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ARAŞTIRMA/RESEARCH

Comparison of cellular responses of parental and epirubicin-resistant non-small cell lung cancer cells against stabilized-ag ion solution induced injury

Stabilize-ag iyon çözeltisinin uyardığı hasara karşı parental ve epirubicin dirençli küçük hücreli dışı akciğer kanseri hücrelerinin hücresel yanıtların karşılaştırılması

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Abstract

Purpose: The aim of this study was to compare the responses of parental and epirubicin-resistant non-small cell lung cancer (NSCLC) cells against stabilized-silver (St-Ag) ion induced oxidative DNA and membrane injury after the cells treated with this solution.

Material and Methods: IC50 values for parental and drug-resistant NSCLC cells as revealed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and resazurin-based assay. Malondialdehyde (MDA) levels in the cells were assayed by using fluorometric method. The genomic DNA samples were used to determine the amount of 8-hydroxy-2'-deoxyguanosine (8-OHdG) with a competitive ELISA kit.

Results: 139 µg/mL for parental cells and 224 µg/mL for resistant cells were calculated as IC50 values by MTT assay. 96 µg/mL and 189 µg/mL respectively were calculated as IC50 values by resazurin-based assay. When the cells were treated with IC50 concentration of St-Ag ion solution, clear signs of oxidative stress, i.e. decreased is glutathione (GSH) (1.5-folds in parental, 2-folds in resistant) and glutathione peroxidase (GPx) (2-folds in parental), as well as increased lipid peroxidation (1.6-folds in parental, 2-folds in resistant) and 8-OHdG formation (1.5-folds in parental, 2-folds in resistant) were seen. Changes in the levels of glutathione-S-transferase (GST) in both cell type and levels GPx in resistant cells were statistically insignificant. The St-Ag ion solution has higher membrane and DNA damaging effect on resistant cells than parental cells even though resistant-cells have high GST and GPx activity.

Conclusion: Thus our data suggest St-Ag ion solution has different potential toxicity on parental and epirubicin-resistant cancer cells depend on concentration that is

Öz

Amaç: Bu çalışmanın amacı, stabilize iyon solüsyonu ile muamele edildikten sonra bu çözeltinin uyardığı oksidatif DNA ve membran hasarına karşı parental ve epirubisin dirençli küçük hücreli dışı akciğer kanseri (NSCLC) hücrelerinin cevaplarını karşılaştırmaktır.

Gereç ve Yöntem: Parental ve ilaca dirençli NSCLC hücreleri için IC50 değerleri 3-(4,5-dimetiltiazol-2-yl) -2,5-difeniltetrazolyum bromür (MTT) ve resazurin-temelli deneyler ile ortaya kondu. Hücrelerdeki malondialdehit (MDA) seviyeleri florometrik yöntemi kullanılarak analiz edilmiştir. Genomik DNA örnekleri rekabetçi ELISA kiti ile 8-hidroksi-2'-deoksiguanozin (8-OHdG) miktarını belirlemek için kullanılmıştır.

Bulgular: MTT deneyi ile IC50 değeri parental hücreleri için 139 µg/mL ve dirençli hücreler için 224 µg/mL olarak hesaplanmıştır. Resazurin-temelli deney ile IC50 değeri sırasıyla 96 µg/ mL ve 189 µg/mL olarak hesaplanmıştır. Hücreler St-Ag iyon çözeltisinin IC50 konsantrasyonu ile muamele edildiğinde oksidatif stresin net işaretlerinden, glutatyon (GSH) (1.5 kat parentalde, 2 kat dirençlide) ve glutatyon peroksidaz (GSH-Px) (2 kat parentalde) azaldığı bunların yanı sıra lipid peroksidasyonun (1.6 kat parentalde, 2 kat dirençlide) ve 8-OHdG oluşumunun (1.5 kat parentalde, 2 kat dirençlide) arttığı görülmüştür. Her iki hücre tipindeki glutatyon S-transferaz (GST) seviyelerindeki ve dirençli hücrelerdeki GSH-Px seviyesindeki değişimler istatistiksel olarak önemli değildir. Dirençli hücrelerin yüksek GST ve GSH-Px aktivitesine sahip olmasına rağmen St-Ag iyon çözeltisi dirençli hücrelerde parental hücrelerden daha yüksek membran ve DNA hasar etkisi vardır.

