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ORIGINAL ARTICLE

The in vitro protective effect of salicylic acid against paclitaxel and cisplatin-induced neurotoxicity

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Abstract Paclitaxel (PAC) and cisplatin (CIS) are two established chemotherapeutic drugs used in combination for the treatment of various solid tumors. However, the usage of PAC and CIS are limited because of the incidence of their moderate or severe neurotoxic side effects. In this study, we aimed to assess the protective role of salicylic acid (SA) against neurotoxicity caused by PAC and CIS. For this purpose, newborn *Sprague Dawley* rats were decapitated in sterile atmosphere and primary cortex neuron cultures were established. On the 10th day SA was added into culture plates. PAC and CIS were added on the 12th day. The cytotoxicity was determined by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Oxidative alterations were assessed using total antioxidant capacity and total oxidative stress assays in rat primary neuron cell cultures. It was shown that both concentrations of PAC and CIS treatments caused neurotoxicity. Although SA decreased the neurotoxicity by CIS and PAC, it was more effective against the toxicity caused by CIS rather than the toxicity caused by PAC. In conclusion it was clearly revealed that SA decreased the neurotoxic effect of CIS and PAC in vitro.

Keywords Paclitaxel · Cisplatin · Salicylic acid · Neurotoxicity · Antioxidant capacity · Oxidative stress

Introduction

A microtubule-binding agent, paclitaxel (PAC, Fig. 1), is widely used to treat several solid tumors including breast, ovarian and lung cancers (Gornstein and Schwarz 2014). Similarly, cisplatin (cis-diamminedichloroplatinum (II), CIS, Fig. 2), one of the most widely used DNA-modifying chemotherapy drug, is commonly used to treat testicular, ovarian, bladder, cervical, esophageal, head, neck and small

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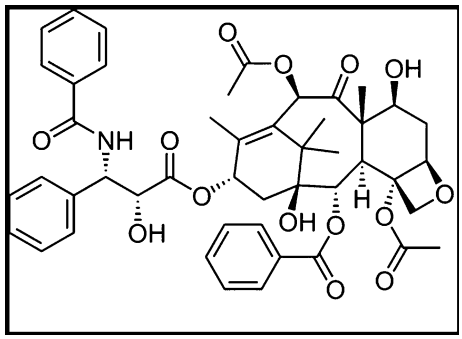

Fig. 1 Chemical structure of paclitaxel

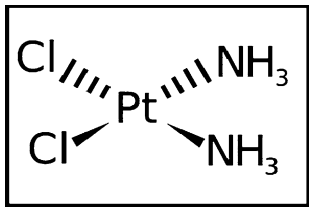

Fig. 2 Chemical structure of cisplatin

cell lung cancers (Jekunen et al. 1994; Huang et al. 2004; Hassan et al. 2014; Song et al. 2014). The treatments with PAC and CIS are known to cause adverse effects such as myelo-suppression and neurotoxicity (Marupudi et al. 2007; Barabas et al. 2008; Wang et al. 2014). Therefore, the usefulness of PAC and CIS are limited due to their neurotoxicity. Up to date, numerous studies have been performed to find protective agents minimizing the neurotoxic effects of PAC and CIS (Openshaw et al. 2004; Chentanez et al. 2009; Turkez et al. 2010; Mao-Ying et al. 2014).

Chemo-protective agents protect healthy tissue from the toxic effects of anticancer drugs. An ideal chemo-protective agent is an agent without side effects, has strong chemo-protective capacities and which does not reduce antitumor activity (Hospers et al. 1999). Reducing the morbidity and mortality of antineoplastic regimens with the concomitant use of chemo-protective agents may lead to more tolerable anti-tumor treatments for patients and may permit for dose-escalation of both radio- and chemotherapy, which could lead to improved survival. Many antineoplastic drugs or chemical agents caused several organ toxicities via oxidative stress. Natural antioxidants or antioxidant featured synthetic compounds have been found to protect various organs from oxidative injuries (Cingolani et al. 2000; Cacciatore et al. 2003; Rispoli et al. 2004; Cacciatore et al. 2005; Geyikoglu and Turkez 2008; Heuking et al. 2009; Di Stefano et al. 2009; Sozio et al. 2010; Turkez and Geyikoglu 2011; Turkez et al. 2012a, b). At this point, salicylic acid (C_6H_4(OH)COOH, SA, Fig. 3) is a phytohormone that regulates many aspects of plant growth and development (Vlot et al. 2009; Rivas-San Vicente and Plasencia 2011; Xue et al. 2013). SA is recognized as an endogenous signal for mediating in plant defense against pathogens by presenting strong antioxidant and antifungal features (Ferger et al. 1999; Shabana et al. 2008; Gören et al. 2009). To the best of our knowledge, the potential protective action of SA against PAC and CIS-induced neurotoxicity in neuronal models has not been studied yet. Therefore, the aim of the present study was to evaluate the cytological (MTT assay) and biochemical effects (TAC and TOS analyses) of SA against PAC and CIS-induced neuronal damage on rat neuron cultures for exploring its neuroprotective potential for the first time.

