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# Effects of aurothiomalate and gold(III) complexes on spontaneous motility of isolated human oviduct

**Slobodan M. Janković · Ana Djeković · Živadin Bugarčić · Snezana V. Janković · Goran Lukić · Marko Folić · Dragan Čanović**

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**Abstract** Organic gold complexes have different biological activity, depending on their potential for interactions with key functional molecules.The aim of this study was to investigate potential of several newly synthesized organic gold complexes to influence spontaneous motility of the Fallopian tubes.The effects of $[Au(bipy)Cl_2]^+$ (dichloride(2,2′-bipyridyl) aurate(III)-ion), aurothiomalate, $[Au(DMSO)_2Cl_2]Cl$ and DMSO on spontaneous motility of Fallopian tubes were tested on the isolated tube segments in vitro. Aurothiomalate (from $2.9 \times 10^{-9}$ to $4.9 \times 10^{-4}$ M/l), $[Au(bipy)Cl_2]Cl$ (from $3.3 \times 10^{-9}$ to $4.2 \times 10^{-5}$ M/l) and DMSO (from $1.9 \times 10^{-8}$ to $1.0 \times 10^{-5}$ M/l) did not affect spontaneous contractions of the isolated Fallopian tube ampulla, while $[Au(DMSO)_2Cl_2]Cl$ (from $2.9 \times 10^{-9}$ to $4.2 \times 10^{-5}$ M/l) showed concentration-dependent increase (stimulation) of spontaneous contractions of the isolated Fallopian tube isthmus, and remained without effect on the isolated ampulla.The drugs designed as organic gold complexes with weaker bonds between the gold itself and organic part of a molecule could adversely affect motility of the Fallopian tubes, and theoretically fertility of women taking such drugs in their reproductive age.

**Keywords** Gold(III) · Complexes · Kinetics · Cytotoxicity · 5′-GMP · Fallopian tubes

S. M. Janković (✉) · S. V. Janković · M. Folić
Department of Pharmacology and Toxicology, Medical Faculty, University of Kragujevac, Ul. Svetozara Markovica 69, 34000 Kragujevac, Serbia
e-mail: slobodan.jankovic@medf.kg.ac.rs

A. Djeković · Ž. Bugarčić (✉)
Department of Chemistry, Faculty of Sciences, University of Kragujevac, R. Domanovića 12, P.O. Box 60, 34000 Kragujevac, Serbia
e-mail: bugarcic@kg.ac.rs

G. Lukić
Clinic for Gynecology and Obstetrics, Medical Faculty, University of Kragujevac, Kragujevac, Serbia

D. Čanović
Clinic for surgery, Medical Faculty, University of Kragujevac, Kragujevac, Serbia

## Introduction

There is long tradition of using gold and platinum drugs in medicine for treatment of various diseases. In particular, during the last 10–20 years, much interest has focused on gold(III) complexes (Messori and Marcon 2004; Dyson and Sava 2006). Presently, platinum drugs are playing a major role as established medical treatments of cancer (Wang and Lippard 2005; Reedijk 2003), while drugs with gold are used mostly in rheumatology. Gold(III) complexes are square-planar $d^8$, isoelectronic and isostructural to Pt(II) complexes. Generally speaking, gold(III) complexes are not very stable under physiological conditions due to their high reduction potential and fast

hydrolysis rate. Therefore, selection of a suitable ligand to enhance the stability became a challenge in the design of gold(III) complexes. The Au(III) is best coordinated by at least two chelating nitrogen donors which lower the reduction potential of metal center and thereby stabilize the complex. Stability of these gold(III) complexes (Giovagnini et al. 2005; Che et al. 2003) in solution was acceptable, which facilitated extensive pharmacological investigation, both in vitro and in vivo (Casina et al. 2008; Tieking 2008; Garza-Ortiz et al. 2007; Casini et al. 2009).

Female patients with rheumatoid arthritis do have decreased fertility rate, which is about 88 % of fertility rate in healthy females of the same age (Wallenius et al. 2011). Although reports on additional decreased fertility in female patients with rheumatoid arthritis who take gold-containing drugs are rare (Janssen and Genta 2000), it cannot be excluded due to paucity of published studies about this topic (Capell 2002).