Sonuç: Böylece verilerimiz St-Ag iyon solüsyonunun oksidatif stresle ilişkili konsantrasyona parental ve

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associated with oxidative stress. Anticancer potential of anticancer drugs for especially drug resistant cells can be increased by combining therapy with stabilized-silver ion solution.

Key words: Stabilized-silver ion solution, antioxidant enzymes, malondialdehyde, lung cancer

epirubisin dirençli kanser hücreleri üzerinde farklı potansiyel toksisiteye sahip olduğunu göstermektedir. Özellikle ilaca dirençli hücreler için anti-kanser ilaçların antikanser potansiyeli stabilize gümüş iyonu çözeltisi ile tedavinin birleştirilmesiyle arttırılabilir.

Anahtar kelimeler: Stabilize-gümüş iyon çözeltisi, antioksidan enzimler, malondialdehit, akciğer kanseri

INTRODUCTION

The discovery of new compounds with antitumor activity has become one of the most important goals in medicinal chemistry. Biological macromolecules present in living organisms, like proteins and DNA, have many metal-binding sites. As a consequence, new metal containing- compounds can react with such cellular components, displaying possible toxic effects.

An interesting group of chemotherapeutic agents used in cancer therapy comprises molecules that interact with DNA after passed cell membrane. The research in this area has revealed a range of DNA recognizing molecules that act as antitumor agents, including oxidizing agents and intercalator compounds[1]. Intercalators are molecules that insert perpendicularly into DNA base pairs, exploiting noncovalent bonds[2]. Recently, it has been reported that silver complexes act as antimicrobial agents, interacting with DNA, and that Ag+ is able to form a metal-mediated base pair complex[3]. Ag(I) mixed ligand complexes showed excellent anticancer activity against Ehrlich's ascites tumor cells (EACs)[4]. Silver and hydrogen peroxide acted synergistically on the viability of E. coli. It appears that the combined toxic effect of silver and hydrogen peroxide may be related with damage to cellular proteins[5]. So, our stabilized-silver (St-Ag) solution can cause DNA and membrane damages by oxidation and display cytotoxic effect on cancer cells. The antioxidant enzymes such as glutathione reductase (GRx) and glutathione peroxidase (GPx) can protect the cells from the effects of oxidative stress. When the H1299 cancer cells preincubated with süperoxide dismutase (SOD) and catalase before oxidant exposure, the GPx activity and glutathione (GSH) amount were found different than control[6]. The purpose of this study was to create new DNA and membrane damaging compounds easily diffusable and targeting remotely implanted tumors. Tumors are heterogeneous in many respects, including chemotherapeutic susceptibility[7].

Resistance to chemotherapeutic agents is a major problem in the treatment of patients with non-small cell lung cancer. A group of drug resistance cells can occur in tumors during chemotherapy. So in this study we investigated the differences between parental and drug-resistant cells responses against St-Ag ion solution induced injury after the cells treated with this solution.

MATERIAL AND METHODS

Preparation of stabilized-silver ion solution

The stabilization of silver ion (St-Ag ion solution) had been optimized previously[8]. Silver nitrate (AgNO3) was dissolved in an isopropyl alcohol /ethyl alcohol/acetone solvent system followed by the dropwise addition of DIAMO (N-(2-aminoethyl)-3- aminopropyltrimethoxysilane) to prepare the St-Ag ion solution. The molar ratios of AgNO3: isopropyl alcohol: ethyl alcohol: acetone: DIAMO were 1:15:6:1.5:5 in the mixture, respectively. This mixture was stirred for 2 h at room temperature.

