Resveratrol may reduce oxidative stress induced by platinum compounds in human plasma, blood platelets and lymphocytes

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Resveratrol (*trans*-3,4′,5-trihydroxystilbene), a polyphenolic compound found in grapes and wine, has been shown to have anti-inflammatory, anti-oxidant, anti-tumor and anti-platelet activities. Using different methods, we show that resveratrol reduces oxidative stress induced by cisplatin (*cis*-diamminedichloroplatinum II) and selenium–cisplatin conjugate ($[NH_3]_2Pt(SeO_3)$, Se-Pt) in human blood platelets, lymphocytes and plasma. Resveratrol decreased the production of 8-epi-prostaglandin F_2 (a biomarker of lipid peroxidation) in control blood platelets and platelets treated with platinum compounds (10 μg/ml), and markedly reduced activities of different anti-oxidative enzymes (glutathione peroxidase, superoxide dismutase and catalase) in these cells. A combined action of resveratrol and Se-Pt evoked a significant decrease of DNA damage (measured by comet assay) in lymphocytes compared with cells treated with Se-Pt only. Resveratrol also caused a distinct reduction of total anti-oxidant level in plasma after incubation with platinum compounds. Therefore, anti-oxidative activity of resveratrol may diminish oxidative stress and damage to cellular biomolecules (lipids, proteins and DNA) induced by platinum compounds. *Anti-Cancer Drugs* 16:659–665 © 2005 Lippincott Williams & Wilkins.

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Introduction

Protection against reactive oxygen species (ROS) is provided by different compounds of the human diet and by enzymatic anti-oxidants, such as glutathione peroxidase (GSH-PX; EC 1.11.1.9), superoxide dismutase (SOD; EC 1.15.6) and catalase (EC 1.11.1.6). The human diet is rich in a great variety of micronutritients with anti-oxidant properties. Dietary anti-oxidants (vitamins, minerals and phenolic compounds) as well as endogenous anti-oxidants may protect against ROS-induced cell damage. An increased dietary intake of anti-oxidants is associated with a reduced risk of some diseases. The consumption of low levels of anti-oxidants in the form of fruit and vegetables has been shown to more than double the incidence of certain cancers [1–3]. Among these anti-oxidants, resveratrol (*trans*-3,4′,5-trihydroxystilbene) plays an important role. This compound exerts multifunctional biological effects, including not only anti-oxidant action [4–7], but also anti-platelet [8–10] and anti-cancer properties [1,2,11,12]. Resveratrol is present naturally in grapes, fruits and in a variety of medicinal plants [13]. Our previous studies show that compounds such as resveratrol and selenium compounds may have a protective action on lipid and protein oxidation of blood platelets treated with platinum compounds, and may minimize the toxicity and side-effects of the chemotherapeutic agent without affecting anti-tumor activity [14,15]. Therefore, the aim of our study was to investigate *in vitro* oxidative stress not only in blood platelets, but also in lymphocytes and plasma after the action of platinum compounds [cisplatin (*cis*-diamminedichloroplatinum II) used in chemotherapy, selenium–cisplatin conjugate ($[NH_3]_2Pt(SeO_3)$), Se-Pt] in the presence of resveratrol. The concentration of resveratrol used in our studies corresponds to the reference of physiological range of resveratrol in plasma and the concentration of platinum compounds used in our experiments is consistent with cisplatin plasma concentrations recorded in cancer patients. Cisplatin is especially useful in the treatment of epithelial malignancies [16]; however, its use is accompanied by several side-effects, including hematological toxicity [14,15, 17–23]. Contrary to cisplatin, Se-Pt conjugate has only a slight toxicity effect on blood platelet function [24,25] and lymphocytes [26].

Materials and methods

Chemicals

Resveratrol, cisplatin, GSH, nicotinamide adenine dinucleotide phosphate (NADPH), 1-butyl peroxide and yeast glutathione reductase (GR) were all purchased from Sigma (St Louis, MO). Stock solution of resveratrol

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was made in 50% dimethylsulfoxide (DMSO) at the concentration of 5 mg/ml and kept frozen. Se-Pt synthesized in the Institute of Pure Chemicals, Lachema, Brno (batch 290592) was a gift obtained from Professor V. Kleinwachter (Institute of Biophysics, Czech Academy of Sciences, Brno). Immunoassay for 8-epi-prostaglandin F_2 (8-EPI) was purchased from Oxis International (Portland, OR). Antioxidant test kit (Microplate luminometer kit ABEL-21M2) was purchased from Knight Scientific (Portland, OR). The RANSOD kit (Randox, Warsaw, Poland) was used for enzymatic analysis of SOD. All other reagents were of analytical grade.

