5 μM.

At concentrations 15.63 μM and 7.81 μM [$AuCl_2$ (en)]Cl, [$AuCl_2$(SMC)]Cl and [$PtCl_2(NH_3)_2$] complexes showed approximately the same cytotoxic effect on 4T1 cells, while at the concentration of 3.9 μM [$AuCl_2$(SMC)]Cl showed higher cytotoxic effect than all other tested complexes (Figure 2).

At low concentration (1.95 μM) [$AuCl_2$(en)]Cl and [$AuCl_2$(SMC)]Cl complexes showed higher cytotoxic effect than [$PtCl_2(NH_3)_2$].

The concentrations from 500 μM to 1.95 μM of [$AuCl_2(DMSO)_2$]Cl showed significantly lower cytotoxic effect in comparison with the same concentrations of all other tested complexes (Figure 2).

The concentration 0.98 μM of all tested gold(III) complexes and cisplatin showed no cytotoxic effect on 4T1 cells (Figure 2).

All 3 tested gold(III) complexes and cisplatin (concentration from 2000 μM to 0.98 μM) showed concentration-dependent cytotoxic effect on 4T1 cells (Figure 3).

The tumors were noticed for the first time on the 8th day of the experiment in one mouse from the control group as well as in two mice that received [$AuCl_2(DMSO)_2$]Cl and in one mouse that received [$AuCl_2$(SMC)]Cl. The tumor was noticed on the 9th day of the experiment in one mice that received [$AuCl_2$(en)] Cl. That mouse was the only mouse from the [$AuCl_2$(en)] Cl group that got tumor (Figure 4).

Till the end of the experiment, a tumor was noticed in all mice from the control group (6/6) and in all mice that received [$AuCl_2$(SMC)]Cl and [$AuCl_2(DMSO)_2$]Cl (6/6). However, tumor was noticed in only one mouse that received [$AuCl_2$(en)]Cl (1/6) (Figure 4).

In order to determine the tumor volume by external caliper, experimental animals were sacrificed on the 21st day and tumors were removed (Figure 5). We noticed that tumors removed from control mice were bigger than tumors removed from mice which received gold(III) complexes (Figure 5). Among the tested gold(III) complexes, [$AuCl_2$(SMC)]Cl most effectively prevented tumor growth. The smallest tumor volume was noticed in mice that received [$AuCl_2$(SMC)]Cl (Figure 5).

Mice from the control group and mice that received [$AuCl_2(DMSO)_2$]Cl, [$AuCl_2$(SMC)]Cl and cisplatin showed weight loss and only mice that received [$AuCl_2$(en)]Cl gained weight during the experiment (Figure 6).

Discussion

The results from the *in vitro* experiment showed that all tested gold(III) complexes and cisplatin displayed cytotoxic effect on 4T1 cells. Decreases in concentrations were followed by markedly decrease in the number of apoptotic cells.

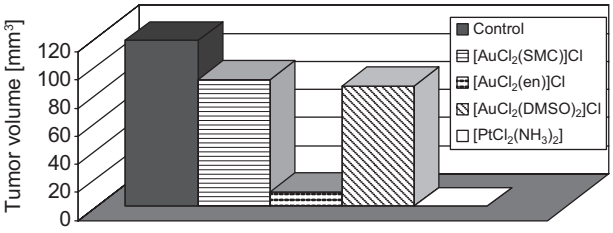

Figure 5. The tumor volume measured on the 21st day of the experiment. Each column represents a mean value of tumor volumes noticed in each group.

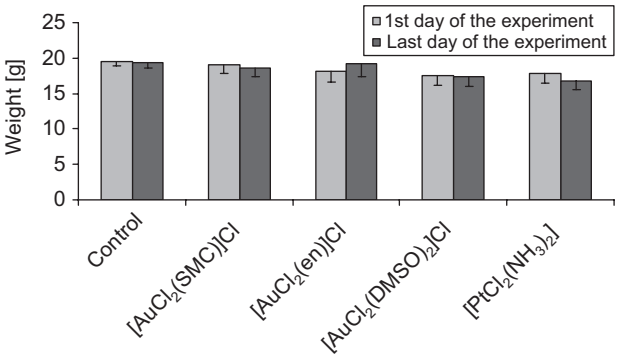

Figure 6. Weight of BALB/c mice during the experiment (measured on the 1st and last day of the experiment). Each column represents a mean value of mice weight from each group.