Cancer cell culture

The H1299 cell line was purchased from American Type Culture Collection (Rockville, MD). Cells were routinely cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640) supplemented with 10% fetal bovine serum, 1% antibiotic-antimycotic solution (penicillin, streptomycin and amphotericin) in a humidified atmosphere containing 5% CO2 at 37°C. For subculturing, cells were harvested after trypsin/ethylenediaminetetraacetic acid (EDTA) treatment at 37°C. Cells were used when monolayer confluence had reached 75%. Epirubicin-resistant H1299 tumor cells were derived from the parental line by stepwise selection in increasing concentrations of epirubicin until the cells were capable of propagating in 220 ng/mL drug, as described previously[9].

Cell viability assays

The cancer cells (10 000 cells/well, monolayer) were plated in a 96-well plate. The next day the cells were treated with different concentrations of St-solution and St-Ag ion solution in the medium for 24 hours. At the end of the incubation period, the cytotoxicity of this solution on cancer cells was determined by the the CellTiter-Blue-Cell Viability Assay and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. The CellTiter-Blue-Cell Viability Assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Nonviable cells rapidly lose metabolic capacity and thus do not generate a fluorescent signal[10]. Following cellular reduction, fluorescence is recorded at 560 nm (excitation) and 590 nm (emission) spectrofluorometrically (PerkinElmer LS 55). The MTT assay, tetrazolium salts such as MTT are metabolized by mitochondrial dehydrogenases to form a blue formazan dye and are, therefore, useful for the measurement of cytotoxicity. Test reagents were added to the culture medium. Briefly, 15% volume of dye solution was added to each well after the appropriate incubation time. After 2 h of incubation at 37°C, an equal volume of solubilization/stop solution (dimethylsulfoxide) was added to each well for additional 1 h incubation. The absorbance of the reaction solution at 490 nm was recorded[11].

The data were expressed as average values obtained from eight wells for each concentration. The concentrations of a compound needed to reduce growth by 50% and 70%, respectively (IC50 and IC70) were calculated using the Linear functions (The equation of a straight line). The reading taken from the wells with cells cultured with the control medium was used as a 100% viability value.

The cells were plated at a density of (5-10) x 10^5 cell/100 mm dishes and incubated with different concentrations (IC_{50} and IC_{70}) of St-Ag ion solution during 24 hours. Cells were scraped off the culture plates with culture medium and were centrifuged 400 × g for 10 minutes. The cell pellets were washed with phosphate-buffered saline (PBS) and then sonicated (3 × 15 sec) in 50 mM potassium phosphate, pH 7.2, containing 1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma) and 1 μg/mL of leupeptin (Sigma) and centrifuged at 150.000 × g for 45 minutes. The supernatant was used for the determination of biochemical parameters.

Determination of malondialdehyde level

Malondialdehyde (MDA) levels in the cells were assayed as described by Wasowicz and his colleagues[12]. This fluorometric method for measuring thiobarbituric acid-reactive substances (TBARS) in supernatant is based on the reaction between malondialdehyde and thiobarbturic acid. The product of this reaction was extracted into butanol and measured at fluorescence spectrometer (525 nm excitation, 547 nm emission). The concentration of proteins was determined by the Bradford method13 with bovine serum albumin as a standard.

Determination of 8-hydroxy-2' -deoxyguanosine level

The cells were plated at a density of (5–10) x 10^5 cell/100 mm dishes and incubated with different concentrations (IC50 and IC70) of St-Ag ion solution during 24 hours. After DNA purification[30] from the cultured cells (Genomic DNA Mini Kit, Invitrogen), the genomic DNA samples were used to determine the amount of 8-hydroxy-2'-deoxyguanosine (8-OHdG) with a competitive ELISA kit (Highly sensitive 8-OHdG Check New, Japan Institute for Control of Aging, Fukuroi, Shizuoka, Japan). Microtiter ELISA plates were precoated with 8-OHdG. Fifty microliters of the sample and primary antibody were added to each well and they were incubated at 4°C overnight. The wells were washed three times. Then 100 microliters of secondary antibody were added to each well and incubated for 1 hour at room temperature. The wells were again washed three times. After that, enzyme substrate solution was added and the wells were incubated at room temperature for 15 minutes. The reaction was stopped by adding the terminating solution. The absorbance was read at a wavelength of 450 nm[14].

