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RESEARCH ARTICLE

Evaluation of reproductive toxicity in male rats treated with novel synthesized ruthenium(II) and gold(I)-NHC complexes

Osman Ciftci[1], Ali Beytur[2], Nigar Vardi[3], and Ismail Ozdemir[4]

[1]Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Inonu University, Malatya, Turkey, [2]Faculty of Medicine, Department of Urology, Inonu University, Malatya, Turkey, [3]Faculty of Medicine, Department of Histology and Embryology, Inonu University, Malatya, Turkey, and [4]Faculty of Science and Art, Department of Chemistry, Inonu University, Malatya, Turkey

Abstract

In this study, we aimed to determine the reproductive toxicity in rat induced by ruthenium(II)-NHC (Ru^{II}) and gold(I)-NHC (Au^{I}) complexes that have anticarcinogenic effects. For this purpose, 35 Sprague–Dawley rats were randomly divided into 5 equal groups. In control group, rats treated with saline, Ru^{II}, and Au^{I} complexes were intraperitoneally given high (10 mg/kg) and low (5 mg/kg) doses to rats via a one-time administration. The animals were sacrificed, and testis tissues were taken on Day 10 of the drug administration for the determination of the biochemical, histopathological, spermatological, and hormonal parameters. It was determined that treatment group that was subjected to treatment using both Ru^{II} and Au^{I} complexes significantly caused oxidative, histopathological, spermatological, and hormonal damage compared to control group. However, the sexual and accessory organ weight did not significantly change when compared to control. In addition, it was shown that Au^{I} treatment generally caused more adverse effects than Ru^{II} treatment in a dose-dependent manner. In conclusion, when these synthesized compounds are used for the treatment of cancer, they could cause toxic effects on male reproductive system and lead to infertility. However, Ru^{II} complex is a more preferable option in cancer treatment, particularly in terms of user safety.

Keywords: $Ruthenium^{II}$, $gold^{I}$, reproductive toxicity, testis tissue, NHC complexes

Introduction

Inorganic elements, including platin, ruthenium, and gold, play important roles in biological and biomedical processes, because they can be used as novel therapeutic and diagnostic agents[1,2]. The clinical usage of metal-based drugs can bring about effective changes in many fields, including the following treatment spheres: anticancer, antiulcer, and antiarthritic[1,2]. Until now, cisplatin, the one first used as an anticarcinogenic inorganic metal complex, was successfully used in treating many types of cancer such as breast, lung, and prostate cancers[3,4]. However, cisplatin's usage was restricted significantly because of its toxic effects such as nephrotoxicity, cardiotoxicity, ototoxicity, and hepatotoxicity[5–7]. Therefore, in recent years, research has focused on identifying and developing new-generation metal complexes that are effective and safe for cancer treatment in order to find an alternative for cisplatin considering its toxic effects. In this context, ruthenium and gold complexes as binding *N*-heterocyclic carbenes (NHCs), one of the most important classes of ligands used in organometallic and coordination chemistry, have anticarcinogenic properties[8].

In the recent years, many studies[9,10] examined to the determination pharmacological properties, including antifungal, antimicrobial, and anticarcinogenic effects of ruthenium agents. Clarke[9] suggested that ruthenium complexes oxidation state 2^+ (Ru^{II}) and 3^+ (Ru^{III}) showed selective and powerful antitumor activity, especially against metastatic cancer. Besides, Aird et al.[11] evaluated Ru^{II} complexes for activity in both *in vitro* and *in vivo* models of human ovarian cancer and cross-resistance profiles established in cisplatin. Gold complexes, especially

Address for Correspondence: Asst. Prof. Osman Ciftci, Department of Pharmaceutical Toxicology, University of Inonu, Faculty of Pharmacy, 44280, Malatya, Turkey. Tel: +90–422 3410660/1836. Fax: +90–422-3411217. E-mail: osmciftci@gmail.com

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Aurofin used for the treatment of rheumatoid arthritis, are well-known pharmaceutical agents[2]. Also, they are active antitumor agents. Mirabelli et al.[12] demonstrated that a novel synthesis and use of Au^{I} and Au^{III} complexes that had phosphine group in their structure was as effective as Aurofin against leukemia P388. They claimed that antitumor activity of these compounds can be as strong as Aurofin and can be as effective against tumor models.

