Asian Journal of Biological and Life Sciences | Original Research

Biological Activity of the Water Soluble Complex trans - dichloro - 1,2 - propylenediamine - N,N,N',N' - tetraacetato ruthenium (III) (RAD)

Fatima Azzahra Delmani[1,3]*, Adnan Jaran[2], José Torreblanca[3], Javier Moreno[3], Gregorio García-Herdugo[3], Rosario Vilaplana[4], Francisco González-Víltchez[4]

1 Department of Biology, Faculty of Science, Jarash University, 26150 Jarash, Jordan.
2 Departamento de BiologíaCelular, Facultad de Biología, Campus Reina Mercedes, Universidad de Sevilla, 41012 Sevilla, Spain.
3 Departamentode QuímicaInorgánica, Laboratorio de QímicaBioinorgánica, Facultad de Química, Universidad de Sevilla, 41071 Sevilla, Spain.
4 Department of Biological Science, Al al-Bayt University, P.O.BOX 130040, Mafraq 25113, Jordan

E-mail : delmanisa@yahoo.com Contact No. : +962 796255433

Submitted : 14.07.2016 Accepted : 11.09.2016 Published : 30.12.2016

Abstract

In this study we investigated the effect of the new antitumor complex *trans*-[$RuLCl_2$]H.4H_2O (RAD) on cultured TG cells (a human ovarian cell line), we found that RAD was cytotoxic to these cells. RAD was shown to interact with DNA of these tumour cells causing damage which was assessed by the comet assay. This interaction was causing a modification in the expression of certain nucleolar proteins, UBF (upstream binding factor) an RNA polymerase I-specific transcription factor involved in the recognition of the ribosomal RNA gene promoter and initiating transcription, and fibrillarinwhich contributes in many post transcriptional processes such as pre-rRNA processing, pre-rRNA methylation, and ribosome assembly.We found,after immunoprecipitating these two proteins, that the 2 isoforms of the protein UBF were expressed differently in TG cell extracts treated with RAD with a major abundance of UBF_1 compared with the control untreated cells, and little expression of UBF_2 when treated with RAD compared with the control. Fibrillarin was present in high quantities in TG protein extracts treated with RAD compared with the control. Comparing these results with the *cis* form of the compound (RAP), we found similar results regarding DNA damage but different results regarding protein expression. Although RAP was more powerful than RAD, the latest could be a good candidate as a potential antitumour drug used in the treatment of cancer.

Key words : Antitumor drugs; RAD; RAP; Comet assay; UBF; Fibrillarin; Immunoprecipitation

INTRODUCTION

Cancer is considered one of the major causes of death in the 21 century; many pharmaceutical companies are working harder to find a cure to the disease by synthesizing and testing new antitumor drugs. Of the widely used drugs, cisplatin and its analogues carboplatin and oxaliplatin, having a platinum metal core,were used against a broad range of malignancies such as colorectal, non-small cell lung, and genitourinary cancers [1-4]. The problem with cisplatin was the severe side effects generated after administration of the drug which encouraged researchers to investigate other metal based drugs with better cytotoxicity and lower side effects.

Ruthenium based drugs (a group of the platinum family) were an example of those anticancer drugs studied extensively in the last decade against a variety of cancers[5-8]. Ruthenium (III) complexes with polyaminocarboxylic molecules as chelating agents were heavily investigated in order to find water-soluble compounds having an antitumor activity; RAP([$RuLCl_2$]H.4H_2O, where L is 1,2-propylenediammine-*N,N,N',N'*-tetraacetic acid (PDTA)) is one of these complexes having an octahedral molecule with two chlorine atoms in a *cis* conformation and two PDTA ligands [9]. RAP showed lower cytotoxicity by interacting with DNA of cancer cells and forming DNA adducts [10]; the drug was tested against different cell lines causing DNA damage expressed by the formation of figures of comets [10, 11] as well as, a change in the expression levels of some proteins involved in rRNA regulation and processing [11, 12]. Other ruthenium (III) complexes were also tested for their antitumor activity, [$RuCl_4$(dmso-S)(histamineH)]·H_2O and [$RuCl_4$(dmso-S)(histamineH)] [13],*trans*-Him[$RuCl_4(Im)_2$] (Im=lmidazole) (ICR), *trans*-Hlnd[$RuCl_4(Ind)_2$] (RuInd) and Na[*trans*-$RuCl_4(Me_2SO)(Im)$] (NAMI) [14].