Cell isolation

Peripheral blood was obtained from young (23–35 years) [collected into ACD solution (citric acid/citrate/dextrose; 5:1; v/v; blood/ACD) or heparin], non-smoking men and lymphocytes were isolated by centrifugation in a density gradient of Gradisol L (280 *g*, 15 min, 4°C). The viability of lymphocytes was measured after isolation by the Trypan blue exclusion assay and was found to be about 90%.

Platelet-rich plasma (PRP) was prepared by centrifugation of fresh human blood at 250 *g* for 10 min at room temperature. Platelets were then sedimented by centrifugation at 500 *g* for 10 min at room temperature, and the platelet pellet was washed twice with Tyrode's buffer containing 10 mM HEPES, 140 mM NaCl, 3 mM KCl, 0.5 mM $MgCl_2$, 5 mM $NaHCO_3$ and 10 mM glucose, pH 7.4. Concentration of platelets in platelet suspensions was estimated spectrophotometrically [27] as 2.5–3.8 $\times$ 10^8/ml.

Suspensions of platelets, lymphocytes or plasma were incubated (30 min, at 37°C) with: (i) resveratrol at the final concentrations of 5, 10, 25, 50 and 100 μg/ml, (ii) cisplatin or Se-Pt conjugate at the final concentration of 10 μg/ml, or (iii) resveratrol (25 μg/ml) plus cisplatin or Se-Pt (10 μg/ml) added together.

Cell viability

The activity of lactic dehydrogenase (marker of platelet lysis) in the extracellular medium after treatment of blood platelets with resveratrol was measured spectrophotometrically according to Wroblewski and La Due [28].

The viability of lymphocytes after treatment with resveratrol was determined by Trypan blue exclusion assay. After incubation for 30 min with resveratrol at concentrations of 5–100 μg/ml, the cells were washed and suspended in a RPMI 1640 medium. An equal volume of 0.4% Trypan blue reagent was added to the cell suspension and the percentage of viable cells was evaluated under a field microscope.

8-EPI quantification

The platelets in suspension were incubated for 30 min at 37°C without (control) or with tested compounds and then samples were activated for 2 min by thrombin (1 U/ml, 37°C). After incubation samples were centrifuged for 2 min in an Eppendorf microcentrifuge. Platelet pellets were resuspended in the previous volume of modified Tyrode's buffer, pH 7.4. For 8-EPI levels, blood platelets were lysed by freezing (−20°C) and thawing (3 times), and then centrifuged at 15 000 *g* for 10 min, and the level of 8-EPI was determined on the supernatants using an immunoassay kit (Oxis International) according to the manufacturer's instructions.

Total anti-oxidant status (TAS)

Estimation of TAS relies on the ability of anti-oxidants present in the plasma to inhibit the oxidation of 2,2′-azinobis(3-ethylbenzthiazoline-sulfonic acid (ABTS) to the radical cation $ABTS^+$ by a peroxidase. Human plasma was incubated for 30 min at 37°C without (control) or with tested compounds and the anti-oxidant test in these samples was performed using a microplate luminometer kit (Randox) according to the manufacturer's instructions.

Enzyme assays

After incubation of platelets with tested compounds, platelets were centrifuged (2 min in an Eppendorf microcentrifuge) and platelet pellets were resuspended in the previous volume of modified Tyrode's buffer, pH 7.4. For activity of GSH-PX, SOD and catalase assays, blood platelets were lysed by freezing (−20°C) and thawing (3 times), and then centrifuged at 15 000 *g* for 30 min, and enzymatic analysis was performed on the supernatants.

GSH-PX activity was measured using a modified version of the method of Paglia and Valentine [29], as reported by Hopkins and Tudhope [30]. The method was based on the reduction of peroxides catalyzed by GSH-PX using glutathione as reducing agent. The activity was measured by coupling the oxidation of NADPH with reduction of oxidized glutathione catalyzed by glutathione peroxidase. The reaction mixture contained 150 mM sodium phosphate buffer (pH 7.0), 15 mM GSH, 4.2 mM NADPH and 0.3 U/ml GR. Platelet supernatants (100 μl) were preincubated for 5 min at 25°C in the assay mixture and the reaction was initiated by the addition of 4 mM *t*-butyl hydroperoxide. Absorbance at 340 nm was recorded in a Unicam Helios spectrophotometer. One milliunit of enzyme activity was defined as 1 nmol NADPH oxidized/min and the results were expressed as mU/min/mg of platelet protein.