Our previous study demonstrated that ruthenium(II)-NHC (Ru^{II}) and gold(I)-NHC (Au^{I}) complexes caused significantly cardiotoxic effect in rats via increased oxidative stress in heart tissue[13]. On the other hand, there is no study about toxic effects of Ru^{II} and Au^{I} on male reproductive system if they are used clinically. For this reason, the present study aims to evaluate the toxic effects of Ru^{II} and Au^{I} complexes on male reproductive system, determine whether there is oxidative and histopathological damage to testis tissue, and evaluate sperm characteristics and testosterone levels.

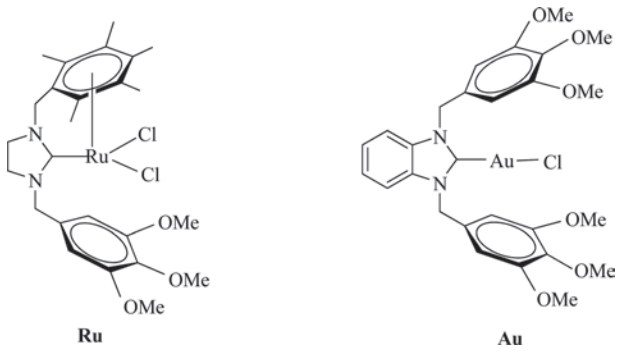
Materials and methods

Chemicals

Ru^{II} and Au^{I} complexes used in this study were synthesized in the Inonu University, Organometallic research laboratory, according to Ciftci et al.[13] (Scheme 1).

Animals and treatment

A total of 35 healthy adult male Sprague–Dawley rats (between 3 and 4 months old and 200 and 300 g in weight) were obtained from Experimental Animal Institute, Malatya-Turkey, for this experiment. Animals were housed in sterilized polypropylene rat cages, in 12-h light-dark cycle, at an ambient temperature of 21°C. Diet and drinking water were given to them via *free feeding*. Rats were randomly divided into five equal groups ($n=7$ in each group), and all groups were exposed to only one dose of drug intraperitoneally. Cisplatin, which is known to cause general toxicity of 5 and 10 mg/kg dose, was added to Ru^{II} and Au^{I} complexes[13]. All drugs were intraperitoneally (i.p.) administered, by only one administration after all chemicals were dissolved in dimethylsulfoxide (DMSO). In the first (control) group, DMSO was administered to rats by a single injection in order to create the same environmental stress. In second and third groups Ru^{II} were given to rats at a dosage of 5 (Ru^{II}-5) and 10 (Ru^{II}-10) mg/kg. In fourth and fifth groups Au^{I} were given rats at the same doses (Au^{I}-5 and Au^{I}-10). The animals were sacrificed under ether anesthesia after 10 days of drug administration. For biochemical analysis, right testis was immediately dissected and weighted. Blood samples were collected from the left ventricle with an injection under anesthesia. Serums were obtained after whole blood centrifugation (3000 g, 20 min, at 4°C). Tissue and serum samples were stored at −45°C in deepfreeze until analysis.



Scheme 1. Chemical structure of Ru(II) and Au(I)-NHC complexes.

Biochemical assay

The homogenization of tissues was carried out in Teflon glass homogenizer with 150 mM KCl (pH 7.4) to obtain 1:10 (w/v) dilution of the whole homogenates. The homogenates were centrifuged at $18.000 \times g$ (4°C) for 30 min to determine thiobarbituric acid–reactive substances (TBARS), total glutathione (GSH) levels and catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities. The levels of TBARS, as an index of lipid peroxidation, were determined by thiobarbituric acid reaction using the method of Yagi[14]. The product was evaluated spectrophotometrically at 532 nm, and results were expressed as nmol/g tissue. The GSH content of the testis homogenate was measured at 412 nm, using the method of Sedlak and Lindsay[15], and was expressed as nmol/ml. SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction resulting from O_2^- generation caused by the xanthine/xanthine oxidase system[16]. The product was evaluated spectrophotometrically at 560 nm, and results were expressed as U/mg protein. CAT activity of tissues was determined according to the method of Aebi[17]. The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The enzyme activities were given in k/mg protein. GPx activity was measured by the method of Paglia and Valentine[18]. The decrease in absorbance at 340 nm was measured as GPx activity expressed as U/mg protein. Tissue protein content was determined according to the method developed by[19] using bovine serum albumin as standard.