The effects of gold complexes on motility of internal hollow organs were rarely investigated in the past. In an early study on isolated guinea pig tracheal rings sodium aurothiomalate and gold chloride inhibited contractile effect of histamine (Suzuki et al. 1983) in micromolar concentrations, but this effect was not reproduced in experiments on isolated proximal and distal canine airway smooth muscles (Ledford et al. 1989). Different effects on smooth muscles were observed for various organic compounds of gold: while auranofin markedly inhibited contractile response of the longitudinal muscle strips from the gastric body of the guinea-pig stomach, elicited by epidermal growth factor—urogastrone, aurothiomalate and aurothioglucose did not have any effect (Itoh et al. 1988). Also, direct vasodilating effect in the patients with rheumatoid arthritis was frequently observed after injection of aurothiomalate, but not after injection of aurothioglucose (Gottlieb and Brown 1977). Effects of gold compounds on smooth muscles probably depend on their ability to bind for enzymes or second messengers, like cGMP, which are involved in processes of contraction or relaxation; the binding of gold alters functioning of these regulatory molecules, and affects motility of hollow organs (Mietens et al. 2012).

The effects of organic gold compounds on smooth muscles of oviduct were not previously investigated at all. However, since aurothiomalate inhibits penetration of sperm into zona pellucida of hamster oocyte (Corselli and Talbot 1987), influence of organic gold compounds on many different aspects of fertilization (including Fallopian tubes motility) could not be excluded. Being highly prevalent disease (overall sex and age adjusted prevalence is 0.72 %) (Myasoedova et al. 2010) with mean age of the patients of around 41 year (Owino et al. 2009), rheumatoid arthritis affects great number of females in their reproductive period. Since organic preparations of gold are still widely used for treatment of rheumatoid arthritis (Gibofsky and Yazici 2010), their eventual influence on process of fertilization could have significant clinical implications. The aim of our study was to investigate whether aurothiomalate and two novel organic compounds of gold influence spontaneous motility of the isolated human Fallopian tubes.

## Materials and methods

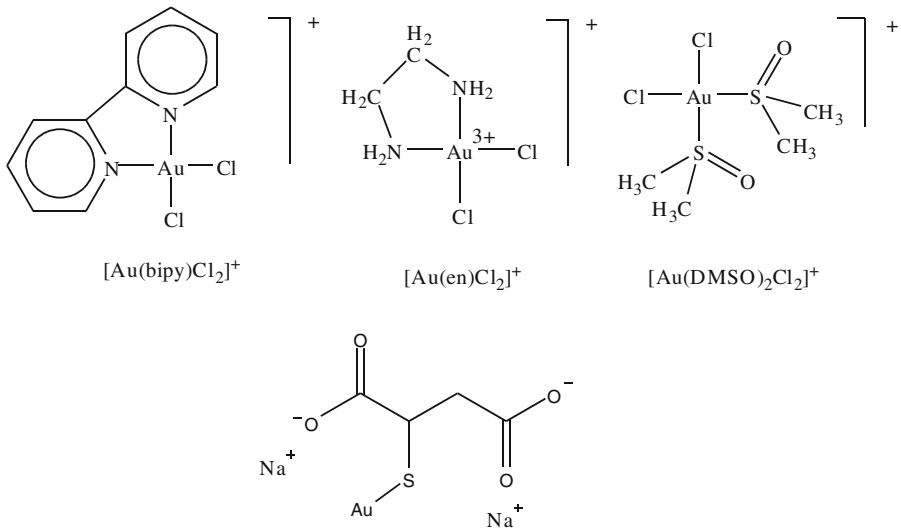
### Chemicals

The ligand 2,2′-bipyridyl(bipy) was obtained from Acros Organics. Starting complex potassium tetrachloridoaurate(III), $K[AuCl_4]$, was purchased from ABCR GmbH & Co. KG, 98 %. Gold sodium thiomalate hydrate (aurothiomalate) was purchased from Sigma-Aldrich, St. Louis, USA). All the other chemicals were of the highest purity commercially available and were used without further purification. Ultra pure water was used in all experiments.

### Synthesis of the complexes

The complex $[Au(bipy)Cl_2]Cl$ was prepared according to the published procedure (Zhu et al. 2006; Skibsted 1986; Milovanovic et al. 2010). Dichloridobis(dimethylsulphoxide)gold(III) chloride, $[Au(DMSO)_2Cl_2]Cl$, was synthesized by dissolving $KAuCl_4$ (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 solution of 0.1 M 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 formed dark yellow crystals were filtrated, washed with cold water 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,

**Fig. 1** Structures of some gold compounds




7.32; S, 7.76 %. Structures of the investigated complexes are shown in Fig. 1.

## Instrumentation

Chemical analyses were performed on a Varian III CHNOS Elemental Analyzer, Elemental Analysensysteme, GmbH. The optical density was measured using microplate multimode detector Zenyth 3100. UV–Vis spectra were recorded on Shimadzu UV 250 and Hewlett-Packard 8452A diode-array spectrophotometers with thermostated 1.00 cm quartz Suprasil cells.