In this work we focused on the antitumor activity of the newly synthesized *trans*[$RuLCl_2$]H.4H_2O, where L is 1,2-propylenediammine-N,N,N',N'-tetraacetic acid (PDTA)) (RAD) (Figure 1A). We compared its antitumor activity *in vitro* with its *cis* isomer RAP (Figure 1B) using a human carcinoma ovarian TG cell line. We investigated the expression patterns of two different nucleolar proteins after treating TG cells with RAD.The first protein was the upstream binding factor (UBF), a specific transcription factor needed for the initiation of RNA polymerase I [15], itrecognizes the ribosomal RNA gene promoter and the transcription factor SLI facilitating the transcription of 5.8S, 18S, and 28S rRNAs[16-18]. UBF was found to activate the transcription of ribosomal RNA genes by binding to elements within the promoter and enhancing elements within the intergenic spacer [19, 20]. It is also known as the nucleolar organizer region (NOR) autoantigen NOR-90 [21] detected by antisera from patients with autoimmune diseases [22].NOR-90/UBF has two isoforms, NOR-97 (UBF_1) andNOR-94 (UBF_2); the 2 isoforms differ in that the NOR-97 lacks 37 amino acids within the second high mobility group (HMG) box [23]. The other protein wasfibrillarin, a 34-kDa protein that was first identified in sera from patients with scleroderma [24, 25], it is a methyltransferase involved in the processing of the primary ribosomal transcript and is mainly found in the FibrillarCenters and Dense FibrillarComponent of the nucleoli where it is directly involved in ribosome biogenesis [26]. Fibrillarin is associated with different small nucleolar RNAs including U3 snoRNArequired for the processing of rRNA[27]; it's also involved in the initiation of ribosomal transcription[28, 29].

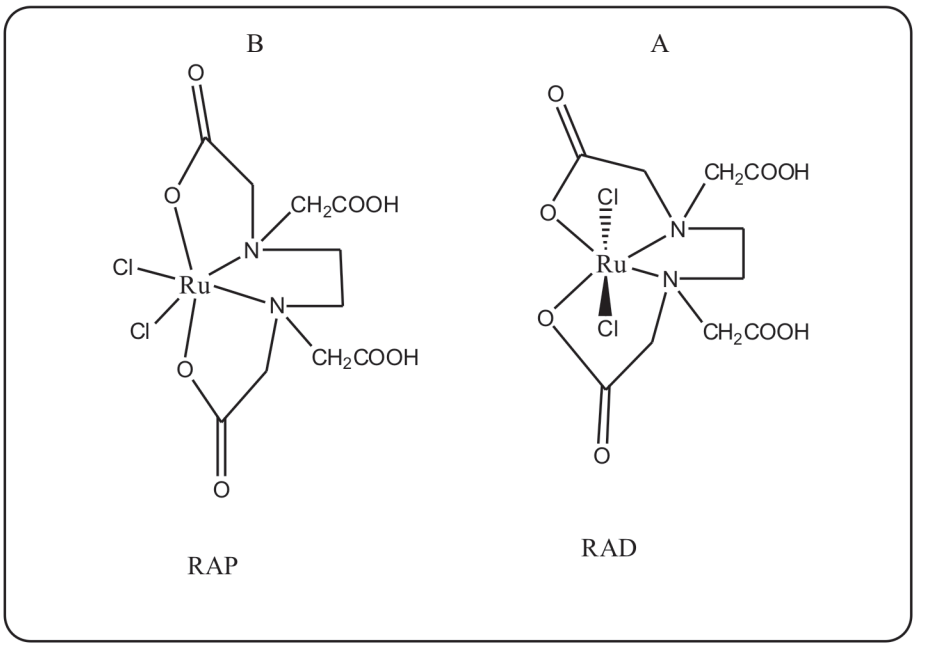

Figure 1 : RAD structure (A) and RAP structure (B).

MATERIAL AND METHODS

Chemicals

RAD compound (Figure 1A) was dissolved in water and stored at 4°C.

Human Sera

NOR-90/UBF antibody was obtained from patients with Rheumatoid arthritis.

Anti-fibrillarin antibody was obtained from patients with Scleroderma.

Cell culture

The human ovarian carcinoma TG cells utilized in this study were grown and maintained in cell culture flasks containing DMEN supplemented with 100.0 U/ml penicillin-streptomycin,10% foetal bovine serum, 2mM glutamine, 1% anti-PPLO,1mM sodium pyruvate and 1% fungizone. RAD was added to the cultured cellsat different concentrations, 1, 10, and 100 µg/ml for 5 days after changing the culture medium in both control and treated cells. At the end of treatment, cells were trypsinized from culture flasks and counted in a hemocytometer. For all the following experiments, cells were treated with RAD at 100 µg/ml for 4 days by changing the culture medium every day. For the immunoprecipitation, ^{35}[S] methionine-cystein (Trans ^{35}S-label) (1000-1100 Ci7 mmol, ICN Radiochemicals, Cleveland, USA) was added at the concentration of 50 µCi/ml on the 3^{d} day of the treatment.