Histopathological examination

For histopathological study, left testis was weighted and fixed in formalin 10%0, dehydrated in ethyl alcohol, cleared in xylol, and embedded in paraffin wax. Sections of 5-μm thickness were cut and stained with hematoxylin and eosin for determining histopathological changes. The diameter of the seminiferous tubule (DST) and germinative cell layer thickness (GCLT) from 20 different areas of each testis were measured using a Leica Q Win Plus Image Analysis System (Leica Micros Imaging Solutions Ltd, Cambridge, UK) at 10 X. The testicular damage severity was semiquantitatively assessed for each of the following parameters: spermatogenic arrest (cease of the spermatogenesis), disintegration of the spermatogenic

layer, disorganization in germinal cells, multinucleated giant cell formation, and degeneration, desquamation, and vacuolization of spermatogenic cells. The degree of damages was identified as absent (0), slight (1), moderate (2), and severe (3), for each criterion. Maximum score is noted as 21, and the microscopic score of each section was calculated as the sum of the scores. Sections were examined using a Leica DFC 280 light microscope at 40× and 100× magnification for overall histologic examination.

Evaluation of sperm parameters

The epididymal sperm concentration was determined with a hemocytometer using a modified method briefly described by Ciftci et al.[20] The right epididymis was finely minced by anatomical scissors in 1 mL of isotonic saline in a Petri dish and allowed to incubate at room temperature. Then the supernatant fluids containing all epididymal sperm cells were counted with the help of a light microscope at 200× magnification. The percentage of forward progressive sperm motility was evaluated using a light microscope with heated (37°C) stage as described by Ciftci et al.[21] The percentage of forward progressive sperm motility was evaluated visually at 400× magnification. Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score. To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin-nigrosin were prepared. The slides were then viewed under a light microscope at 400× magnification. A total of 300 sperm cells was examined on each slide (2,100 cells in each group), and the head, tail, and total abnormality rates of spermatozoa were expressed as a percentage[20,21].

Hormonal analysis

Serum testosterone level was determined by enzyme-linked immunosorbent assay (ELISA) using antirat ELISA commercial kits from Cayman Chemical Company (Ann Arbor, MI, USA) according to the manufacturer's instructions. The plates were read at 405 nm using the CA-2000 ELISA microplate reader (CIOM Medical Co., Ltd. in China). Testosterone quantities in the samples were calculated from standard curves of testosterone using a linear regression method.

Statistical analysis

All values were presented as mean ± SEM. Differences were considered to be significant at $p < 0.05$. A computer program SPSS 11.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. For biochemical values, statistical analyses were performed using one-way ANOVA and post hoc Tukey's honestly significant difference test. Histopathological results were compared with Kruskal–Wallis variance analysis. Where differences among the groups were detected, group means were compared using the Mann–Whitney *U* test.

Results

Biochemical evaluation

The testis CAT, SOD, and GPx activities and TBARS and GSH levels for Ru^{II} and Au^{I} given at the doses of 5 mg/kg (low dose) or 10 mg/ kg (high dose) are presented in Table 1. The results showed that Ru^{II} and Au^{I} complexes caused a significant increase TBARS levels in all treatment groups (both low and high doses) compared to that of control group. However, the CAT, SOD, and GPx activities and GSH levels in testis tissue of rats were significantly increased in the experimental groups compared to control groups. Also, the current study observed that treatment of Ru^{II} and Au^{I} altered SOD, CAT, and GPx activities and TBARS and GSH levels in a dose-dependent manner. Generally, the high-dose administration of Ru^{II} and Au^{I} led to a decrease of antioxidant enzymes and an increase of TBARS levels in testicular tissue compared to low-dose administration.

On the other hand, when compared to low and high dose of Ru^{II} and Au^{I} administration, in low-dose application, it was found that only the CAT activity changed significantly and that Au^{I} treatment caused more decrease in the levels of CAT than Ru^{II} did. However, there were no significant changes in SOD, GPx activities, and GSH and TBARS levels between Ru^{II} and Au^{I} at low-dose application. In Au^{I} treatment (high-dose administration), TBARS levels significantly increased, but antioxidant enzyme activities and GSH levels did not significantly change compared to Ru^{II} treatment at the same dose.