Catalase activity was measured spectrophotometrically according to Bartosz [31] using 30% H_2O_2 as substrate in

a 30-ml reaction mixture containing 50–200 μg of protein in 0.05 M phosphate buffer (pH 6.8) at 25°C. The results were expressed as mU/min/mg of platelet protein

Enzymatic analysis of SOD was performed on the supernatants using the RANSOD kit according to the manufacturer's instructions. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was measured by the degree of inhibition of this reaction.

Comet assay

The comet assay was performed at pH > 13, essentially according to the procedure of Singh *et al.* [32] with modifications [33–35] as described previously [36]. A freshly prepared suspension of human lymphocytes ($1–3 \times 10^5$ cells/ml) in 0.75% LMP agarose dissolved in PBS was spread onto microscope slides (Superior; Paul Marienfeld, Lauda-Konigshofen, Germany) precoated with 0.5% NMP agarose. The cells were then lysed for 1 h at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100 and 10 mM Tris, pH 10. After the lysis, the slides were placed in an electrophoresis unit, DNA was allowed to unwind for 20 min in the electrophoretic solution consisting of 300 mM NaOH and 1 mM EDTA, pH > 13. Electrophoresis was conducted at an ambient temperature of 4°C (the temperature of the running buffer did not exceed 12°C) for 20 min at an electric field strength of 0.73 V/cm (28 mA). The slides were then washed in water, drained, stained with 2 μg/ml DAPI and covered with cover slips.

The comets were observed at × 200 magnification using an Eclipse fluorescence microscope (Nikon, Tokyo, Japan) attached to a COHU 4910 video camera (Cohu, San Diego, CA) equipped with a UV-1 filter block (an excitation filter of 359 nm and a barrier filter of 461 nm) and connected to a personal computer-based image analysis system Lucia-Comet version 4.51 (Laboratory Imaging, Praha, Czech Republic). Images were randomly selected from each sample. Percentage of DNA in the tail was used as a metric of DNA damage.

Data analysis

Statistical analysis was performed by several tests. In order to eliminate uncertain data, both the Q-Dixon and Grubbs tests were performed. All the values in this study were expressed as mean ± SD. The statistically significant differences between variations were found (Snedecor–Fisher test) so the differences between means were assessed by applying the Cochran–Cox test. Regression lines were calculated by means of the least-squares method.

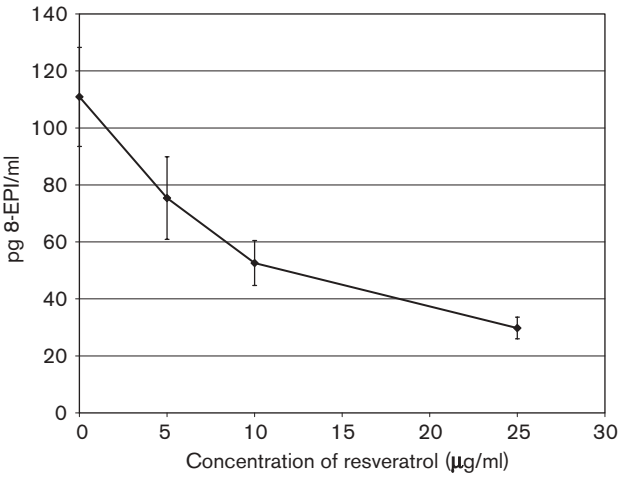
Results

The cytotoxicity of resveratrol to human blood platelets and lymphocytes was evaluated. We noticed that the tested doses of resveratrol did not cause the lysis of platelets determined as a leakage of lactic dehydrogenase into the extracellular medium. Resveratrol evoked a concentration-independent slight decrease in the viability of human peripheral blood lymphocytes. At the maximum applied concentration of resveratrol, 100 μg/ml, the viability of lymphocytes was about 83% (data not shown).