Before each experiment, cells were trypsinized, washed with PBS (2.68 mMKCl, 136 mMNaCl, 1.46 mM KH_2PO_4, and 6.7mM Na_2HPO_4) and pelleted after a mild centrifugation. Cells were then counted in a hemocytometer to have the same starting number of cells in both control and RAD treated cells. Data were analyzed statistically using the student's t-test. A difference of P< 0.001 was considered statistically significant.

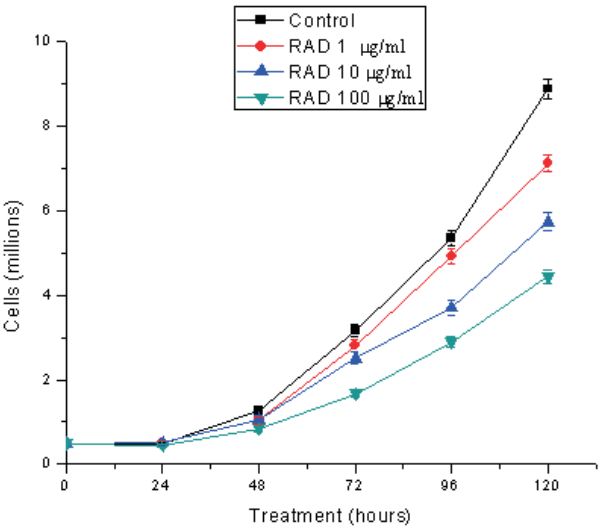

Figure 2 : Effect excerted by RAD on the kinetic of proliferating TG cells.

Single cell gel electrophoresis (Comet assay)

DNA lesions were detected using an adaptation of the method of Singh et al.[30].The alkaline treatment of cells followed by electrophoresis at high pH results in the flow of cellular DNA toward the anode, giving to cells the appearance of a comet, as a consequence of the presence of lesions in DNA which are converted to breaks under alkaline conditions. The intensity of diamidino-2-phenylindole (DAPI) (Sigma) stain was taken as a measure of DNA breakage [31].

TG cells were treated with RAD at 100 µg/ml for 4 days, suspended in low melting point agarose in PBS, pH 7.4 at 37 °C and then transferend onto frosted microscope slides pre-coated

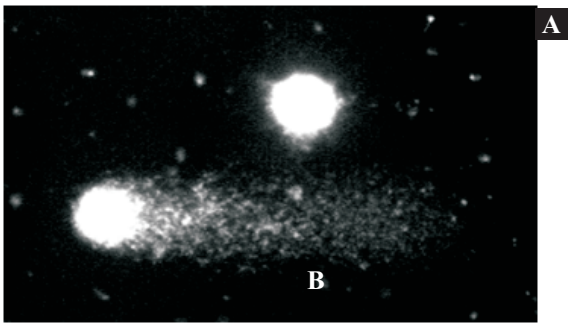

Figure 3 : Figure 3.Human TG cells. Cells were isolated and subjected to a comet assay. Following electrophoresis, cells were stained with DAPI, (A) control TG cells, (B) RAD treated TG cells.

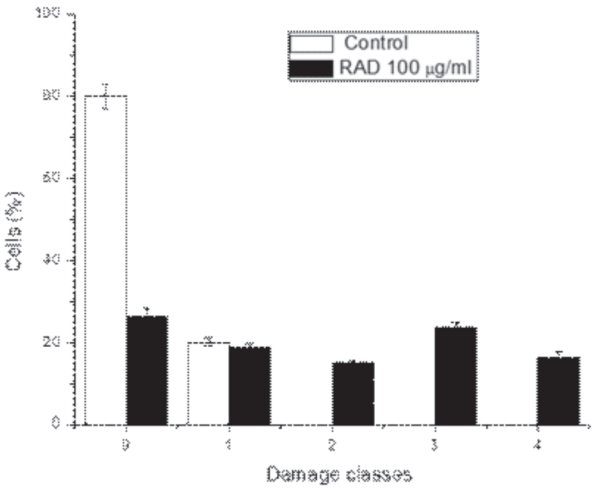

Figure 4 : Comet assay; DNA damage observed in human TG cells control and treated with RAD at 100 µg/ml for 4 days. Control (white columns) and RAD (black columns).

with a layer of normal melting point agarose. Slides were chilled on ice for 10 min then immersed in lysis buffer (100 mM Na_2EDTA, 2.5 M NaCl, 0.2 mMNaOH, 10 mMTris-HCl, pH 10.0, % Triton 10%, and DMSO) at 4 °C for 1h to remove cellular proteins. Slides were then placed in an electrophoresis tank containing 1 mM Na_2EDTA, pH 10.0 and 300 mMNaOH for 30 min. Electrophoresis was run at 27V for 20 min at 12 ºC. The slides were then washed 3 times, 5 min each, with 0.4 M Tris-HCl, pH 7.5 at room temperature. When dried, the slides were fixed in absolute methanol for 15 sec followed by a stainingwith 5 µg/ml DAPI. Slides were examined under a fluorescent microscope for the presence of DNA breaks; 20 cells from the control and RAD treatment were digitalized and analyzed using an image analysis software (PCBas Software). The stored images were used to estimate the DNA content in the individual nuclei and to evaluate the degree of DNA damage representing the fraction of total DNA in the tail. Cells were classified into five classes: 0 (< 7% of the DNA in the tail, undamaged), 1 (8-15%), 2 (16-22%), 3 (23-30%), and 4 (> 30%, maximally damaged cells).