Hormonal results

Serum testosterone levels are given in Figure 1. We determined that serum testosterone levels at both doses of Ru^{II} and Au^{I} decreased significantly compared to control groups. In addition, the testosterone levels did not significantly change in a dose-dependent manner in all groups. Also, there were no significant changes between same dose of Ru^{II} and Au^{I}.

Table 1. Changes in SOD, CAT, and GPx activities and GSH and TBARS levels in testis tissue of rats (value ± SEM, $n = 7$).

	Control	Ru^{II}-5	Ru^{II}-10	Au^{I}-5	Au^{I}-10
TBARS nmol/g tissue	12.3 ± 0.33	15.9 ± 0.89*[a]	20.9 ± 1.11*[b]	16.7 ± 0.45*[a]	24.6 ± 0.30*
SOD U/mg protein	39.8 ± 1.46	35.9 ± 1.35[a]	24.3 ± 0.48*	30.6 ± 0.86*[a]	23.8 ± 1.32*
CAT k/mg protein	0.78 ± 0.18	0.57 ± 0.13*[ab]	0.38 ± 0.21*	0.49 ± 0.19 *[a]	0.33 ± 0.13*
GSH nmol/ml	24.1 ± 1.11	18.5 ± 0.92*[a]	14.1 ± 0.89*	16.0 ± 0.74*[a]	11.5 ± 0.57*
GPx U/mg protein	687.6 ± 15.0	574.8 ± 17.1*[ab]	428.1 ± 11.5*	506.3 ± 9.5*[a]	372.4 ± 14.5*

* indicates means significantly different from control group ($p < 0.05$); [a] indicates significant difference between low- and high-dose treatment in the same drug ($p < 0.05$); [b] indicates significant difference between same dose treatment of CP and Pt-NHC ($p < 0.05$).

Organ weights and sperm characteristics

The value of organ weights (testis, epididymis, seminal vesicles, and prostate weight) and sperm characteristics (epididymal sperm concentration, sperm motility, and abnormal sperm rate) in rats treated with Ru^{II} and Au^{I} are given in Table 2. The results demonstrated that the weight of testis, epididymis, seminal vesicles, and prostate did not significantly change with Ru^{II} and Au^{I} treatment at low and high doses compared to control group. Besides, in all doses, Ru^{II} and Au^{I} caused a significant decrease in epididymal sperm concentration and sperm motility. However, there was not a significant change in abnormal sperm rate. Also, it was determined that these changes of epididymal sperm concentration and sperm motility generally occurred in a dose-dependent manner and increased correspondingly with an increase in dose. Besides, there were no significant differences between Ru^{II} and Au^{I} treatment in low-dose application groups. However, in high-dose-administered groups, Au^{I} treatment caused severe side effects compared to Ru^{II} treatment at the same dose.

Histopathological changes

Morphological damage ranged from none (control) to slight (Au^{I}-5) to moderate (Ru^{II}-5) and to severe (Au^{I}-10 and Ru^{II}-10). In sections of control rats, the seminiferous tubules were intact (Figure 2). The histology of testis of Au^{I}-5 was similar to that of the control group except for the disintegration of spermatogenic layer observed in some of the tubules (Figure 2). However, in the Ru^{II}-5 groups, more obvious damages were present, such as spermatogenic arrest and disorganization of germinal cells (Figure 2). On the other hand, testicular damage increased in a dose-dependent manner. As a matter of fact, seminiferous tubules containing arrested spermatogenic cells at various stages of division and degenerative changes in germinal cells were observed in Au^{I}-10 and Ru^{II}-10 groups (Figure 3). Also in Au^{I}-10 group, multifocal nodules were observed among the seminiferous tubules (Figure 3). The cells within nodules showed the light microscopic features of Leydig cells, such as vacuolated and eosinophilic cytoplasm. These nodules were not observed in any of groups, except for the Au^{I}-10 group.

