Acta Biologica Turcica 32 (4) 198-205 2019 Received 07 Mar. 2019; Accepted 19 Jun. 2019

ACTA BIOLOGICA TURCICA
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E-ISSN: 2458-7893, http://www.actabiologicaturcica.com

Original research

Effect of resveratrol against cisplatin-induced clastogenicity

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Abstract: Cisplatin (cDDP) is a widely used chemotherapeutic agent for treating a variety of cancers. The drug can also induce chromosomal aberrations (clastogenicity) in healthy cells in cancer patients. Resveratrol (RES) is a natural polyphenol and has been claimed that it has a high protection against clastogenicity. In this study, we aimed to investigate the effect of resveratrol on cDDP-induced chromosome aberrations in bone marrow cells of Wistar albino male rats. Six groups with six animals in each were orginized. Two different resveratrol doses (12.5 ve 25 mg/kg body weight) were used and injected intraperitoneally (ip). Resveratrol doses were applied 5 times during the 24 hours study period to coincide with the schedule for the cDDP-RES combination groups. With regard to the experimental schedule, cDDP-RES groups received cDDP (5 mg/kg bw) and RES at either 12.5 or 25 mg/kg bw, 30 min before, concurrently, and then at every 6 h after cDDP administration. RES caused a significant reduction in the frequency of chromosome aberrations compared to those induced with cDDP alone. As a result, repeatedly applied RES doses (12.5 or 25 mg/kg bw) during 24h caused statistically significant reduction at the frequency of cisplatin induced chromosome aberrations. In conclusion, it is evaluated that resveratrol can be an option as an adjuvant treatment.

Keywords: Cisplatin, Chromosome Aberration, Resveratrol, Anticlastogenic

Citing: Bingöl, G., Gülkaç, M.D., Dillioğlugil, M.Ö., & Özön Kanlı, A., 2019. Effect of resveratrol against cisplatin-induced clastogenicity. *Acta Biologica Turcica, 32*(4): 198-205.

Introduction

Resveratrol (RES) is an exogenous source of antioxidant taken from the outside through the foods. RES (trans-3,5,4'-trihydroxystilbene), found abundantly in the crusts of grape beans, is a natural, polyphenolic compound in the stilbene structure. It has also protective effects on plants against various damages caused by environmental factors such as drought, ultraviolet rays, and fungal infections (Langcake and Pryce, 1976). Its molecular formula is $C_{14}H_{12}O_3$, and molecular weight is 228.25 g/mol. It is metabolized 30-60 minutes after oral ingestion and is often converted to glucuronide and sulfate metabolites by conjugation in the liver and duodenum (Wenzel and Somoza, 2005).

Antioxidant action mechanisms of RES have not yet been fully elucidated. Phenolic antioxidants can act as donors of hydrogen atoms or electron donors, during their reactions with free radicals. Resveratrol is often stated to be a hydrogen atom donor (Tang et al., 2011). On the other hand, the hydroxyl groups in the resveratrol structure may also have important functions, within the context of the antioxidant activity (Wang et al., 1998). In some studies, RES was found to sweep hydroxyl radicals (OH) (Leonard et al., 2003) and reduce the reactive oxygen deposits (Sgambato et al., 2001). RES was also found to inhibit

lipid peroxidation (Leonard et al., 2003). Moreover, in a study, RES was shown to regulate the oxidative stress and detoxification mechanisms, in particular, catalase, superoxide dismutase (SOD), glutathione peroxidase, NADPH-quinone oxidoreductase and glutathione S transferase enzymes, and reduce oxidative damage (Rubilio et al., 2008).