Immunoprecipitation

We treated TG cells for 4 days with RAD at 100 µg/ml, on the 3^d day of treatment, methionine-cystein labelled with ^{35}[S] was added to the cells. Immunoprecipitation was carried out using protein A-bound Sepharose CL-4B (Sigma). Extracts from ^{35}[S] methionine-cystein labelled TG cells were prepared using NET buffer (400 mMNaCl,50 mMTris-HCl, pH 8, 5 mM Na_2EDTA, 0.05% azide, and1% Triton X-100) containing protease inhibitors (aprotinin,soybean trypsin inhibitor, CLAP mixture, benzamidine, and 100 mM PMSF in methanol), the mixture was sonicated for 1-2 min in order to break cell membranes. The extracts were incubated for 90 min with the anti-NOR90/UBF or the anti-fibrillarin antibodies coupled to Sepharose. The immunoprecipitates were washed 3 times in NET buffer, 3 times in NET buffer containing 800 mM NaCl and twice in a solution containing 1mM Na_2EDTA, and 10 mMTris-HCl, pH 8. The antibodies were eluted by adding SDS-PAGE sample buffer heated at 98 °Cfor 10 min and dithiotreitol (Sigma). Samples were subjected to an electrophoresis according toLaemmli[32] on a 12%

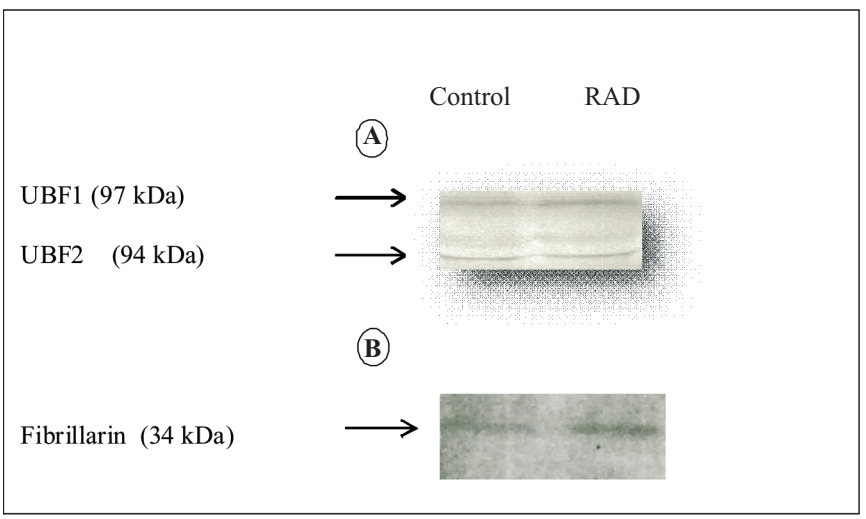

Figure 5. Immunoprecipitation of the human transcription factor hUBF and fibrillarin. TG cells were treated with 100 µg/ml RAD for 4 days; cells were incubated on the 3d day with methionine-cystein labelled with 35[S]; cellular lysates were immunoprecipitated and run on an SDS-PAGE electrophoresis gel. Gels were dried and exposed in an imaging plate (20-40) for 2 hours. Quantification of the bands was performed using a PCBas software computer. (A) TG cell extracts incubated with anti-NOR/UBF, (B) TG cell extracts incubated with anti-fibrillarin.

Table 1: Optical density of the two bands corresponding to UBF. Values are mean ±SEM (standard error of the mean). Proteins from whole TG cell extracts (control and RAD treated cells) were immunoprecipitated and analyzed following electrophoresis. The corresponding bands on the gels were quantified.

	Control	RAD treated cells
UBF1	94023 ± 8699.13	142501 ± 11786.01
UBF2	56432.53 ± 4356.98	39647.32 ± 5521.97

Table 2: Optical density of the band corresponding to fibri.llarin. Values are mean ±SEM ((standard error of the mean). Proteins from whole TG cell extracts (control and RAD treated cells) were immunoprecipitated and analyzed following electrophoresis. The corresponding bands on the gels were quantified.