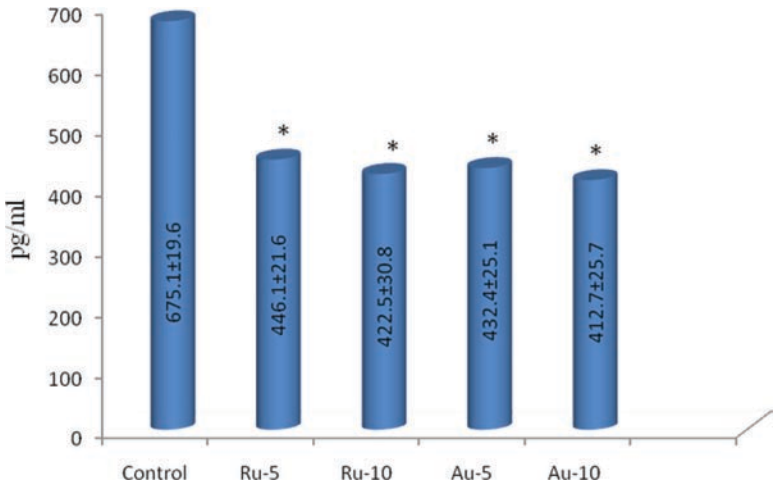


Figure 1. Serum testosterone levels in rates (pg/ml ± SEM). Asterisk indicates means significantly from control group ($p \leq 0.01$).

Table 2. Reproductive organ weights, epididymal sperm concentration, sperm motility, and abnormal sperm rate in rats after treatment of CP and Pt-NHC ($n = 7$).

Examining organs		Control	Ru^{II}-5	Ru^{II}-10	Au^{I}-5	Au^{I}-10
Testes weight (g)	Right	1.29 ± 0.041	1.380 ± 0.046	1.395 ± 0.023	1.285 ± 0.033	1.432 ± 0.021
	Left	1.33 ± 0.027	1.330 ± 0.046	1.380 ± 0.013	1.260 ± 0.030	1.389 ± 0.043
Epididymis weight (g)	Right	0.492 ± 0.045	0.542 ± 0.033	0.531 ± 0.022	0.489 ± 0.013	0.542 ± 0.025
	Left	0.532 ± 0.021	0.526 ± 0.022	0.532 ± 0.024	0.481 ± 0.021	0.529 ± 0.028
Seminal vesicles (g)		1.182 ± 0.216	1.318 ± 0.139	1.347 ± 0.225	1.158 ± 0.095	1.047 ± 0.118
Prostate (g)		0.190 ± 0.030	0.225 ± 0.032	0.194 ± 0.029	0.219 ± 0.038	0.215 ± 0.017
Sperm concentration (million/cauda)		79.07 ± 2.89	67.00 ± 5.52*[a]	60.00 ± 4.52*[b]	63.90 ± 5.03*[a]	46.30 ± 5.30*
Sperm motility (%)		83.52 ± 1.24	66.66 ± 2.70*[a]	59.66 ± 2.06*[b]	63.66 ± 1.69*[a]	52.66 ± 3.23*
Abnormal sperm rate (%)	Head	5.70 ± 0.27	5.80 ± 0.49	7.00 ± 0.44	6.80 ± 0.37	6.20 ± 0.37
	Tail	6.20 ± 0.21	6.40 ± 0.67	7.20 ± 0.54	6.80 ± 0.58	7.60 ± 0.24
	Total	11.90 ± 0.48	12.20 ± 1.11	14.20 ± 0.94	13.60 ± 0.58	13.80 ± 0.81

* indicates means significantly different from control group ($p < 0.05$); [a] indicates significant difference between low- and high-dose treatment in the same drug ($p < 0.05$); [b] indicates significant difference between same dose treatment of CP and Pt-NHC ($p < 0.05$).

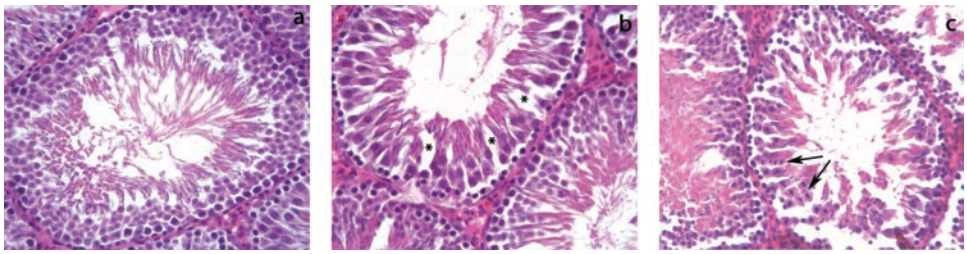


Figure 2. (A) Normal histopathological view of seminiferous tubules in control group, H-E X132. (B) Seminiferous tubules show slight histopathological changes in the Au^{I}-5 group. Testicular histology appears normal, except for the disintegration of spermatogenic layer of some tubules (*), H-E X132. (C) Moderate damage of seminiferous epithelium in Ru^{II}-5 group. Degeneration of germinal cells and spermatogenic arrest (arrows) are evident, H-E X132.