Cisplatin (cis-diamminedichloroplatinum(II), cDDP) is an antineoplastic drug that has been used since the 1970s to treat numerous types of cancer. It is used in the treatment of many types of adulthood and childhood period tumors such as neuroblastoma, hepatoblastoma, osteosarcoma, and also other cancer types as especially head and neck, ovary, chest, esophagus, testis, and breast (Ndagi et al., 2017).

cDDP is an inorganic molecule with chlorine and ammonium atoms around the central platinum atom. The molecular formula of cDDP is $Cl_{12}H_6N_2Pt$, and its molecular weight is 300.1 g/mol. According to the mechanisms of action on the cells, it is counted within the group of alkylating agents. Alkylating agents are the agents that cause DNA strand breaks, inhibition of DNA synthesis, and cells' being drawn into apoptosis (Chamber et al., 2001).

cDDP may show its genotoxic effect by direct binding to DNA. Because it can be converted into active compounds in aqueous media and these compounds can react with the nucleophilic region on DNA. The drug can bind directly to DNA, as well as establishing cross-links such as DNA-DNA and DNA-protein (Sanderson et al., 1996, Vogel et al., 1991). All of these events can cause the formation of lesions inside the DNA, thus causing cell cycle arrest, cell death, or mutations.

On the other hand, cDDP indirectly causes DNA mutagenesis by inducing the formation of reactive oxygen species (ROS) (Deavall et al., 2012). This action results in aberrations on chromosomes. From studies desingned as in vivo and in vitro, cDDP has been reported to cause sister chromatid exchange, chromosome aberrations and micronucleus formation in normal cells (Adler and El-Taras, 1989; Antunes et al., 2000; Blasko et al., 1987; Edelweiss et al., 1995; Kliesh and Adler, 1987). Production of ROS causes an increase in lipid peroxidation, a decrease in GSH levels as well as a decrease in antioxidant activity in tissues and organs (Amaral et al., 2008). Among toxicities caused by high dose cDDP usage are ototoxicity, nephrotoxicity, hepatotoxicity, and neurotoxicity (Barabas et al., 2008). Although the therapeutic effect of cisplatin increases with the increment of the dose, especially nephrotoxicity is a dose-limiting factor in this drug use. The therapeutic dose of cDDP for cancer patients is 40-175 mg/m2 intravenously (Weijl et al., 1997).

As it is known, cDDP is a drug with an antitumoral effect, however, it is mutagenic because it causes secondary malignancies in humans. This drug has serious toxic effects on many organs and tissues, as well as its mutagenic effects at the DNA level. All these drawbacks cause some limitations for this medicine usage. Although many prophylactic administrations have been taken to overcome the side effects of chemotherapeutic drugs, they seem to be inadequate. Antioxidants play an important role in preventing cellular damage caused by cDDP. In this study, it was aimed to investigate the modulatory effect of resveratrol and of its combination with cDDP on the clastogenic action of cDDP in Wistar rat bone marrow cells. The results to be obtained will shed light on the debates over the need for an adjuvant therapy with chemotherapeutic drugs.

Materials and Methods

Animals

In this study, Wistar albino male rats, having 6 weeks of age and sexual maturity, with the weight of 150-200 gr were used. The reason for the selection of the rats aged 6 weeks is that this period, as the first step of the animal's cycle of maturity, will eliminate the assessment mistakes that may be led by the chromosomal damage that may occur due to old age (Antunes and Takahashi, 1998).

The animals used in the experiments were provided by the Experimental Medical Research and Application Unit (Kocaeli Üniversitesi Deneysel Tıp Araştırma ve Uygulama Birimi - DETAB) of Kocaeli University with the approval of the Ethics Committee of Kocaeli University Faculty of Medicine.

While the general maintenance of the experimental animals was carried out by DETAB, the surveillance and maintenance of the animals taken in the experiment were undertaken by the researchers theirs. During the study, animals in the experimental and control groups were kept in the standard conditions of the unit at 22 ± 5 °C, under 50 ± 20% relative humidity, in 12 hours dark-12 hours light cycle, and the rats were fed with standard rat feed and tap water.

A total of 6 groups each consisting of 6 animals were formed as the control and experimental groups (Table 1).