	Control	RAD treated cells
Fibrillarin	89962 ± 10176.28	126690 ± 14006.85

SDS-polyacrilamide gel using the mini Protean II electrophoresis cell (Bio-Rad, Richmond, CA).Gels were fixed inasolution containing distilled water-isopropanol-acetic acid (65:25:10) for 1h with low agitation. In order to detect radiolabelled proteins, gels were soaked in Amplify (Amersham International, Buckinghamshire, England) with agitation for 30 min. Gels were then dried under vacuum at 80 °C using a model 583 gel dryer and exposed in an imaging plate (20-40) for 2h approximately. The plate was read in an IP-Autoradiography System Bio-Imaging Analyser (Fujix, Bas1000). Quantification of the bands was performed using PCBas Software.

RESULTS

Cytotoxicity assay

TG cells were treated with different concentrations of RAD, 1, 10, and 100 µg/ml for 5 day by changing the culture medium every day. Figure 2 shows the evolution with time of the total number of cultured TG cells in the absence (control cells) and presence of RAD (treated cells). At 1 µg/ml and 10 µg/ml concentrations of RAD, the treated cells did not show any clear significance with the untreated cells during the first days of treatment, after 120 hours of treatment with RAD at 1 µg/ml and 96 hours of treatment with RAD at 10 µg/ml,we were able to see a statistical significance compared with the controls. At a concentration of 100 µg/ml of RAD, a statistically significant difference was shown after 48 hours of RAD treatment compared with the control TG cells (Figure 2).

DNA damage caused by RAD on human TG cell

To see the extend of the DNA damage caused by RAD on human cultured TG cells, we carried out the comet assay, a method used to analyse and quantify DNA damage in individual cells [30]. The DNA damage detected by alkaline treatment can be caused by different mechanisms, DNA single strand breaks, DNA:DNA cross links, , incomplete excision repair sites, and alkali labile sites present at the time of lysis [30]. For each cell, wescored the total and the nuclear fluorescence to calculate the percentage of staining in the tail. The percentage of DNA in the tail was reported to be a good parameter for assessing DNA damage[31]. Figure 3 shows a picture of two TG cells, control (Figure 3A) and treated with RAD at 100 µg/ml (Figure 3B). The treated cells show a figure of a comet with its tail representing the fraction of damaged DNA that migrated when subjected to electrophoresis. Cells were distributed according to the percentage of damaged DNA in five classes ranging from 0 (undamaged cells) to 4 (maximally damaged cells). Figure 4 shows the distribution of cells according to their damaged DNA; in the control, most cells were undamaged (80.0%); after continuous treatment with RAD at 100 µg/ml for 4 days, we could see that most cells were damaged with 18.75 % of them with class 1 damage (8-15 %), 15.0 % with class 2 damage (16-22 %), 23.75 % with class 3 damage (23-30 %) and 16.25 % with class 4 damage (> 30 %) (Figure 4).

Immunprecipitation

We carried out an immunoprecipitation assay of two nucleolar proteins, the first protein was the NOR-90/hUBF corresponding to the nucleolar transcription factor hUBF (human upstream binding factor), this protein exists in two isoforms: UBF_1 and UBF_2having a molecular weight of 97 and 94 kDa respectively. The second protein was fibrillarinwith a molecular weight of 34 kDa. TG cells were treated with RAD for 4 days; cells were radiolabelled with *trans* [^{35}S] methionine-cystein before proceeding to the immunoprecipitation and electrophoresis. After analyzing the images, we found that the band corresponding to UBF_1 in RAD treated cell extracts was denser and the band corresponding to UBF_2 was lighter compared with the control cell extracts (Figure 5A). Table 1 shows the optical density (O.D.) of the bands corresponding to UBF_1 and UBF_2 compared with the control immunoprecipitated cell extracts, UBF_1 in the control extracts had an O.D. of 94023compared with 142501 in the RAD treated ones. UBF_2 showed an O.D. of 56432.53 in the control cell extracts compared with 39647.32 in the RAD treated ones. These results showed an increase in UBF_1 expression with 1.5-fold higher and a decrease in UBF_2 expression with 0.7-fold lower in RAD treated cells compared with the controls (table 1).

For fibrillarin, we found that the band corresponding to that protein in the control TG cell extracts was less dense than the one corresponding to the RAD treated ones (Figure 5B). The O.D. of the band corresponding to fibrillarin in both control and RAD treated cells showed a 1.4-fold increase in the protein expression in RAD treated cells (O.D. 126690) compared with the controls (O.D. 89962) (table 2).