## Patients

Fallopian tubes were taken from 18 female patients (one tube from each patient) during abdominal hysterectomy with adnexectomy. All patients underwent surgery because of extensive uterine fibroids which were causing prolonged uterine bleeding. The patients were unable to identify regular menstrual bleeding for 3 months prior to hospital admission. The mean age of the patients was 44.6 ± 5.4 years, with the range from 36 to 56 years. The study was approved by Ethics Committee of Clinical Center "Kragujevac", and the patient signed the informed consent forms.

All patients underwent surgery from 2008 to 2010 in the Gynecological Clinic of Clinical Centre "Kragujevac" in Kragujevac, Serbia. The time span of sample collection was 22 months, due to difficulties in obtaining undamaged samples of the same size. None of the patients received sex hormones for one and a half months prior to the operation. The operations were performed under general anesthesia produced by gas $N_2O$, opioid fentanyl and neuroleptic droperidol. The anesthesia was induced by intravenous injection of thiopental sodium, and muscle relaxation achieved initially by succinyl-choline and later on by pancuronium. All patients were pre-medicated with 0.5 mg of atropine subcutaneously.

After clamping the blood supply and resecting a Fallopian tube it was placed in 250 ml dish filled with De Jalons solution (154 mM NaCl, 5.95 mM $NaHCO_3$, 5.63 mM KCl, 0.54 mM $CaCl_2{\cdot}2H_2O$, 2.78 mM glucose) which was gassed (95 % $O_2$ and 5 % $CO_2$, 5 ml/min) and transported to the laboratory.

## Isolated preparations

About fifteen minutes after taking a Fallopian tube in the operating room the isolated preparations were mounted in an isolated organ bath. Two types of Fallopian tube preparations were isolated: isthmus and ampulla. The serosa was removed from both the isthmic and the ampullar preparations. The isthmic preparations with following measures were used in the experiments: 4 cm in length, wall thickness 1.2 mm and the lumen diameter 1 mm. Also, the ampullar

preparations with following measures were used in the experiments: 5 cm in length, wall thickness 1.2 mm and the lumen diameter 5–6 mm. Both types of preparations were mounted in an organ bath longitudinally, analogous with Magnus preparations of rat ileum (Magnus 1904). Opposite walls of the preparation were attached to the bath base and the transducer, respectively.

### The bath and the transducer

The isolated preparations were mounted in 75 ml isolated organ bath, filled with De Jalons solution (154 mM NaCl, 5.95 mM $NaHCO_3$, 5.63 mM KCl, 0.54 mM $CaCl_2 \cdot 2H_2O$, 2.78 mM glucose). The bath solution was maintained at 37 °C and aerated with 95 % $O_2$ and 5 % $CO_2$. One end of the isolated preparation was attached to the bath base, and the other to the lever of the isometric transducer. The tension of the isolated preparations was continuously recorded with the isometric transducer (Palmer Bio Science, Los Angeles, CA, USA) and registered on personal computer using Majk Electronic interface and software (Majk Electronic, Mladenovac, Serbia and Montenegro). The isolated preparations were given a passive load of 1 mN and allowed to equilibrate for 1 h before an experiment started.

### The agonists

The spontaneous contractions of isolated preparations were measured as area under the curve (AUC). The effects of experimental substances on area under the contraction curve were measured.

In the beginning of each experiment, at least one hour of spontaneous activity was recorded, in order to observe for spontaneous changes of phasic contractions. The experimental substances were added to the isolated organ bath cumulatively, without washing between the subsequent doses. The interval between two adjacent doses was always 5–6 min. After cumulating all doses of a substance, the bath was washed three times, and the isolated preparation was allowed to rest for further 30 min. The effect of each experimental substance was observed on at least four isolated preparations, taken from different individuals.

### Statistics

The effect of each concentration of a test-substance on spontaneous contractions was expressed as a percentage of the maximum effect obtained with that agonist, and used for construction of concentration–response curves. The concentration–response relationship was determined by linear regression on logarithmically transformed data calculated according to the method of least squares. The range of values used for the linear regression was from 15 to 85 % of the maximal response, in the more linear part of the curve. The concentration of an agonist eliciting 50 % of its own maximum response ($EC_{50}$) and its confidence limits (1.96 × standard error) were determined graphically for each curve by linear interpolation (Bowman and Rand 1980; Kenakin 1984).

The significance of changes in phasic activity of the isolated preparations was tested by one-way analysis of variance.