Table 1. Control and experimental groups

Groups	Treatments	Dose
1	Control	2 cc bidistillated water
2	RES12.5	12.5 mg/kg, b.w. RES
3	RES25	25 mg/kg, b.w. RES
4	cDDP	5 mg/kg, b.w. cDDP
5	cDDP+RES12.5	5 mg/kg, b.w. cDDP + 12.5 mg/kg, b.w. RES
6	cDDP+RES25	5 mg/kg, b.w. cDDP + 25 mg/kg, b.w. RES

RES resveratrol, *cDDP* cisplatin

Chemicals and Administrations

cDDP (Platinil, Cas no: 15663-27-1) was purchased from a local pharmacy (Kocaeli) as 50 mg/100 ml vials. 50 mg/100 ml of solution was injected into the animals as ip in 5 mg/kg bw doses. It has been reported that the dose of cDDP effective in inducing chromosome aberrations in rat bone marrow is 5 mg/kg bw (Antunes et al., 2000).

Resveratrol in molecular purity (trans 3-4 ', 5-trihydroxystilbene, Sigma, Cas no: 501-36-0) was purchased from Interlab Firm (Istanbul) and was dissolved in 95% ethyl alcohol. The stock solution with ethyl alcohol was prepared in the density of 50 mg/ml. RES was diluted with bidistilled water before the injections. Before application, 0.05 ml of this stock solution was diluted to 0.5 ml of by adding 0.45 ml distilled water to give a final concentration of 5 mg/ml. We did the injection of intraperitoneal RES according to the experimental procedure that we used in our previous study (Bingöl et al., 2014). According to this, RES was administered as ip in a total of 5 doses (cumulative dose 62.5-125 mg / kg bw) half an hour prior to administration of cDDP, together with cDDP, and at every 6th hours following that.

0.08 g colchicine was weighed in a sterile environment and was diluted in 100 ml distilled water. The rat was administered intraperitoneally 70 minutes prior to cervical dislocation, with 0.01 ml of stock solution per gram (Ford and Hamerton, 1956). All animals were sacrificed 24 hours after cDDP administration. 75 min before the sacrification, colchicine (Sigma, C9754) was administered intraperitoneally to the animals as 0.01 ml per gram. Hypotonic shock technique was used in collecting the bone marrow.

Statistical Analysis

Statistical analysis of the differences in frequencies of chromosome aberrations, abnormal metaphases (cell with chromosome aberrations), and mitotic-index between groups were evaluated by a one-way ANOVA test. Data for each group were expressed as mean ± SD. Since structural chromosome anomalies and abnormal metaphase distributions examined in the control and experimental groups did not have a normal distribution and were not homogeneously distributed, the statistical significance between the groups was evaluated by the Kruskal Wallis ANOVA test, which is a non-parametric test. Mann Whitney U test was used to compare the frequencies of chromosomal anomalies between the two groups. The significance level was accepted as $p<0.05$ for the statistics. When chromatid and isochromatid type fractures, complex exchange, triraial and quadriradial figures were included in the calculation of the total chromosomal anomalies, while gaps were not evaluated within this scope.

Results

The results of the rat bone marrow chromosome aberration analysis following treatment with RES alone, cDDP alone, and cDDP+RES are presented in Table 2. Total six structural chromosome aberrations were identified in the control and experimental groups (Savage, 1976). Treatment with RES (12.5 or 25.0 mg/kg bw) alone did not induce a significant increase in either the frequency of chromosome aberrations or the frequency of abnormal metaphases, as compared with control values. These findings show that RES, at these doses, not clastogenic in rat bone marrow cells.

There were no statistically significant differences among the control, RES12.50, RES25, cDDP and cDDP+RES12.50 and cDDP+RES25 groups in terms of isochromatoid type fracture, triradial figure and quadriradial figure ($p>0.05$).