Among the tested Au(III) complexes, $[AuCl_2(en)]Cl$ showed the best cytotoxic effects *in vitro*. The cytotoxic effects of $[AuCl_2(en)]Cl$ and $[PtCl_2(NH_3)_2]$ were similar at all concentrations. Only at the concentration of 3.9 μM $[AuCl_2(SMC)]Cl$ complex showed higher cytotoxic effect than $[AuCl_2(en)]Cl$ and $[PtCl_2(NH_3)_2]$, while at very low concentration (1.95 μM) both $[AuCl_2(en)]Cl$ and $[AuCl_2(SMC)]Cl$ complexes showed higher cytotoxic effect than cisplatin.

All 3 tested gold(III) complexes and cisplatin (at all tested concentrations) showed concentration-dependent cytotoxic effect on 4T1 cells.

The data from the *in vivo* experiment showed that $[AuCl_2(en)]Cl$ can prevent the primary breast tumor growth in BALB/c mice (Figures 4 and 5). Only one from 6 mice that received $[AuCl_2(en)]Cl$ got tumor and the tumor in that mouse appeared later than the tumors which appeared in mice in the control group and in mice in the $[AuCl_2(SMC)]Cl$ and $[AuCl_2(DMSO)_2]Cl$-groups (Figure 4).

So, the *in vivo* experiment confirmed the data obtained *in vitro* that $[AuCl_2(en)]Cl$ has shown the best cytotoxic effects on 4T1 cells among all tested gold(III) complexes.

Although no tumor was noticed in mice that received cisplatin (0/6), 2 mice that received cisplatin died during the experiment. One mouse died on the 9th day and the second mouse died on the 20th day of the experiment, while all mice that received $[AuCl_2(en)]Cl$, $[AuCl_2(DMSO)_2]Cl$ and $[AuCl_2(SMC)]Cl$ stayed alive till the end of the experiment (Figure 4).

$[AuCl_2(en)]Cl$ was tolerated well and much better than $[AuCl_2(DMSO)_2]Cl$, $[AuCl_2(SMC)]Cl$ and cisplatin; this was confirmed by weight gain in mice that received $[AuCl_2(en)]Cl$ (Figure 6) and by better survival time in mice that received $[AuCl_2(en)]Cl$ in comparison with mice that received cisplatin. The possible reason why $[AuCl_2(en)]Cl$ was less toxic than cisplatin might be the different anticancer mechanisms utilized by gold complexes compared to cisplatin. Cisplatin exerts its anticancer effect by interacting with DNA and forming adducts that interfere with transcription and replication, followed by apoptosis [36]. The interactions of cisplatin with DNA result in Pt-GG intrastrand crosslink that is the critical lesion leading to cisplatin toxicity dominantly manifested by dysfunction of gastrointestinal and hematological systems [36,37]. Although the main biological targets for gold(III) complexes and the precise mechanisms responsible for gold-induced cytotoxicity are still unknown, some recently published data suggest that their mechanisms of action, such as modification of surface protein residues and inhibition of proteasome function [30], are substantially different from that of the Pt(II) complexes [38].

Several lines of evidence suggested that proteins rather than DNA are mainly targeted by gold complexes. The selective modification of surface protein residues by gold(III) complexes in a defined coordination geometry could be the molecular basis for their biological effects. Proteins containing exposed cysteine residues might be proper targets for gold(III) complexes that might cleave the disulfide bond of cystine and oxidize methionine and glycine suggesting the possibility that amino terminus of peptides and proteins could be deaminated by gold(III) complexes. In addition, it has been recently shown that proteasome inhibition by gold(III) complexes is associated with apoptosis of breast cancer cells *in vitro* and *in vivo* [30].