DISCUSSION

Ruthenium complexes have been intensively studied over the last two decades, and still are, as potential anticancer drugs. We reported previously that one of the ruthenium isomer compounds, RAP (Fig. 1.*B*), was found to interact with DNA of tumour cells by being significantly cytotoxic to them [10], also, RAP was found to cause DNA damage in a number of human cell lines by interacting with their DNA leading to the formation of DNA adducts [11,33]. In this study we examined the cytotoxic behaviour of the *trans* form of RAP, RAD (*trans*-dichloro-1,2-propylenediamine-N,N,N',N'-tetraacetato ruthenium (III) (Figure 1.A). We found that RAD (at a concentration of 100 µg/ml was cytotoxic to the human ovarian carcinoma TG cell line by inducing a significant decrease in the proliferation rate of these cells after just 48 hours of continuous treatment. In order to confirm that this delay in the cell cycle of TG cells was related to the interaction of RAD with DNA, we carried out the comet assay, a method used to evaluate the extent of damage in the DNA caused by antitumor drugs [31]. From the comet assay, we found that RAD effectively induced DNA damage in TG cells with damage ranging from 8% to more than 30% (approximately 74% of cells were damaged), the obtained results are comparable with those found for the *cis* isomer, RAP, where high levels of damage to cells *in vitro*were reported but with stronger impact on cells than RAD [10]; these results show that RAD effectively targets DNA of cancer cells.

To further investigate the effect of RAD on tumor TG cells, we chose 2 nucleolar proteins involved in rRNA synthesis, rRNA 18S and rRNA 28S,and mRNAs of ribosomal proteins. Ribosomal RNA genes are arranged in tandem repeats in the NORs (nucleolar organizing regions) and are transcribed by RNA polymerase I in conjunction with associated factors including UBF (upstream binding transcription factor, encoded by UBTF) and SL1 proteins [16, 17, 34-36]. rDNAs encode precursor transcripts which are processed to form 18S, 28S, and 5.8S RNAs [37]. UBF is localized in the nucleoli in interphase cells and it regulates RNA polymerase 1 following acetylation [38- 40]. UBF is present in 2 isoforms UBF_1 and UBF_2, we found after the immunoprecipitation of this protein that RAD affected the expression of these two isoforms in a different manner, it caused a high expression of the 1st isoform UBF_1 with 1.5 fold higher than the control, and a low expression of the 2d isoform UBF_2 with 0.7 fold lower than the untreated cells. UBF_1 has more affinity to DNA adducts formed because of the presence of RAD than UBF_2, this could be explained by the fact that UBF_1 has a high affinity to bind rDNA than UBF_2, also the absence of the 2d HMG box motif in UBF_2 could be responsible for its low affinity to bind rDNA [23, 41]. The *cis*isomer RAP was found to increase the expression levels of the two isoforms of this transcription factor in a number of cell lines including TG cells used in this study with high expression of UBF_1 compared to UBF_2 in both control and treated cells [33, 11].Previous data showed that UBF levels were affected by treatment of cells with cisplatin leading to its displacement from the nucleolus where it's usually present to other nuclear compartments leading to cell death [42].

Fibrillarin, was found in high quantities in TG cells treated with RAD after its immunoprecipitation, about 1.4-fold increase in its expression compared with the control; this over expression could be explained by an alteration of the protein behaviour due to RAD treatment. Studies done by Legartova and his colleagues treating HeLa cells with ellargic acid, which was shown to affect the epigenome of mammalian cells, as well as, having an anti-proliferative effect, showed an increase in the expression of both UBF1/2 and fibrillarin[43]. Bo Geun Kim and his group showed that Chijongdan, an antitumor herbal drug, induces apoptosis and inhibits ribosomal biogenesis proteins including UBF and fibrillarin by suppressing them via caspase activation[44].

Previously, it was reported that the cis isomer RAP leads to an over expression of UBF1/2 and a lower expression of fibrillarin[33, 11], in this study, RAD was also found to cause an increase in the expression of UBF_1 but not UBF_2, and an increase in the expression of fibrillarin; these results could be attributed to differences in the binding ability of the *cis* form of this compound RAP compared with its *trans* form RAD. These results show that the *cis* isomer RAP is highly cytotoxic to human cells *in vitro* compared with its *trans*isomer RAD. Finally, in comparing RAD to the platinum compounds (*cis* and *Trans* isomers), transplatin was found to be inactive clinically and do not cause any cytotoxicity to cells *in vitro*[45] compared to cisplatin, however the new ruthenium (III) compounds, RAP and RAD, were found to be cytotoxic *in vitro*. Further studies need to be done *in vitro* on other cell lines as well as *in vivo*.

CONCLUSION

Our data suggest that RAD was cytotoxic to TG cells by causing a signification decrease in the proliferation rate of these cells; this was explained by a possible interaction of RAD with DNA of TG cells leading to the formation of RAD-DNA adducts confirmed by the comet assay.These adducts were found to cause a modification in the expression levels of the two proteins studied in this work, UBF and fibrillarin. The study of the UBF was based on its role in activating transcription and the up-regulation of this protein, as a result of RAD-DNA interaction, could be an indicative of an eventual repair process needing more UBF to initiate the transcription machinery of the cell, although UBF_2 was not increased as a result of RAD treatment compared to its *cis* isomer RAP. Fibrillarin was also found to be expressed in highquantities in TG cells treated with RAD which was explained by a possible alteration of the protein behaviour due to RAD treatment.