Animals applied with a single dose of cDDP (5 mg/kg bw) showed an increased frequency of total chromosome aberrations, and abnormal metaphases compared to the control group ($p < 0.05$). Chromatid type aberrations values were found to be significantly higher when the cDDP group was compared to the healthy control, RES12.50 and RES25 groups ($p=0.003$, $p=0.003$, $p = 0.002$, respectively). Complex exchange values were significantly higher in the cDDP group than in the healthy

control, RES12.50 and RES25 groups (p=0.002, p=0.002, p=0.002, respectively). In the cDDP group, chromatid-type breaks were observed at the highest frequency, followed by complex exchanges.

Repeated administration of RES at 12.5 mg/kg in the cDDP+RES 12.5 group caused statistically significant reduction in the frequency of total chromosome aberrations and abnormal metaphases induced by cDDP ($p < 0.05$). In the cDDP+RES 12.50 group, the chromatid type aberrations were significantly lower than the cDDP group (p=0.006), but significantly higher than the control group (p=0.003). Complex exchange values were significantly higher in cDDP+RES12.5 group when compared with control (p=0.007), while no significant difference was seen with cDDP alone (p>0.05).

In the cDDP+RES25 group, animals treated RES at 25 mg/kg had a statistically significant reduction in total chromosome aberrations and abnormal metaphases induced by cDDP when compared to animal treated with cDDP alone ($p < 0.05$). 25 mg/kg significantly reduced cDDP-induced chromatid breaks and complex exchange compared to the cDDP group (p=0.004, p=0.013 respectively). There were no significant differences in complex exchange and chromatid break values between cDDP+RES25 and control groups (p>0.05). When cDDP+RES 25 group and cDDP+RES 12.50 group were compared, chromatid breaks were significantly lower ($p = 0.012$), but no difference in complex exchange was seen (p>0.05)

A significant difference in mitotic index values was not observed between the control group and RES12.5 or RES25 groups. The animals treated with a single dose of cDDP showed a significantly lower mitotic index compared to the controls ($p < 0.05$) (Table 2). The rats in cDDP+RES12.5 and cDDP+RES25 groups had a significantly higher mitotic index than the cDDP group ($p<0.05$). There was no significant difference in mitotic index values when cDDP+RES12.50 and cDDP+RES25 groups were compared (p>0.05).

Discussion

Bioavailability of resveratrol taken orally or intravenously is low due to the low plasma concentration and rapid metabolism to glucuronide and sulfate conjugates. Measurements with marked resveratrol (^{14}C-resveratrol) have shown that the plasma half-life of 25 mg resveratrol is 9.2 hours when it's given orally, and it is 11.4 hours when 0.2 mg is administered intravenously (Walle et al., 2004). Whichever way it is given, the low half-life and rapid metabolizm reduce the bioavailability of resveratrol. In some studies, bioavailability of resveratrol has been shown to be rise when it is given in increased and repeated doses (Almeida et al., 2009, Nunes et al., 2009).

In a study investigating effective concentrations of resveratrol at the cellular level, oral doses of 50 mg/kg bw was reported to reach concentrations of 25-30 μM in mouse tissues, and this dose was indicated to be effective on molecular targets (Vitrac et al., 2003). From a human cell culture study, it was reported that the effective dose of inducing cellular antioxidant levels of resveratrol was 10 μM (Kode et al., 2008) (Table 2).

Table 2. Mitotic index and distributions of the different types of chromosomal aberrations and abnormal metaphases observed with Wistar rat bone marrow cells after treatment with resveratrol (RES; 12.5 or 25 mg/kg bw) alone or in combination with cisplatin (cDDP; 5mg/kg bw).