In our *in vivo* experiment we showed that $[AuCl_2(en)]Cl$ complex is more stable under physiological conditions than $[AuCl_2(SMC)]Cl$ and $[AuCl_2(DMSO)_2]Cl$. The possible explanation is that $[AuCl_2(SMC)]Cl$ and $[AuCl_2(DMSO)_2]Cl$ have S donor atom in the coordination sphere and, because of that, these complexes are less stable and probably less active under physiological conditions than $[AuCl_2(en)]Cl$ complex [19].

In line with all these results, $[AuCl_2(en)]Cl$ complex seems to be a good candidate for future pharmacological evaluation in the field of breast cancer research and treatment.

References

1. Hollingshead MG. Antitumor efficacy testing in rodents. J Natl Cancer Inst 2008; 100: 1500-1510.
2. Bibby MC. Orthotopic models of cancer for preclinical drug evaluation: advantages and disadvantages. Eur J Cancer 2004; 40: 852-857.
3. Dediu M, Median D, Alexandru A, Vremes G, Gal C, Gongu M. Adjuvant therapy with aromatase inhibitors in postmenopausal, estrogen receptor- positive breast cancer patients: up-

front or sequential? J BUON 2009; 14: 375-379.
4. Karaca B, Atmaca H, Uzunoglu S, Karabulut B, Sanli UA, Uslu R. Enhancement of taxane-induced cytotoxicity and apoptosis by gossypol in human breast cancer cell line MCF-7. J BUON 2009; 14: 479-485.
5. Miller FR, Miller BE, Heppner GH. Characterization of metastatic heterogeneity among subpopulations of a single mouse mammary tumor: heterogeneity in phenotypic stability. Invasion Metastasis 1983; 3: 22-31.
6. Miller FR. Tumor subpopulation interactions in metastasis. Invasion Metastasis 1983; 3: 234-242.
7. Tao K, Fang M, Alroy J, Sahagian GG. Imageable 4T1 model for the study of late stage breast cancer. BMC Cancer 2008; 8: 228.
8. Yoneda T, Michigami T, Yi B, Williams PJ, Niewolna M, Hiraga T. Actions of bisphosphonate on bone metastasis in animal models of breast carcinoma. Cancer 2000; 88: 2979-2988.
9. Aslakson CJ, Miller FR. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. Cancer Res 1992; 52: 1399-1405.
10. Pulaski BA, Ostrand-Rosenberg S. Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. Cancer Res 1998; 58: 1486-1493.
11. Eckhardt BL, Parker BS, van Laar RK et al. Genomic analysis of a spontaneous model of breast cancer metastasis to bone reveals a role for the extracellular matrix. Mol Cancer Res 2005; 3: 1-13.
12. Tomasevic Z, Radosevic-Jelic Lj, Jovanovic D. Brain metastases as late breast cancer relapse. Single institution experience and review of the literature. J BUON 2009; 14: 225-228.
13. Chavdarova L, Piperkova E, Tsonevska A, Timcheva K, Dimitrova M. Bone scintigraphy in the monitoring of treatment effect of bisphosphonates in bone metastatic breast cancer. J BUON 2006; 11: 499-504.
14. Alexandrova E, Sergieva S, Nikolova V, Danon S. Bone marrow micrometastases as a prognostic factor in early breast cancer patients. J BUON 2003; 8: 133-137.
15. Tsakonas G, Kosmas C. Integration of novel targeted therapies into the systemic treatment of breast cancer-a review. J BUON 2007; 12: 319-327.
16. Eniu A. Integrating biological agents into systemic therapy of breast cancer: trastuzumab, lapatinib, bevacizumab. J BUON 2007; 12 (Suppl 1): S119-126.
17. Milacic V, Dou QP. The tumor proteasome as a novel target for gold(III) complexes: implications for breast cancer therapy. Coord Chem Rev 2009; 253: 1649-1660.
18. Shaw CF. Gold-Based Therapeutic Agents. Chem Rev 1999; 99: 2589-2600.