Resveratrol reduced the 8-EPI generation in thrombin-stimulated blood platelets; the action of resveratrol was not dose dependent (Fig. 1). In the presence of the highest dose of resveratrol (25 μg/ml) generation of 8-EPI in stimulated cells was inhibited by about 70% (Fig. 1 and Table 1). Contrary to resveratrol, platinum compounds (cisplatin, Se-Pt; 10 μg/ml) had a significant stimulatory effect on the production of 8-EPI in blood platelets stimulated by thrombin (Table 1). Treatment of cells simultaneously with cisplatin and resveratrol decreased this process (about 40%) (Table 1). The same effect was observed when platelets were treated with Se-Pt and resveratrol (Table 1).

Treatment of blood platelets with cisplatin and Se-Pt (for 30 min) resulted in the decreased activity of different anti-oxidative enzymes (GSH-PX, SOD and catalase) (Table 2). We noticed that resveratrol alone did not change the activity of these enzymes (Table 3). There were no statistical differences in the glutathione

Fig. 1



The effects of resveratrol (30 min, 37°C) on the 8-EPI production in human blood platelets activated by thrombin (1 U/ml, 2 min, 37°C). Each experiment was carried out using three independent measurements ($n = 3$, $p < 0.05$, with respect to resveratrol-untreated platelets).

peroxidase activity between control and resveratrol-treated platelets at concentrations of 5, 10 and 25 μg/ml (Table 3). After the exposure of platelets to resveratrol (25 μg/ml) the inhibitory effect of cisplatin (10 μg/ml) on the activity of catalase was decreased to around 20% (Table 2). The inhibitory effect of Se-Pt (10 μg/ml) on catalase activity was also reduced by resveratrol (Table 2). GSH-PX activity was significantly lower in platelets incubated with cisplatin than Se-Pt (Table 2). The decrease of GSH-PX activity was about 35 and 20%, respectively. After exposure of blood platelets to resveratrol (25μg/ml) and platinum compounds (10 μg/ml), the inhibitory effect of cisplatin and Se-Pt on the activity of GSH-PX was decreased to about 10% (Table 2). A similar protective effect of resveratrol was observed, when we measured the SOD activity in these cells (Table 2).

Figure 2 presents percentage of DNA in the tail of lymphocytes exposed to resveratrol. In these cells, resveratrol at tested concentrations (5–50 μg/ml) did not induce significant changes of comet tail DNA. However, we observed a significant increase of tail DNA in the presence of 10 μg/ml of Se-Pt (Fig. 3). Figure 3 shows slight DNA damage of the lymphocytes exposed to Se-Pt (10 μg/ml) in the presence of resveratrol (25 μg/ml). A combined action of resveratrol and Se-Pt evoked a significant decrease of DNA damage in comparison with DNA damage of lymphocytes treated only with Se-Pt (Fig. 3).

TAS was measured in plasma according to a kit. TAS of human plasma treated with platinum compounds was distinctly increased compared with control. We observed a distinct reduction of TAS values in the presence of resveratrol (Fig. 4).

Table 1 The effect of resveratrol (25 μg/ml, 30 min, 37°C) and platinum compounds (10 μg/ml, 30 min, 37°C) on 8-EPI production in human blood platelets activated by thrombin (1 U/ml, 2 min, 37°C)

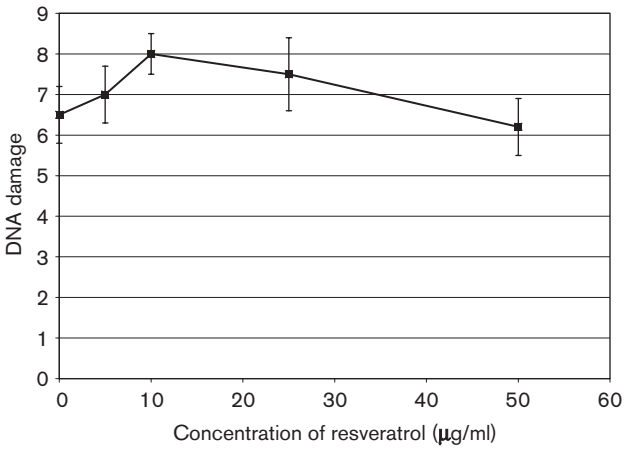
Blood platelets treated with	8-EPI (pg/ml)
(Control)	110.9 ± 17.4
Resveratrol	29.8 ± 3.8
Cisplatin	174.4 ± 25.5
Cisplatin + resveratrol	114.8 ± 20.2
Se-Pt	140.2 ± 13.4
Se-Pt + resveratrol	104.2 ± 17.1

Results are means ± SD of three experiments ($p < 0.05$, with respect to tested compound-untreated platelets).