Groups	MI (%)[b]	Chromosomal aberrations[a]						TCA	(TCA/rat))	AM (AM/rat)
		Gaps	Breaks		E	T	Q			
			CB	ICB						
1	2.68 ± 0.69	2	1	0	0	0	0	1	0.17 ± 0.41	0.17 ± 0.41
2	2.71 ± 0.80	3	2	0	0	0	0	2	0.33 ± 0.52	0.33 ± 0.52
3	2.73 ± 0.62	1	0	0	0	0	0	0	0.00	0.00
4	1.62 ± 0.48[d]	7	39	1	14	3	1	58	9.67 ± 2.58[d]	8.83 ± 1.47[d]
5	2.60 ± 0.68[e]	9	18	0	8	1	1	28	4.67 ± 1.37[d,e]	4.33 ± 1.03[d,e]
6	2.70 ± 0.49[e]	3	8	0	4	1	4	17	2.83 ± 3.19[e]	1.67 ± 1.37 [e,f]

chromatid break; ICB, isochromatid break; E, complex exchange; T, triradial figure; Q, quadriradial figure; AM, abnormal metaphase; TCA, total chromosome aberrations. [a]Numbers of chromosome aberrations. Each chromosomal aberration has been counted by analyzing 100 cells/animal (6 animals/group, for a total of 600 cells/treatment), [b]Mitotic index has been calculated by analyzing 1000 cells/animal (for a total of 6000 cells/treatment) and percentage of the mitotic cells calculated for each treatment group, [c]Gaps were not included in total chromosomal aberration, [d] Significantly different from the control, RES12.5 and RES25 groups ($p < 0.05$), [e] Significantly different from the cDDP group ($p < 0.05$)., [f] Significantly different from the cDDP+RES12.5 group ($p < 0.05$)

Resveratrol is toxic at high doses. In rats, a dose of 3000 mg/kg bw has been shown to cause clinical toxicity such as decreased body weight and nutrient intake, elevated BUN, creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin and albumin, hemoglobin, hematocrit and red blood cell counts; lesions and nephropathy in the kidneys; whereas no side effects have been identified for the dose of 300 mg/kg bw per day (Crowel et al., 2004).

In our study, resveratrol was given intraperitoneally at frequent and repeated doses because of its low bioavailability and rapid metabolizing. Resveratrol, at two different doses, 12.50 and 25 mg/kg bw, was administered half an hour prior to cDDP administration, with cDDP, and every 24 hours thereafter (62.5-125 mg/kg bw daily cumulative dose, respectively). In our study, it was seen that chromosomal aberrations induced by cDDP in bone marrow cells was significantly reduced when frequent and repeat dose application of resveratrol was applied.

In a study showing that resveratrol reduced the genotoxic effects induced by cDDP in normal cells, resveratrol was administered to male Swiss albino rats at doses of 50 mg/kg bw and 100 mg/kg bw for 14 days, as a single dose every day (Attia, 2012). Attia used alkaline comet assay and found that frequency of DNA fractures in resveratrol-treated cisplatin group was significantly lower than cDDP group ($p < 0.01$). In our study, a 24-hour periodic effect of resveratrol was investigated and bone marrow aspiration was performed 24 hours after administration of 5mg/kg cisplatin. The reason for this is that most chromosomal aberrations occur 24 hours after the administration of a single dose of cisplatin (5 mg/kg bw) (Rosselli et al., 1990, Antunes et al., 2000).

In our study, it was found that chromatid breaks, complex exchange, total chromosome aberrations and abnormal metaphase mean values were significantly different between the groups ($p<0.05$). The chromatid breaks, complex exchange, total chromosomal aberrations and abnormal metaphase mean values were found to be significantly higher as expected ($p<0.05$) in cDDP group when compared to the control, cDDP+RES12.50 and cDDP+RES25 groups. Chromatid break and complex exchange values of cDDP+RES12.5 group were significantly higher than that of control group ($p<0.05$); while only chromatid break values were significantly lower when compared to the cDDP group ($p<0.05$). In cDDP+RES 25 group, the frequencies of complex exchange and chromatid breaks were not different from control group ($p>0.05$), while significantly lower than the cDDP group ($p < 0.05$). In our study, in cDDP+RES12.50 group, frequencies of total chromosome aberrations and abnormal metaphase were significantly higher than control group ($p<0.05$) while significantly lower than cDDP group ($p<0.05$). There was no statistically significant difference between the cDDP+RES25 group and the control group ($p>0.05$). In cDDP+RES25 group, abnormal metaphase values were found to be significantly lower when compared to the cDDP+RES12.50 group ($p<0.05$), while there was no statistically significant difference between the two groups in terms of total chromosomal aberrations values ($p>0.05$). Our results showed that repeated doses of resveratrol significantly decreased the mean values of chromatid breaks, complex exchange aberrations, total chromosome aberrations and abnormal metaphases caused by cDDP in a concentration dependent manner ($p < 0.05$). In addition, it was observed that the decrease in mitotic index caused by cDDP was significantly increased with the usage of repeated resveratrol doses ($p<0.05$). In conclusion, in our study, when compared with cDDP group, total chromosomal aberrations were reduced by 3.42 times with repeated doses of resveratrol, and values were not difference from the control group ($p>0.05$).