Determination of enzymes activity and total glutathione (GSH) level

Glutathione S-transferase (GST) was determined using 1-chloro-2,4-dinitrophenol as a substrate15. One unit of enzyme activity results in the binding of one mmole GSH/min. Glutathione peroxidase (GPx) was determined with t-butyl hydroperoxide as a substrate[16]. The assay based on determination of is nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. One unit enzyme activity

results in the oxidation of 1 mmol GSH/min. Total glutathione (GSH) content was determined spectrophotometrically by following the reduction of 5.5'-dithiobis (2-nitrobenzoic) acid by NADPH in the presence of glutathione reductase (GSSG-Rx)17. The concentration of proteins was determined by the Bradford method[13] with bovine serum albumin as a standard.

Data analysis

The results of the replicates were pooled and expressed as mean ± standard error. Analysis of variance (ANOVA) was carried out. The ANOVA was used to determine whether there are any significant differences between the means of three or more independent (unrelated) groups on some variable. Significance was accepted at $p \leq 0.05$[18].

RESULTS

The purpose of this study was to create new DNA and membrane damaging compounds easily diffusable and targeting remotely implanted tumor cells, especially Epirubicin-resistant tumor cells and compare the responses of parental and resistant cells against this new compounds St-Ag solution.

The cytotoxic effect of stabilized silver ion solution (St-Ag ion solution) on parental and Epirubicin-resistant H1299 cells was evaluated after 24 hour exposure time. For two assays, MTT Assay (Figure 1) and The CellTiter-Blue-Cell Viability Assay (Figure 2), a dose dependent response was observed and an IC_{50} values were calculated. The CellTiter-Blue-Cell Viability Assay was found to be more sensitive than MTT assay in both cells. In one study MTT assay was found to be the most sensitive assay among the four assays alamar blue (AB), neutral red (NR), coomassie brilliant (CB) and MTT[19]. The concentrations of St-Ag ion solution needed to reduce growth by 50% (IC_{50}) were calculated by using results obtained from CellTiter-Blue-Cell Viability Assay and MTT assay. The IC_{50} values of parental and Epirubicin-resistant cells from CellTiter-Blue-Cell Viability Assay were slightly lower as compared to MTT assay values after 24 h of exposure. The IC_{50} value from MTT assay was found as 224 µg/mL (5.74 microliter) for resistant cells and 139 µg/mL (3.53 microliter) for parental cells (Figure 1). But for the CellTiter-Blue-Cell Viability Assay, the IC_{50} value was found as 189 µg/mL (4.84 microliter) and 96 µg/mL (2.44 microliter) for Epirubicin-resistant and parental cell respectively (Figure 2). The viability of the cells decreased when the cells were exposed to the St-Ag ion solution at increasing concentrations between 2 microliter and 8 microliter and did not change at concentrations of 10 microliter or higher (Figure 2). The St-solution which is used for preparation of St-Ag ion solution didn't show any cytotoxic effect on the cells (Figure 1 and 2).

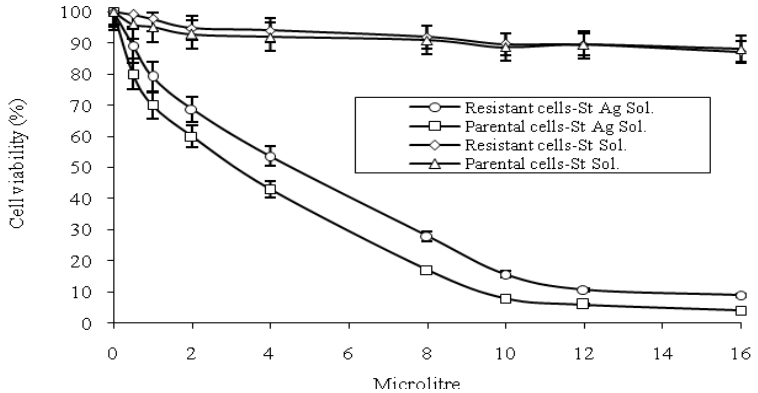

Figure 1. Cytotoxic effect of stabilized-Ag solution on parental and Epirubicin-resistant H1299 cells by MTT assay.