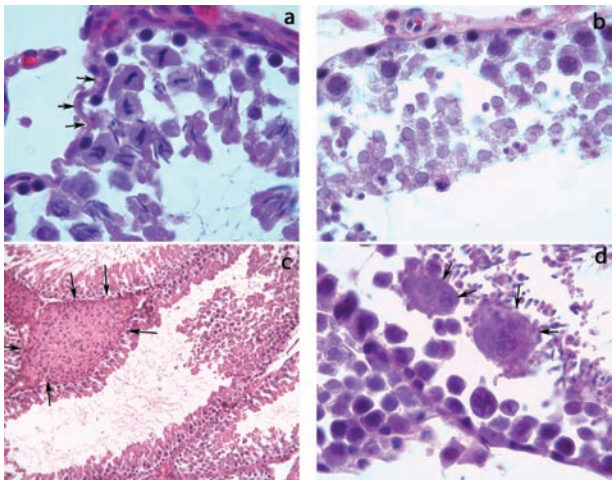


Figure 3. (A) Peritubular tissue in surrounding the epithelium are irregular and convolute (arrows) in Au^{I}-10 group H-EX330. (B) Loss of the germ cells is noted in Ru^{II} group, H-E X132. (C) Nodule is seen among seminiferous tubules (arrows) in Au^{I}-10 group, H-EX66. (D) A giant multinucleated cells derivative from round spermatids (arrows) are evident in Ru^{II}-10 group, H-EX330.

The multinucleated giant cells, which form as a result of the fusion of spermatids, were observed in the some of the seminiferous tubules, but only in Ru^{II}-10 group (Figure 3). Diameters seminiferous tubules (DST), germinal cell layer thickness (GCLT), and histopathological score (HIS) are given in Table 3.

Discussion

Elemental medicine is an important aspect mainly in the treatment of cancer and others illnesses such as arthritis and ulcer[1]. Cisplatin, which is one of the most used chemotherapeutic agents, had many side effects; therefore, its clinical usage was terminated in a phased manner, that is, progressively. For cancer therapy, new metal complexes, including Ru^{II} and Au^{I}, were synthesized and examined to assess their effectiveness as well as toxicity. In the present study, it was determined that the novel synthesized Ru^{II} and Au^{I} complexes treatment not only caused reproductive toxicity but also induced oxidative, histopathological, spermatological, and hormonal imbalances in the male reproductive system of rats.

Oxidative and histopathological damage

Oxidative stress is an imbalance between TBARS levels, an indicator of lipid peroxidation; it causes irreversible cell damage and diminishes the capacity of antioxidant defense system consisting of CAT, SOD, and GPx activities and GSH levels. Testes are the major target organs for oxidative stress because of a high content of polyunsaturated membrane lipids cells equipped with an antioxidant system[22,23]. Our results showed that Ru^{II} and Au^{I} complexes not only produced dose-dependent increase in TBARS levels but also induced lipid peroxidation in testis tissue of rats. On the other hand, the CAT, SOD, and GPx activities and GSH levels significantly decreased with Ru^{II} and Au^{I} treatment in a dose-dependent manner. Besides, it was demonstrated that Au^{I} treatment caused more oxidative stress than Ru^{II} did at the same doses. Au^{I} can affect antioxidant defense system in testis tissue much more seriously than Ru^{II} can. These compounds were novel syntheses, and, as far as we are aware, there was no study in the past that focused on these compounds to evaluate their capacity to cause oxidative damage in testis tissue. On the other hand, it was thought that cisplatin, being a metal-based drug, can also trigger oxidative stress on testis tissue, similar to Ru^{II} and Au^{I}. In this context, toxic effects of cisplatin on testis are well known. Many previous studies[6,7,24] showed that cisplatin induced oxidative damage in testis via an increase in TBARS levels and a decrease in CAT, SOD, and GPx activities and GSH levels. These results generally agree with and confirm our results. Metal drugs such as cisplatin showed their effects via binding DNA or unsaturated membrane lipids[2]. It was thought the oxidative effects of Ru^{II} and Au^{I} may lead to DNA binding or unsaturated membrane lipids as would other metal complexes. For checking this effect mechanism of Ru^{II} and Au^{I}, their use should form part of molecular experiment in future. We believe that oxidative damage has a key role to play in the reproductive toxicity caused by Ru^{II} and Au^{I} in rats.