19. Messori L, Abbate F, Marcon G et al. Gold(III) complexes as potential antitumor agents: solution chemistry and cytotoxic properties of some selected gold(III) compounds. J Med Chem 2000; 43: 3541-3548.
20. Buckley RG, Elsome AM, Fricker SP et al. Antitumor properties of some 2-[(dimethylamino)methyl]phenylgold(III) complexes. J Med Chem 1996; 39: 5208-5214.
21. Calamai P, Carotti S, Guerri A et al. Cytotoxic effects of gold(III) complexes on established human tumor cell lines sensitive and resistant to cisplatin. Anticancer Drug Des 1998; 13: 67-80.
22. Giovagnini L, Ronconi L, Aldinucci D, Lorenzon D, Sitran S, Fregona D. Synthesis, characterization, and comparative in vitro cytotoxicity studies of platinum(II), palladium(II), and gold(III) methylsarcosinedithiocarbamate complexes. J Med Chem 2005; 48: 1588-1595.
23. Che CM, Sun RW, Yu WY, Ko CB, Zhu N, Sun H. Gold(III) porphyrins as a new class of anticancer drugs: cytotoxicity, DNA binding and induction of apoptosis in human cervix epithelioid cancer cells. Chem Commun (Camb) 2003; 1718-1719.
24. Casini A, Hartinger C, Gabbiani C et al. Gold(III) compounds as anticancer agents: relevance of gold-protein interactions for their mechanism of action. J Inorg Biochem 2008; 102: 564-575.
25. Tiekink ER. Anti-cancer potential of gold complexes. Inflammopharmacology 2008; 16: 138-142.
26. Messori L, Orioli P, Tempi C, Marcon G. Interactions of selected gold(III) complexes with calf thymus DNA. Biochem Biophys Res Commun 2001; 281: 352-360.
27. Garza-Ortiz A, den Dulk H, Brouwer J, Kooijman H, Spek AL, Reedijk J. The synthesis, chemical and biological properties of dichlorido(azpy)gold(III) chloride (azpy=2-(phenylazo)pyridine) and the gold-induced conversion of the azpy ligand to the chloride of the novel tricyclic pyrido[2,1-c][1,2,4]benzotriazin-11-ium cation. J Inorg Biochem 2007; 101: 1922-1930.
28. Casini A, Kelter G, Gabbiani C et al. Chemistry, antiproliferative properties, tumor selectivity, and molecular mechanisms of novel gold(III) compounds for cancer treatment: a systematic study. J Biol Inorg Chem 2009; 14: 1139-1149.
29. Ericson A, Arthur J, Coleman R, Elding L, Elmroth S. Kinetics of interaction of the thione unit in 4-thio-2-deoxyuridine and 4-thiouridine 5-monophosphate with rapidly reacting gold(III) complexes. J Chem Soc Dalton Tran 1998; 10: 1687-1692.
30. Ronconi L, Marzano C, Zanello P et al. Gold(III) dithiocarbamate derivatives for the treatment of cancer: solution chemistry, DNA binding, and hemolytic properties. J Med Chem 2006 9; 49: 1648-1657.
31. Zhu S, Gorski W, Powell DR, Walmsley JA. Synthesis, structures, and electrochemistry of gold(III) ethylenediamine complexes and interactions with guanosine 5'-monophosphate. Inorg Chem 2006; 45: 2688-2694.
32. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55-63.
33. Euhus DM, Hudd C, LaRegina MC, Johnson FE. Tumor measurement in the nude mouse. J Surg Oncol 1986; 31: 229-234.
34. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol 1989; 24: 148-154.
35. Eckhardt S. Recent progress in the development of anticancer agents. Curr Med Chem Anticancer Agents 2002; 2: 419-439.
36. Abrams MJ, Murrer BA. Metal compounds in therapy and diagnosis. Science 1993; 261: 725-730.
37. Ott I, Gust R. Non platinum metal complexes as anti-cancer drugs. Arch Pharm (Weinheim) 2007; 340: 117-126.
38. Marzano C, Bettio F, Baccichetti F, Trevisan A, Giovagnini L, Fregona D. Antitumor activity of a new platinum(II) complex with low nephrotoxicity and genotoxicity. Chem Biol Interact 2004; 148: 37-48.