Discussion

ROS and damage caused by these species are implicated in the pathogenesis of a variety of diseases, including cancer. Although ROS may be generated as byproducts of aerobic metabolism, they are essential for various defense mechanisms in the cells or are involved in signaling processes. They can also cause oxidative damage to DNA, proteins and lipids. During cancer therapy (radiotherapy or chemotherapy), in patients undergoing treatment with cisplatin, ROS may be generated. The toxic side-effects of chemotherapy may be associated also with damage to different cellular components by ROS. Cisplatin is a widely used anti-cancer drug, but its application is limited due to severe side-effects [16]. To reduce these side-effects, many other platinum drugs have been synthesized. Our study presents the comparative analysis

Fig. 2



DNA damage, measured as the comet tail DNA in the alkaline (pH > 13) comet assay of human lymphocytes incubated for 30 min at 37°C with resveratrol. The number of cells scored for each treatment was 50. Each experiment was carried out using three independent measurements ($n = 3$, $p > 0.05$, with respect to resveratrol-untreated lymphocytes).

Table 2 The effect of resveratrol (25 μg/ml, 30 min, 37°C) and platinum compounds (10 μg/ml, 30 min, 37°C) on the activity of different anti-oxidative enzymes in human blood platelets

Blood platelets treated with	Activity of catalase (mU/min/mg)	Activity of GSH-PX (mU/min/mg)	Activity of SOD (mU/mg)
(Control)	74.4 ± 17.4	73.9 ± 9.4	393.9 ± 32.4
Resveratrol [$p > 0.05$, with respect to resveratrol-untreated platelets (control)]	84.5 ± 20.1	74.0 ± 11.1	388.4 ± 12.5
Cisplatin [$p < 0.05$, with respect to cisplatin-untreated platelets (control)]	39.9 ± 14.7	48.5 ± 12.2	202.5 ± 20.9
Cisplatin + resveratrol [$p < 0.05$, with respect to cisplatin-treated platelets]	59.4 ± 12.6	66.3 ± 9.9	298.7 ± 21.4
Se-Pt [$p < 0.05$, with respect to Se-Pt-untreated platelets (control)]	54.2 ± 14.4	57.2 ± 7.7	266.8 ± 18.5
Se-Pt + resveratrol [$p < 0.05$, with respect to Se-Pt-treated platelets]	69.7 ± 12.4	68.4 ± 6.5	312.4 ± 22.8

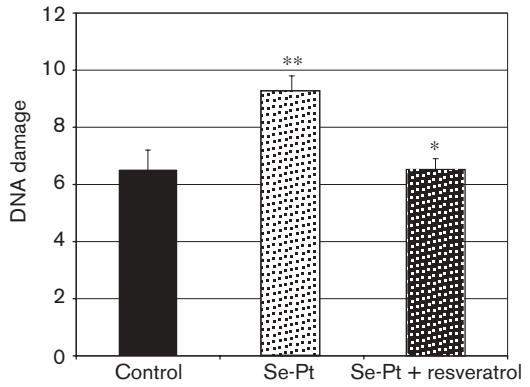
Results are means ± SD of three experiments.

Table 3 The effect of resveratrol (30 min, 37°C) on the activity of different anti-oxidative enzymes in human blood platelets

Concentration of resveratrol (μg/ml)	Activity of catalase (mU/min/mg)	Activity of GSH-PX (mU/min/mg)	Activity of SOD (mU/mg)
0	74.4 ± 17.4	73.9 ± 9.4	393.9 ± 32.4
5	75.3 ± 17.8	70.8 ± 8.5	404.5 ± 13.7
10	78.4 ± 15.5	71.9 ± 9.3	389.6 ± 10.8
25	84.5 ± 20.1	74.0 ± 11.1	388.4 ± 12.5

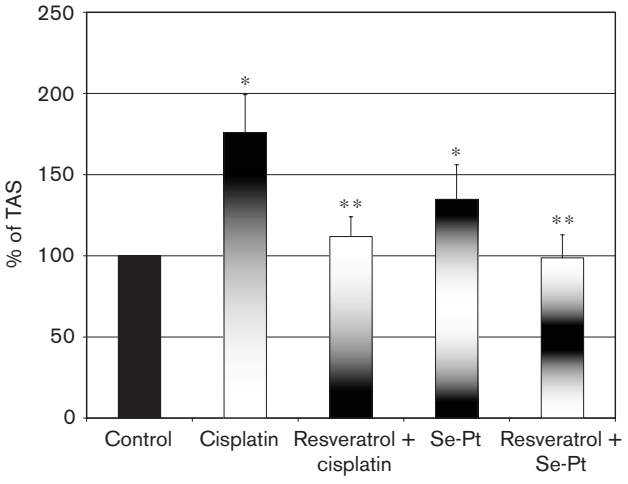
Results are means ± SD of five experiments ($p > 0.05$, with respect to resveratrol-untreated platelets).