Histopathological results indicated that Ru^{II} and Au^{I} complexes caused testicular damage in a dose-dependent manner. They could lead to spermatogenic arrest, disorganization of germinal cells, multifocal nodules, and vacuolated and eosinophilic cytoplasm in Leydig cells. Besides, it was shown that Au^{I} caused more

Table 3. Diameters seminiferous tubules (DST), germinal cell layer thickness (GCLT), and histopathological score (HIS).

	Control	Ru^{II}-5	Ru^{II}-10	Au^{I}-5	Au^{I}-10	*P* values
D ST	210.9 ± 5.8	161.5 ± 7*[b]	139.5 ± 6.2*[b]	226.8 ± 5[a]	207 ± 9.6	≤0.001
GCLT	45 ± 1.3	28.8 ± 1.8*[b]	29.5 ± 1.4*	48.9 ± 1.3[a]	26.8 ± 1.4*	<0.001
HIS	0.4 ± 0.2	6.6 ± 0.9*[ab]	13.0 ± 1.0*	3.2 ± 0.2*[a]	10 ± 1.5*	≤0.01

* indicates means significantly different from control group; [a] indicates significant difference between low- and high-dose treatment in the same drug; [b] indicates significant difference between same dose treatment of CP and Pt-NHC.

histopathological damage in testis tissue than Ru^{II} did, at the high dose. However, for low dose, this situation is the opposite of the high-dose application. There is no study about how Ru^{II} and Au^{I} affect testis-tissue histopathology. However, previous studies determined that testicular damages were typically associated with cisplatin therapy in rats[7,25]. It is thought that the histopathological toxic effects of Ru^{II} and Au^{I} on testis may be due to their oxidative effects, which may lead to infertility in rats.

Organ weight and spermatological and hormonal changes

In the present study it was determined that the treatment using Ru^{II} and Au^{I} led to a significant decrease in sperm motility and concentration compared to control group. On the other hand, organ weight (testis, epididymis, seminal vesicles, and prostate weight) and abnormal sperm rate did not significantly change between all groups. In addition, the current study demonstrated that toxic effects of metal complexes are proportionate to the dosage used and increase with high-dose treatment. Also, we showed that Au^{I} treatment was more toxic than Ru^{II} treatment in terms of effects on sperm characteristics. Many studies[7,24] and our unpublished data showed that cisplatin and novel platin–NHC complex caused significant decrease in sperm motility and concentration and did not change accessory organ weight (epididymis, seminal vesicles, and prostate weight). These results agree with our findings. By contrast, there were other past studies[24,26] that indicated that cisplatin and novel platin–NHC complexes significantly increased abnormal sperm rate and decreased testis weight in rats. We thought that this disagreement may be due to the fact that Au^{I} and Ru^{II} complexes have a different chemical structure. The adverse effects of cisplatin on serum testosterone level were previously documented, and it was determined[26] that testosterone levels significantly lowered with cisplatin treatment at a dose of 7 mg/kg. There is no study about how Ru^{II} and Au^{I} affect serum testosterone level in rats. First, it was demonstrated that Ru^{II} and Au^{I} treatment led to a significant decrease in serum testosterone levels compared to that of the control group. On the other hand, between experimental groups there was no significant change observed posttreatment. Previous studies[25,26] paralleled and confirmed our findings. It became obvious that oxidative and histopathological damage in testis tissue led to toxicity in sperm characteristics and testosterone levels. The present results indicated that Ru^{II} and Au^{I} may affect fertility adversely.

Conclusion

In conclusion, it was clearly determined that both Ru^{II} and Au^{I} complexes induced oxidative and histopathological damages in rat testis tissue. In addition, they can cause infertility via a decrease in epididymal sperm concentration, sperm motility, and testosterone level. On the other hand, Au^{I} treatment caused more testicular and spermatological damage in testis tissue than Ru^{II} did. These results indicated that when Ru^{II} and Au^{I} are used in cancer treatment, they can lead to severe toxic effects on male reproductive system and can cause infertility, similar to the effects observed in rats studied in our experiment.