## Results

### Isolated preparations of ampulla

Preparations from all patients showed spontaneous activity comprised of slow phasic contractions with amplitude of $10.7 \pm 3.9$ μN (3.9 μN = standard deviation [SD]) and frequency of 3–7 cycles per minute.

The spontaneous change in phasic activity of isolated ampulla was not observed after 2 h of follow-up ($F = 0.057, df_1 = 10, df_2 = 32, p > 0.05$).

Aurothiomalate (from $2.9 \times 10^{-9}$ to $4.9 \times 10^{-4}$ M/l), $[Au(bipy)Cl_2]Cl$ (from $3.3 \times 10^{-9}$ to $4.2 \times 10^{-5}$ M/l), $[Au(DMSO)_2Cl_2]Cl$ (from $1.9 \times 10^{-8}$ to $1.0 \times 10^{-5}$ M/l) and DMSO (from $1.9 \times 10^{-8}$ to $1.0 \times 10^{-5}$ M/l) did not affect spontaneous contractions of the isolated Fallopian tube ampulla ($F = 0.873$, $df_1 = 8$, $df_2 = 27$, $p > 0.05$; $F = 1.573$, $df_1 = 7$, $df_2 = 18$, $p > 0.05$; $F = 1.279$, $df_1 = 7$, $df_2 = 24$, $p > 0.05$; and $F = 0.636$, $df_1 = 7$, $df_2 = 23$, $p > 0.05$ respectively).

### Isolated preparations of isthmus

Preparations from all patients showed spontaneous activity comprised of slow phasic contractions with

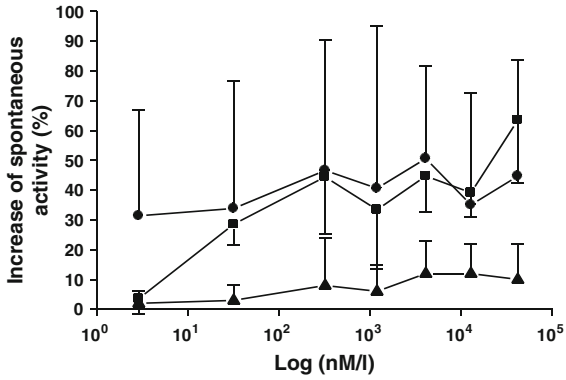



**Fig. 2** Semi-logarithmic plot of stimulatory effects of [Au(DMSO)$_2$Cl$_2$]Cl (*open square*), aurothiomalate (*black up-pointing triangle*) and [Au(bipy)Cl$_2$]Cl (*black circle*) on spontaneous contractions of isolated human oviduct isthmus. Each point represents mean effect obtained from experiments on isolated preparations taken from four different persons. *Error bars* standard deviations. Half of the *error bars* was omitted for the sake of clarity

amplitude of 7.6 ± 2.8 μN (2.8 μN = standard deviation [SD]) and frequency of 3–8 cycles per minute.

The spontaneous change in phasic activity of isolated isthmus was not observed after 2 h of follow-up ($F = 0.395$, $df_1 = 7$, $df_2 = 22$, $p > 0.05$).

[Au(DMSO)$_2$Cl$_2$]Cl (from $2.9 \times 10^{-9}$ to $4.2 \times 10^{-5}$ M/l) showed concentration-dependent increase (stimulation) of spontaneous contractions of the isolated isthmus. ($EC_{50} = 8.62 \pm 5.08 \times 10^{-6}$ M/l, $r = 0.620$, $p < 0.05$) (Fig. 2).

Aurothiomalate (from $2.9 \times 10^{-9}$ to $4.9 \times 10^{-4}$ M/l), [Au(bipy)Cl$_2$]Cl (from $3.3 \times 10^{-9}$ to $4.2 \times 10^{-5}$ M/l) and DMSO (from $1.9 \times 10^{-8}$ to $1.0 \times 10^{-5}$ M/l) did not affect spontaneous contractions of the isolated Fallopian tube isthmus ($F = 0.900$, $df_1 = 8$, $df_2 = 36$, $p > 0.05$; $F = 0.130$, $df_1 = 6$, $df_2 = 21$, $p > 0.05$; and $F = 0.290$, $df_1 = 10$, $df_2 = 44$, $p > 0.05$, respectively) in a concentration-dependent manner (Fig. 2).