Fig. 3



DNA damage, measured as the comet tail DNA in the alkaline (pH > 13) comet assay of human lymphocytes incubated for 30 min at 37°C with Se-Pt (10 μg/ml) in the presence of resveratrol (25 μg/ml). The number of cells scored for each treatment was 50. Each experiment was carried independently in triplicate ($n = 3$, $^*p < 0.05$, with respect to Se-Pt-treated lymphocytes; $^{**}p < 0.001$, with respect to Se-Pt-untreated lymphocytes).

Fig. 4



The effects of resveratrol (25 μg/ml, 30 min, 37°C) and platinum compounds (10 μg/ml, 30 min, 37°C) on the total anti-oxidant status in human plasma. Each experiment was carried out using three independent measurements ($n = 3$, $^*p < 0.05$, with respect to cisplatin or Se-Pt-untreated plasma; $^{**}p < 0.05$, with respect to cisplatin or Se-Pt-treated plasma).

of the toxicity of cisplatin and a conjugate Se-Pt in the presence and absence of anti-oxidant (resveratrol) in terms of cell viability, DNA damage in human lymphocytes, production of 8-EPI in human blood platelets, activity of platelet anti-oxidative enzymes and total anti-oxidant status in plasma. Recently, Hakimuddin *et al.* [3] observed that flavonoids present in red grape wine showed a selective cytotoxicity towards breast cancer cells, but are marginally cytostatic towards normal cells. This agrees with our experiments, because resveratrol (also present in red wine at high concentration) did not induce lysis of blood platelets and caused only a slight decrease in the viability of lymphocytes (data not shown). Yen *et al.* [37] also observed that resveratrol shows no cytotoxicity to human lymphocytes.

Cisplatin causes hematological toxic effects inducing oxidative stress and changes of the biological function of blood cells. It has an inhibitory effect on blood platelet activation [20,24,38], induces platelet lipid and protein peroxidation, and causes the generation of ROS in these cells [14,15,23,25]. Thiols are involved in the action of platinum compounds on platelets. Our previous studies show that after incubation of blood platelets with cisplatin the amount of glutathione (GSH) decreased, and the complex of cisplatin with GSH (GS–cisplatin) was formed (via reaction catalyzed by glutathione S-transferases) [21,22,38,39]. We suggest that not only cisplatin alone but also the GS–cisplatin complex formed in blood platelets may be responsible for the toxic effects of cisplatin in hemostasis and in suppressing platelet functions [21–23]. Blood platelets are the smallest unucleated blood cells. They have no DNA, but possess a high concentration of unsaturated fatty acids. In this study we showed that platinum compounds (cisplatin and Se-Pt) caused production of isoprostanes in blood platelets (Table 1). Isoprostanes are mainly synthesized through non-enzymatic free radical-catalyzed oxidation of arachidonic acid. The first demonstration that prostaglandin F_2-like compounds termed isoprostanes from arachidonic acid are produced in humans was shown by Morrow *et al.* [40] in 1990. Since then, F_2-isoprostanes have been used extensively as clinical markers of lipid peroxidation and oxidative stress *in vivo* in human diseases [41–43]. Among these F_2-isoprostanes, 8-EPI (also named isoprostaglandin $F_{2\alpha}$ type III [44] or 15-F_{2t}-IsoP [45]) is extensively used as a sensitive clinical