In our study, among control group, only RES12.50 and only RES25 groups, there were no statistically significant differences in the frequencies of total chromosome aberrations and abnormal metaphase ($p>0.05$). In a study showing the genotoxic effect of resveratrol, resveratrol at the concentrations of 1-60 μM was reported to cause micronucleus formation in the L5178Y mouse lymphoma and Chinese hamster V79 cells (Schmitt et al., 2002). We did not use cancerous cells in our study. In a study, it was stated that cancer cell line response to the resveratrol could be different from normal cells; for example, resveratrol showed pro-apoptotic effect in tumor cells, but not in normal cells (Lu et al., 2001). Moreover, in vitro mutagenicity test (Ames, 1989) result for resveratrol has been determined as negative (Matsuoka et al., 2001). Besides, an in vivo study investigating the clastogenic effect of resveratrol obtained anegative result up to 2000 mg/kg bw in rats (Williams et al., 2009). Other studies performed within in vivo models also showed that resveratrol did not have genototic effect in normal cells (Edwards et al., 2011; Attia, 2012; Carsten et al., 2008).

Concordance with these studies, our study also showed that resveratrol doses as 12.5 and 25 mg/kg bw with repeated intervals within 24 h time period did not lead to chromosomal aberrations and genotoxicity in groups applied resveratrol alone.

In our previous study, in which we applied the same technique, we found that repeated resveratrol dose (12.5 and 25 mg/kg bw, every 6 h for a day) caused statistically significant reductions in chromosome aberrations and abnormal methapse frequencies caused by cDDP (Bingöl et al., 2014). The mechanism by which the anticlastogenic effect of resveratrol is exerted on chromosome aberrations caused by chemotherapeutic agents is not fully understood. However, we can say that the antioxidant function of resveratrol may have contributed to the reduction of chromosome damage that occurs. Chemotherapeutic drugs contribute to the formation of oxidative stress in the cells and thus cause breakage and oxidative lesions on the DNA. Resveratrol can show antioxidant function by decreasing ROS formations and increasing antioxidant activity (Leonard et al., 2003; Sgambato et al. 2001; Sengottuvelan et al., 2009).

In this study, we investigated the protective effect of resveratrol with repeated doses against the chromosomal changes induced by cDDP. Our work is different from other studies in terms of resveratrol application and method. After single dose cDDP administration, chromosomal damage and oxidative stress are seen at the highest level after 24 hours. Since the half-life of resveratrol is much shorter than 24 hours, administration of a single dose of resveratrol can lead to weaken its antioxidant effect against clastogenic and oxidative stress caused by cDDP. In this study, it was aimed to strengthen the protective effect of resveratrol with repeated doses to combat with this problem. This study determined that repeated doses resveratrol reduces the chromosomal damage caused by cDDP to a significant extent, depending on concentration, at the doses of 12.5 and 25 mg/bw. As a conclusion, we have found that resveratrol has an important effect in reducing chromosomal damage caused by cDDP, but the need to apply resveratrol therapy with antineoplastic agents should be addressed with more extensive research.

Acknowledgments

This investigation was supported by Scientific Research Unit, Kocaeli University (2010/103).

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