Declaration of interest

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References:

1. Guo Z, Sadler PJ. (1999). Metals in medicine. Angew Chem Int Ed, 38:1512–1531.
2. Zhang CX, Lippard SJ. (2003). New metal complexes as potential therapeutics. Curr Opin Chem Biol, 7:481–489.
3. Hara M, Yoshida M, Nishijima H, Yokosuka M, Iigo M, Ohtani-Kaneko R et al. (2001). Melatonin, a pineal secretory product with antioxidant properties, protects against cisplatin-induced nephrotoxicity in rats. J Pineal Res, 30:129–138.
4. Mansour MA, Mostafa AM, Nagi MN, Khattab MM, Al-Shabanah OA. (2002). Protective effect of aminoguanidine against nephrotoxicity induced by cisplatin in normal rats. Comp Biochem Physiol C Toxicol Pharmacol, 132:123–128.
5. Antunes LM, Darin JD, Bianchi Nde L. (2001). Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol Res, 43:145–150.
6. Ciftci O, Ozdemir I, Vardi N, Gurbuz N. (2010). Novel platinum-N-heterocyclic carbene complex is more cardiotoxic than cis-platin in rats. Hum Exp Toxicol, doi: 10.1177/0960327110390064.
7. Türk G, Atessahin A, Sönmez M, Ceribasi AO, Yüce A. (2008). Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. Fertil Steril, 89:1474–1481.
8. Teyssot ML, Jarrousse AS, Manin M, Chevry A, Roche S, Norre F et al. (2009). Metal-NHC complexes: a survey of anti-cancer properties. Dalton Trans, 21:6894–6902.
9. Clarke, M.J. (2003). Ruthenium metallopharmaceuticals. Coord Chem Rev, 236:209–233.
10. Li H, Qian ZM. (2002). Transferrin/transferrin receptor-mediated drug delivery. Med Res Rev, 22:225–250.

11. Aird RE, Cummings J, Ritchie AA, Muir M, Morris RE, Chen H et al. (2002). *In vitro* and *in vivo* activity and cross resistance profiles of novel ruthenium (II) organometallic arene complexes in human ovarian cancer. Br J Cancer, 86:1652–1657.
12. Mirabelli CK, Hill DT, Faucette LF, McCabe FL, Girard GR, Bryan DB et al. (1987). Antitumor activity of bis(diphenylphosphino)alkanes, their gold(I) coordination complexes, and related compounds. J Med Chem, 30:2181–2190.
13. Ciftci O, Ozdemir I, Cakir, O, Demir S. (2011). The determination of oxidative damage in heart tissue of rats caused by ruthenium(II) and gold(I) N-heterocyclic carbene complexes. Toxicol Ind Health, doi: 10.1177/0748233710395993.
14. Yagi K. (1998). Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol, 108:101–106.
15. Sedlak J, Lindsay RH. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem, 25:192–205.
16. Sun Y, Oberley LW, Li Y. (1988). A simple method for clinical assay of superoxide dismutase. Clin Chem, 34:497–500.
17. Aebi H. (1974). Catalase. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis. New York: Academic Press, 673–771.
18. Paglia DE, Valentine WN. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med, 70:158–169.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem, 193:265–275.
20. Ciftci O, Aydin M, Ozdemir I, Vardi N. (2011). Quercetin prevents 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced testicular damage in rats. Andrologia, doi: 10.1111/j.1439-0272.2010.01126.
21. Ciftci O, Ozdemir İ,, Aydin M, Beytur A. (2011). Beneficial effects of chrysin on the reproductive system of adult male rats. Andrologia, doi: 10.1111/j.1439-0272.2010.01127.
22. Chainy GB, Samantaray S, Samanta L. (1997). Testosterone-induced changes in testicular antioxidant system. Andrologia, 29:343–349.
23. Chandra AK, Chatterjee A, Ghosh R, Sarkar M. (2010). Vitamin E-supplementation protect chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats. Food Chem Toxicol, 48:972–979.
24. Atessahin A, Karahan I, Türk G, Gür S, Yilmaz S, Ceribasi AO. (2006). Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Reprod Toxicol, 21:42–47.
25. Silici S, Ekmekcioglu O, Eraslan G, Demirtas A. (2009). Antioxidative effect of royal jelly in cisplatin-induced testes damage. Urology, 74:545–551.
26. Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A, Somay A. (2009). Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways. Hum Reprod, 24:1717–1725.