Results are presented as viability ratio compared with the control group (treated with with only the medium-untreated cells). Values were expressed as the mean of three separate experiments (n=3).

The parental cells was found to be more sensitive to St-Ag ion solution cytotoxic effects than Epirubicin-resistant cells after we compared the results of both assays. The Epirubicin-resistant H1299 cells was

found to be less sensitive to the toxic effect of St-Ag ion solution with IC_{50} values of 189 µg/mL (4.84 microliter) and IC_{70} values of 263 µg/mL (6.67 microliter) than parental cell in the CellTiter-Blue-Cell Viability Assay. On the other hand, parental H1299 cells showed almost 2 times higher sensitivity to St-Ag ion solution toxicity with IC50 values of 96 µg/mL (2.44 microliter) and IC_{70} values of 140 µg/mL (3.45 microliter) than Epirubicin-resistant cells (Figure 2).

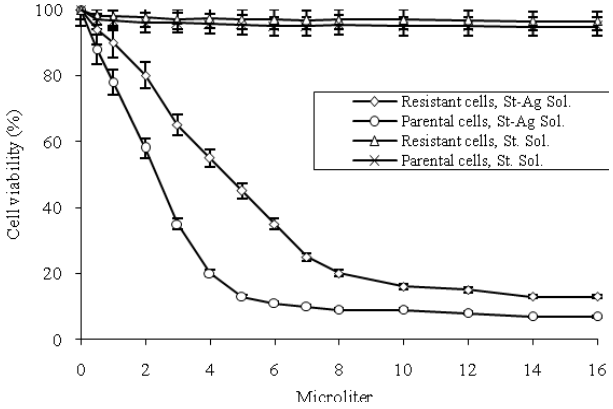

Figure 2. Cytotoxic effect of stabilized-Ag solution on parental and Epirubicin-resistant H1299 cells by CellTiter-Blue viability assay.
Results are presented as viability ratio compared with the control group (treated with with only the medium-untreated cells).

When the cells were challenged with IC_{50} concentration of St-Ag ion solution (189 µg/mL for resistant cells and 96 µg/mL and parental cell), clear signs of oxidative stress, i.e. decreased GSH (1.5-folds in parental, 2-folds in resistant) and GPx (2-folds in parental), as well as increased lipid peroxidation (1.6-folds in parental, 2-folds in resistant) and 8-OHdG formation (1.5-folds in parental, 2-folds in resistant) were seen. Changes in the levels of GST in both cell type and levels GPx in resistant cells were statistically insignificant (Table 1). The St-Ag ion solution has higher membrane and DNA damaging effect on resistant cells than parental cells even though resistant-cells have high GST and GPx activity levels than parental cells.

Table 1. Effect of Stablized-Ag solution on MDA, 8-OHdG and GSH level and antioxidant enzymes activities of parental and Epirubicin-resistant H1299 cells.

Concentrations	MDA level (X±SE)	8-OHdG level (X±SE)	GSH level (X±SE)	GSH-Px (X±SE)	GST (X±SE)
Control (P)	0.90 ± 0.07 a	0.80 ± 0.01 a	53 + 3.1 f	8 + 2.2 ab	43 + 2.0 e
IC50 St-Ag (P)	1.40 ± 0.26 ab	1.20 ± 0.06 ab	35 + 3.0 d	4 + 1.4 a	42 + 1.4 e
IC70 St-Ag (P)	2.80 ± 0.31 c	1.60 ± 0.06 b	20 + 2.3 c	2 + 1.2 a	41 + 1.9 e
Stab. sol.(P)	0.95 ± 0.06 a	0.81 ± 0.02 a	52 + 2.8 f	7 + 2.1 ab	44 + 2.1 e
Control (R)	0.80 ± 0.06 a	0.85 ± 0.03 a	26 + 3.1 cd	14 + 1.8 b	134 + 1.1 n
IC50 St-Ag (R)	1.70 ± 0.41 b	1.70 ± 0.06 b	13 + 2.5 b	14 + 1.1 b	133 + 1.4 n
IC70 St-Ag (R)	4.03 ± 0.55 d	4.00 ± 0.06 d	7 + 1.9 ab	13 + 2.0 b	131 + 1.4 n
Stab. sol .(R)	0.85 ± 0.08 a	0.90 ± 0.02 a	26 + 3.1 cd	15 + 1.8 b	133 + 2.1 n