REFERENCES

1. Keys HM, Bundy BN, Stehman FB, Muderspach L, Chafe WE, et al. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. N. Engl. J. Med. 1999: 340: 11541161.

2. Horwich A, Shipley J, Huddart R. Testicular germ-cell cancer. Lancet.2006:367: 754765.

3. Kelland L. The resurgence of platinum-based cancer chemotherapy. Nat. Rev. Cancer. 2007:7:573584.

4. Cepeda, V, Fuertes MA. Castilla J, Alonso C, Quevedo C, et al. Biochemical Mechanisms of Cisplatin Cytotoxicity. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry - Anti-Cancer Agents). 2007:7(1):3-18.

5. Reedijk J. New clues for platinum antitumor chemistry: kinetically controlled metal binding to DNA. Proc. Natl. Acad. Sci. USA. 2003:100:3611.

6. Alessio E. Synthesis and reactivity of Ru-, Os-, Rh-, and Ir-halide-sulfoxide complexes. Chem. Rev. 2004:104:4203.

7. Gonzalez-Vilchez F, Vilaplan R (2005) Metal compounds in cancer chemotherapy. In: Perez-Martin JM, editor. Research Signpost, Kerala, India.

8. Du J, Kang Y, Zhao Y, Zheng W, Zhang Y, et al. Synthesis, Characterization, and in Vitro Antitumor Activity of Ruthenium(II) Polypyridyl complexes Tethering EGFR-Inhibiting 4-Anilinoquinazolines. Inorg. Chem. 2016:55(9): 4595-605.

9. Vilaplana R, Romero MA, QuiroS M, Salas JM, Gonzalez-Vilchez F. Synthesis, structure and antitumour properties of a new 1,2-propylene diaminetetraacetate - ruthenium (III) compound. Metal Based Drugs. 1995:2:211-219.

10. Vilaplana RA, Delmani F, Manteca C, Torreblanca J, Moreno J, et al. Synthesis, interaction with double-helical DNA and biological activity of the water soluble complex cis-dichloro-1,2-propylene diamine - N,N,N',N'-tetraacetato ruthenium (III) (RAP). J.Inorg.Biochem.2006:100:1834.

11. Delmani F, Torreblanca J, Moreno J, Garcia-Herdugo G, Vilaplana R, et al. Biological activity and redistribution of nucleolar proteins of two different cell lines treated with cis-dichloro-1,2-propylenediamine-N,N,N',N'-tetraacetato ruthenium (III) (RAP). Ind. J. Exp. Bio. 2014:52:579-588.

12. Gallori E, Vettori C, Alessio E, Gonzalez-Viltchez F, Vilaplana R, et al. DNA as a possible target for antitumor ruthenium (III) complexes: A spectroscopic and molecular biology study of the interactions of two representative antineoplastic ruthenium (III) complexes with DNA. Arch. Biochem. Biophys. 2000:1:156162.

13. Kljun J, Petricek S, Zigon D, Hudej R, Miklavcic D, et al. Synthesis and Characterization of Novel Ruthenium (III) Complexes with Histamine. Bioinorg. Chem. Applic. 2010:2010:183097-183103.

14. Messori L, Orioli P, Vullo D, Alessio E, Iengo E. A spectroscopic study of the reaction of NAMI, a novel ruthenium (III) anti-neoplastic complex, with bovine serum albumin. Eur. J. Biochem. 2000: 267:1206-13.

15. Jantzen HM, Admon A, Bell SP, Tjian R. Nucleolar transcription factor hUBF contains a DNA binding motif with homology to HMG proteins. Nature (Lond.). 1990:344:830836.

16. Bell SP, Learned LM, Jantzen HM, Tjian R. Functional cooperativity between transcription factors UBF1 and SL1 mediates human ribosomal synthesis. Science. 1988: 241: 11921197.

17. McKeown PC, Shaw PJ. Chromatin: linking structure and function in the nucleolus. Chromosoma. 2009:118:1123.

18. Hamdane N, Stefanovsky VY, Tremblay MG, Nemeth A, Paquet E, et al. Conditional inactivation of Upstream Binding Factor reveals its epigenetic functions and the existence of a somatic nucleolar precursor body. PLoS Genet.2014:10: e1004505.

19. Larson DE, Zahradka P, Sells BH. Control points in eukaryotic ribosome biogenesis. Biochem. Cell Biol. 1991:69:522.

20. Moss T, Stefanovsky VY. Promotion and regulation of ribosomal transcription in eukaryotes by RNA polymerase I. Prog Nucleic Acid Res. Mol. Biol.1995:50:2566.

21. Chan EK, Imai H, Hamel JC, Tan EM. Human autoantibody to RNA polymerase I transcription factor hUBF. Molecular identity of nucleolus organizer region autoantigen NOR-90 and ribosomal transcription upstream binding factor. J. Exp. Med.1991:174:12391244.