marker of lipid peroxidation and biomarker of oxidative stress. Elevated levels of 8-EPI have been described in different disorders and levels of it increased by up to 200-fold in animal models of oxidative injury. Increased levels of 8-EPI were also described in patients with hypercholesterolemia, diabetes mellitus, hepatorenal syndrome, scleroderma, in smokers, and in neurological disorders such as Alzheimer's disease, Parkinson's disease and Creutzfeldt–Jakob disease [41–43]. However, Wiswedel *et al.* [46] showed that dietary flavonol, using cocoa drink as an example, can lower the plasma level of isoprostanes. Here, we show that resveratrol, a natural anti-oxidant, also reduced the 8-EPI concentration in human blood platelets (Fig. 2 and Table 1). Isoprostanes have been measured in biological fluids such as urine or plasma [47], and in different cells or tissues [43]. Isoprostanes are also generated in blood platelets when ROS mediate the peroxidation of arachidonic acid. We measured the level of 8-EPI in blood platelets treated with platinum compounds in the presence (or not) of resveratrol (Table 1). We observed an increased level of 8-EPI in platelets treated with platinum compounds (Table 1); moreover, platinum compounds had distinct inhibitory effects on the activity of platelet anti-oxidative enzymes (Table 2).

Using the comet assay we showed that platinum compounds may oxidize not only lipids, but also other cellular components [36,48]. We observed previously that cisplatin did not cause a significant change in the viability of the lymphocytes, but that the conjugate Se-Pt dramatically diminished viability. Se-Pt bound to isolated DNA was about 100 times weaker than the remaining cisplatin. Cisplatin formed cross-links with DNA in lymphocytes, whereas the conjugate induced DNA strand breaks. The lesions evoked by cisplatin were slowly removed, but damage induced by Se-Pt was not repaired after 5 h even at a drug concentration of 10 μM [36,48].

Because exposure of cells to ROS is associated with cell damage, we examined whether the dietary anti-oxidant resveratrol can help maintain an appropriate oxidative status and can ameliorate toxic effects of platinum compounds. Resveratrol shows various biological activities, including anti-platelet, anti-cancer, anti-mutagenic, anti-fungal, anti-inflammatory and anti-oxidant properties. The anti-oxidative effect of resveratrol requires the presence of the 4′-hydroxyl group in ring B and the meta-hydroxyl configuration in ring A. Our previous studies show that compounds present in the human diet (resveratrol, selenium compounds and vitamin C) may minimize the toxicity and side-effects of the chemotherapeutic agent (without affecting anti-tumor activity) on blood platelets [14,15] and lymphocytes [48]. The present study provides more information about the anti-oxidant activity of resveratrol. The range of resveratrol concentrations (5–100 μg/ml) was similar to that used in studies of other authors [49]. The results obtained in this study indicate that resveratrol distinctly reduced the level of 8-EPI in control platelets and platelets treated with platinum compounds (Table 1), and markedly diminished inhibition of activities of platelet anti-oxidative enzymes (glutathione peroxidase, catalase and SOD) caused by platinum compounds (Table 2). Resveratrol evoked a slight decrease in the lymphocyte viability, and distinctly reduced the extent of DNA damage in lymphocytes incubated with Se-Pt (measured with the comet assay) (Fig. 3). Yen *et al.* [37] also observed that the inhibition of resveratrol on oxidative DNA damage in human lymphocytes might be attributed to an increase of glutathione level and modulation of anti-oxidant enzymes (glutathione peroxidase, glutathione reductase and glutathione S-transferase). Moreover, results of our earlier studies suggest that resveratrol protects against changes in glutathione metabolism in blood platelets induced by platinum compounds [14]. Recently, it was shown that different flavonoids may modulate the toxicity of drugs. van Acker *et al.* [50] demonstrated that a new synthetic flavonoid 3,7-disubstituted-2(3′,4′-dihydroxyphenyl) is a potent protector against doxorubicin-induced cardiotoxicity.

Our results indicate that resveratrol may change anti-oxidant status (which is the balance between pro-oxidant and anti-oxidant systems) in plasma treated with platinum compounds (Fig. 4). The cisplatin concentration used in our experiments (10 μg/ml) is considered suitable with the clinically achievable plasma concentrations. The anti-oxidant status is affected either from increased dietary supply of anti-oxidants or from their endogenous production and is also affected by the production of ROS, which causes increased utilization of anti-oxidants.

Due to anti-oxidative properties of resveratrol, the oxidative stress of studied cells and damage to cell components induced by platinum compounds may be limited. Consuming dietary anti-oxidant supplements (with resveratrol and other phenolics) during anti-cancer therapy with cisplatin seems to have protective effects against undesirable side-effects induced by platinum compounds. Resveratrol can be considered as a potential protective agent against side-effects of anti-tumor drugs, but further research with both normal and cancer cells is needed to clarify this point.

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