Results are means of eight different experiments. MDA (nmol/mg protein), 8-OHdG (ng/ml). Enzyme activities (nmol of product formed/mg of protein/min). SE: Standard Error. X: Arithmetic mean. P; parental cell. R; resistant cells.
Means in the table followed by different letters within each column present significant differences at the $p \leq 0.05$ level.

Various markers of oxidative damage have been identified[31]. In the past, the most popular markers were designed for lipid peroxidation, such as malondialdehye (MDA), oxidized low density lipoprotein (LDL), MDA-modified LDL, auto-antibodies against oxidized LDL and MDA-

modified LDL, F2-isoprostane, and conjugated diene. The detection of a new carbonyl group, dityrosine and oxidized histidine has been measured to indicate protein oxidation. Markers for DNA oxidation were few. Only in recent years has 8-hydroxy-2'-deoxyguanosine (8-OHdG, or 8-oxodG) emerged as a marker of oxidative stress[32]. Among all purine and pyridine bases, guanine is most prone to oxidation. Upon oxidation a hydroxyl group is added to the 8th position of the guanine molecule and the oxidatively modified product 8-OHdG is one of the predominant forms of free radical-induced lesions of DNA. Oxidative modified DNA in the form of 8-OHdG can be quantified to indicate the extent of DNA damage. The induction of cytotoxic cell death can be accompanied by membrane and DNA damage. The St-Ag ion solution induced membrane and DNA damage at IC_{50} and IC_{70} concentrations (Table 1) than those that mediate its anticancer activities.

The St-Ag ion solution caused increasing malondialdehyde level (MDA), an end product of lipid peroxidation of membrane and 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation, a product of oxidative DNA damages flowing specific enzymic cleavage after 8-hydroxylation of guanine base. Generally, the MDA and 8-OHdG amount in IC_{50} and IC_{70} St-Ag ion solution concentrations exposed cells was found to be statistically different from the control cells ($p \leq 0.05$). The membrane and DNA damaging increased when the cells were exposed to the St-Ag ion solution at increasing concentrations (Table 1).

The highest membrane and DNA damages were caused by St-Ag ion solution at IC_{70} concentration in Epirubicin-resistant cells. The cell membrane damage increased 38.8% in parental cells and 91% in resistant cells at IC70 concentration compare to IC_{50} concentration. Also, DNA damage increased 88% in parental cells and 91% in resistant cells at IC^{70} concentration compare to IC^{50} concentration. The Epirubicin-resistant cells was found to be more sensitive to St-Ag ion solution injury at all concentration compare to parental cells and control cells.

DISCUSSION

In this study we investigated the differences between parental and drug-resistant cells responses against St-Ag ion solution induced injury after the cells treated with this solution in this study. Such studies on toxic effects of silver are on cancer cells very few. A group of drug resistance cells can occur in tumors during chemotherapy. The responses of cells to chemotherapy are different. These differences play a role in therapy failures in many tumors[7]. The difference in the sensitivity of different cell lines can be understood in terms of their natural antioxidant levels, which is a key behind their natural defense mechanism during oxidative stress. Therefore, this probably governs the sensitivity of the cell lines upon same St-Ag ion solution exposure. In one study showed that Epirubicin-resistant H1299 cells had more GPx and GST activity, but less GSH amounts than parental H1299 cells[9].