22. White B. Immunologic aspects of scleroderma. Curr.Opin.Rheumatol.1995:7:541545.

23. Kuhn A, Voit R, Stefanovsky V, Evers R, Bianchi M, et al. Functional differences between the two splices variants of the nucleolar transcription factor UBF: The second HMG box determines specificity of DNA binding and transcriptional activity. EMBO J. 1994:13:416 424.

24. Ochs RL, Lischwe MA, Spohn WH, Busch H.Fibrillarin: A new protein of the nucleolus identified by autoimmune sera. Biol. Cell. 1985:54:123133.

25. Raska I, Reimer G, Jarnı ´k M, Kostrouch Z, RaskaJrK. Does the synthesis of ribosomal RNA take place within nucleolar fibrillarcenters or dense fibrillar components? Biol. Cell.1989:65:7982.

26. Loza-Muller L, Rodríguez-Corona U, Sobol M, Rodríguez-Zapata LC, Hozak P, et al. Fibrillarin from Archaea to human. Biol. Cell. 2015:107:159-74.

27. Smith CM, Steitz JA.Sno storm in the nucleolus: New roles for myriad small RNPs. Cell. 1997:89:669672.

28. Sobol M, Yildirim S, Philimonenko VV, Marasek P, Castano E, et al. UBF complexes with phosphatidylinositol 4,5-bisphosphate in nucleolar organizer regions regardless of ongoing RNA polymerase I activity. Nucleus. 2013:4:478486.

29. Yildirim S, Castano E, Sobol M, Philimonenko VV, Dzijak R, et al. Involvement of phosphatidylinositol 4,5-bisphosphate in RNA polymerase I transcription. J. Cell Sci.2013:126:27302739.

30. Singh NP, MacCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell. 1988:175:184-191.

31. Tice RR, Strauss GH. The single cell gel electrophoresis/comet assay: a potential tool for detecting radiation-induced DNA damage in humans. Stem Cells. 1995:13:207-214.

32. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature.1970:227:3932.

33. Delmani F, Torreblanca J, Moreno J, Garcia-Herdugo G, Vilaplana R, et al. In vitro expression and redistribution of nucleolar proteins following treatment with cis-dichloro-1-2,-propylenediammine-N,N,N',N'-tetraacetato ruthenium (III) (RAP). Oncology Res.2009:17: 425.

34. Bell SP, Pikaard CS, Reeder RH, Tjian R. Molecular mechanisms governing species-specific transcription of ribosomal RNA. Cell. 1989:59:48997.

35. Lamond AI, Sleeman JE. Nuclear substructure and dynamics. Curr. Biol.2003:13:825-8.

36. Panov KI, Friedrich JK, Russell J, Zomerdijk J. UBF activates RNA polymerase I transcription by stimulating promoter scape. EMBO J.2006:25:331022.

37. Brock GJR, Bird A. Mosaic methylation of the repeat unit of the human RNA genes. Hum. Mol. Genet. 1997:6:4516.

38. Roussel P, Andre C, Masson C, Géraud G, Hernández-

Verdun D. Localization of the RNA polymerase I transcription factor UBF during the cell cycle. J. Cell Sci.1993:104:32737.

39. Mais C, Wright JE, Prieto JL, Ragett SL, McStay B. UBF-binding site arrays form pseudo-NORs and sequester the RNA polymerase I transcription machinery. Genes Dev.2005:19:5064.

40. Meraner J, Lechner M, Loidl A, Goralik-Schramel M, Voit R, et al. Acetylation of UBF changes during the cell cycle and regulates the interaction of UBF with RNA polymerase 1. Nucl. Acids Res.2006:34:1798806.

41. Jacob ST. Regulation of ribosomal gene transcription. Biochem. J.1995:306:617626.

42. Hamdane N, Herdman C, Mars JC, Stefanovsky V, Tremblay MG, et al. Depletion of the cisplatin targeted HMGB-box factor UBF selectively induces p53-independent apoptotic death in transformed cells. Oncotarget. 2015:6(29):27519-36.

43. Legartova S, Sbardella G, Kozubek S, Bartova E.Ellagic Acid-Changed Epigenome of Ribosomal Genes and Condensed RPA194-Positive Regions of Nucleoli in Tumour Cells. Folia Biologica. 2016:61:49-59.

44. Figueiredo VC, Roberts LA, Markworth JF, Barnett MPG, Coombes JS, et al. Impact of resistance exercise on ribosome biogenesis is acutely regulated by post-exercise recovery strategies. Physiological Repots. 2016:4:12670.

45. Vrana O, Novohradsky V, Medrikova Z, Burdikova J, Stuchlikova O, et al. Internalization of Ineffective Platinum Complex in Nanocapsules Renders It Cytotoxic. Chemistry. 2016:22:2728-35.