## Discussion

Gold(III) complexes react much faster than Pt(II) complexes with the same nucleophiles (Bugarcić et al. 2008). Nucleotides (like 5′-GMP) have a higher affinity for gold(III) complex than chloride, which may have important biological implications. In our previous study (Arsenijevic et al. 2012) we have shown that Au(III) readily binds to guanosine 5′-monophosphate (5′-GMP) via nucleophilic attack on N7 atom of guanine (Zhu et al. 2006; Skibsted 1986; Milovanovic et al. 2010) similar interaction could be expected from Au(III) and physiological precursor of 5′-GMP, guanosine-3′, 5′-cyclic monophosphate (cGMP). Decrease in free intracellular cGMP in human semen is associated with decreased sperm motility (Zhang and Zheng 1996; Mostafa 2007), decreased Leydig cell secretory function, decreased motility of epididymis, decreased prostatic secretory function and male infertility (Dimitriadis et al. 2009). On the other hand, increase in free intracellular cGMP is associated with increased viability of human granulosa luteinized cells, better development of pre-ovulatory follicles and increased fertilization rate of oocytes (Dineva et al. 2011). Au(III) complexes and other drugs containing gold which binds to the cGMP could adversely influence both male and female fertility, through decrease of intracellular free cGMP which should be available for normal signaling in reproductive tissues.

The type of coordinated inert ligand has a large effect on reaction rate. The [Au(bipy)Cl$_2$]$^+$ complex is more reactive than [Au(en)Cl$_2$]$^+$. The activation parameters for all studied reactions suggest an associative substitution mechanism. In the second step of the reaction, another chloride ion from the starting complex is substituted, and 1:2 complexes are formed. Both the first and the second steps of the substitution of [Au(bipy)Cl$_2$]$^+$ complex are faster than in the case of [Au(en)Cl$_2$]$^+$ complexes suggesting higher biological activity of [Au(bipy)Cl$_2$]$^+$, similar to other labile gold complexes, like [Au(DMSO)$_2$Cl$_2$]Cl (Milovanovic et al. 2010).

Gold complexes could be good candidates for future pharmacological evaluation as new therapeutic agents in the pre-clinical studies for treatment of lung carcinoma, considering their recently described cytotoxic effect in vitro (Milovanovic et al. 2010). However, their adverse effects profile is also important, including interference with motility of hollow organs. It was shown that several metals (zinc, copper and titanium) may inhibit contractility of human airway smooth muscle cells, without affecting their viability (Berntsen et al. 2010). Although mechanism of their action remains unclear, it is unlikely to be mediated by some non-specific pathway, like

metal-induced generation of reactive oxygen species (Berntsen et al. 2010; Ozturk et al. 2009; Vaziri 2008). On the other hand, some heavy metals like lead induce contraction of vascular smooth muscle cells through activation of protein kinase C (Ozturk et al. 2009; Vaziri 2008). Incubation of isolated smooth muscle strips from aorta with cadmium led to augmentation of 5-hydroxytryptamine-stimulated inositol monophosphate accumulation and contraction (Sakurada and Wakabayashi 1999). All these experimental data suggest significant influence of metals, including gold, on tone and spontaneous activity of smooth muscle organs in human body.

In our study only one of the gold(III) preparations used, $[Au(DMSO)_2Cl_2]Cl$, produced stimulation of spontaneous activity of isolated oviduct. This effect should be attributed to the gold itself, since in our experiments with DMSO alone no changes in spontaneous motility of Fallopian tubes happened. Since gold is more easily released from this organic compound than from aurothiomalate or $[Au(bipy)Cl_2]Cl$, this could explain better contact of gold with smooth muscle cells and observed differences in the effects. Other studies have also confirmed higher biological activity of organic gold compounds with weaker bonds between the gold itself and organic part of a molecule (Suzuki et al. 1983; Ledford et al. 1989; Itoh et al. 1988; Gottlieb and Brown 1977). The difference in stability of organic gold compounds and subsequent difference in biological activity should be taken into account during design of novel drugs that contain gold.

Our patients were at the and of their reproductive period, and phase of their menstrual cycle could not be determined due to long period of irregular bleeding caused by uterine fibroids. This prevents extension of our findings to all phases of menstrual cycle, creating need for additional studies with gold complexes on isolated isthmus taken from women which unequivocally were in follicular or luteal phase.

## Conclusion

In conclusion this work demonstrated that gold(III) complexes with weaker bonds between the gold itself and organic part of a molecule more readily influence motility of the Fallopian tubes' isthmus, causing stimulation. Excessive stimulation of the Fallopian tubes spontaneous motility by gold may hamper its important role in the process of fertilization, leading to decreased fertilization rate in women taking drugs containing gold. Therefore, drugs designed as organic gold complexes with stronger bonds between the gold itself and organic part of a molecule could be better tolerated by women in their reproductive age.

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