In the present study, when St-Ag ion solution induced oxidative stress as a mechanism(s) of toxicity was assessed depletion of GSH (1.5-folds in parental, 2-folds in resistant) and GPx (2-folds in parental) was seen which probably results in the shift of overall redox balance towards oxidation, leading to functional damage of cells and enhanced lipid peroxidation (1.6-folds in parental, 2-folds in resistant) and DNA oxidation (1.5-folds in parental, 2-folds in resistant). These results also suggest oxidative damage to cells after exposure to St-Ag ion solution. Results obtained by us clearly indicated that St-Ag ion solution could not inhibit GPx activity in parental cells by any direct mechanism. Therefore, the observed GPx inactivation might be due to generation of peroxyradicals after St-Ag ion solution exposure. Similar observations were reported inactivation of superoxide dismutase (SOD) due to carbon tetrachloride induced oxidative stress in rat liver and kidney cells21.

Statistically insignificant changes observed in the levels of GST in both parental and epirubicin resistant cells after treatment with St-Ag ion solution in this work is suggestive of a differential and less pronounced response by these cellular defense mechanisms as compared to GSH, GPx, lipid peroxidation and DNA oxidation. On the whole, data obtained by us clearly suggest that oxidative stress is the cause of ensuing cytotoxicity in case of St-Ag ion solution-exposed parental and resistant cells, at IC50 concentrations. Many studies showed that, drug resistant cells can resists to oxidants or anticancer agent, oxidized molecule in the cells, with their higher antioxidant enzymes[20]. The cellular redox status was determined from the reduced

glutathione to oxidized glutathione ratio (GSH/GSSG) and the enzymes involved in glutathione metabolism, including glutathione peroxidase (GPx), glutathione S-transferase (GST) etc. Epirubicin resistant cells was found to be more sensitive to St-Ag ion solution induced membrane and DNA damage due its weak antioxidant defense mechanism. It can be caused by less GSH amount of drug resistant cells indirect mechanisms.

Consistent with previous reports silver containing solutions provoke oxidative stress22, 23. Recent studies have shown that silver nanoparticles (AgNPs) accumulation in the liver could induce cytotoxicity via oxidative cell damage[24,25]. One study shows that the Ag nanoparticle was noted to induce elevated levels of oxidative stress, glutathione depletion and damage to the cell membrane in human dermal and cervical cancer cell lines[19]. Studies on rat liver derived cell line (BRL 3A) showed that there was a significant increase in ROS and decrease in GSH levels at 25 and 50 g/mL of Ag (15, 100 nm)[26]. A significant elevation of lipid peroxidation and marginal GSH depletion was demonstrated in a fish model upon exposure to fullerenes[27]. Upon exposure to silver nanoparticles, GSH amount and SOD activity decreased while MDA amount increase in HT1080 and A431 cells. Changes in the levels of catalase and GPx in A431 cells were statistically insignificant in both cell types[28]. AgNO3 induced cytotoxicity by reactive oxygen species (ROS) generation and suppression of reduced glutathione (GSH) in human Chang liver cells[29]. Present study was found consistent with previous studies.

Our results showed that St-Ag ion solution acted as an oxidizing agent on membrane and DNA causing elevation of MDA level as a result of membrane lipid peroxidation and 8-OHdG level as a result of oxidation of DNA and of depletion GSH and GPx in the cells. The responses of parental and resistant H1299 cells are different against St-Ag ion solution induced injury. Epirubicin-resistant cells having more GSH depletion were found more sensitive to St-Ag solution induced membrane and DNA damages. This result is more important, because acquired multidrug resistance is the main obstacle for the cure of lung cancer. In our previous study, the activities of enzymes involved in detoxification and antioxidant mechanisms were found higher in epirubicin-resistant H1299 cells than in parental cells[9].

The St-Ag ion solution has more membrane and DNA damaging effect on drug-resistance cells, which make potentially the St-Ag ion solution interesting for cancer treatment as a anticancer drug or adjuvant agent.

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