Materials and methods

Test compounds and chemicals

Taxol® (6 mg/mL vial) was purchased from Bristol-Myers Squibb (New York, NY, USA) and Cisplatin® (10 mg/mL flakon) was purchased from Pharmachemie BV (Haarlem, Holland). Salicylic acid (CAS Number 69-72-7), Dulbecco's Modified Eagle's Medium (DMEM), Hanks' Balanced Salt solution (HBSS) and DNase type 1 were purchased from Sigma Aldrich® (Steinheim, Germany). Neurobasal medium (NBM) and fetal calf serum (FCS) were purchased

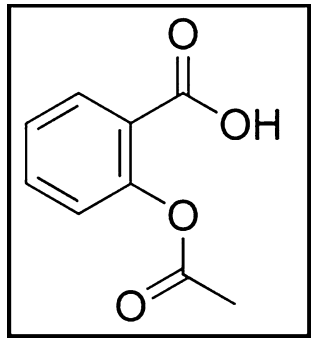

Fig. 3 Chemical structure of salicylic acid

from Gibco-Life Technologies (Australia Pty Ltd, Mulgrave, VIC, Australia).

Rat primary neuron cell cultures

This study was conducted at the Medical Experimental Research Center of the Atatürk University (Erzurum, Turkey). The Ethical Committee of the Atatürk University approved the study by the protocol B.30.2.ATA.0.01.02/5515. Primary rat cerebral cortex neuron cultures were prepared using rat foetuses as described previously (Daikhin and Yudkoff 2000; Aydın et al. 2014; Togar et al. 2014). Briefly, a total of nine newborn Sprague-Dawley rats were used in the study. Making a cervical fracture in the cervical midline, the rats were decapitated and the cerebral cortexes were dissected and removed. The cerebral cortex was placed into 5 mL of HBSS, which had already been placed in a sterile petri dish and macromerotomy was performed with two lancets. The cerebral cortices were dissociated with HBSS, were pulled into a syringe and treated at 37 °C for 25–30 min in 5 mL HBSS plus 2 mL Trypsin– EDTA (0.25 % trypsin–0.02 % EDTA) and chemical decomposition was achieved. 8 µl of DNase type 1 (120 U/mL), was added to this solution and treated for 1–2 min, and centrifuged at 800 rpm for 3 min. After the supernatant was removed, 31.5 mL of neurobasal medium (NBM) and 3.5 mL fetal calf serum (FCS) were added to the pellet. The cells were seeded in 48-well cell culture plates. Plates were left in the CO_2 incubator (5 % CO_2 at 37 °C). Every 3 days, the medium were refreshed with fresh medium at the rate of half their volumes, until the cells reached a certain maturity. In vitro experiments were performed 10 days later. On the 10th day, SA (10 and 100 µM) was added into culture flask. At the end of the 12th day PAC (10^{-1} and 10^{-2} µM) and CIS (50 and 100 µM) were applied. 150 µL isotonic saline solution (ISS) added group was used as controls.

MTT assay

MTT assay (MTT Cell Proliferation Kit, Cayman Chemical Company, Ann Arbor, MI, USA) was used to determine cell viability. Rat cortex neuron cells were seeded in 48-well plates (0.3 mL). Then 10 µL MTT solution (5 mg/ml) was added to each well and incubated for 4 h at 37 °C. After this step, 100 µL crystal dissolving solution (DMSO) was added to each well to dissolve the formed formazan crystals. Finally, the absorbance was recorded at 570 nm using an ELISA plate reader (Thermo LabSystem, Helsinki, Finland) (Turkez et al. 2012a, b; Aydın et al. 2014). The cell viability was then calculated using the following equation: cell viability (%) = (A treated/A control) × 100 (A...absorbance).

TAC and TOS analysis

Antioxidants in the sample reduce dark blue-green colored ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related to the total antioxidant level of sample. The TAS assay is calibrated with a stable antioxidant standard solution that is traditionally named as Trolox Equivalent, which is a vitamin E analog (Kusano and Ferrari 2008). Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion reacts to a colored complex with a chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Equiv./L). TOS assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Equiv./L). Both TAC and TOS assays were carried out with commercially available kits (Rel Assay Diagnostics®, Gaziantep, Turkey) (Erel 2004; Turkez et al. 2014; Dirican and Turkez 2014).

Statistics

For statistical analysis, we used SPSS for Windows 18.0 (SPSS Inc., Chicago, IL, USA). The experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for multiple comparisons. Results are presented as mean ± standard deviation (SD) and values $p < 0.05$ were regarded as statistically significant.

Results

The neurotoxicity of PAC was examined using MTT assay. Both concentrations of PAC exhibited statistically significant ($p < 0.05$) neurotoxicity on cultured rat neuron cells. In fact, the 10^{-1} and 10^{-2} μM of PAC caused cytotoxicity at levels of 36.6 and 28.5 %, respectively. On the contrary, the treatments with different concentrations of SA (10 and 100 μM) for 48 h did not change the viability. However, when MTT assay was carried out after SA and PAC treatment, it was observed that 100 μM SA but not 10 μM SA provided a slight protection against the 10^{-1} μM PAC-induced neurotoxicity. However both SA concentrations did not exhibit a protective action against neurotoxicity by PAC at 10^{-2} μM (Table 1).

As seen from Table 2, concentrations of 50 μM and 100 μM CIS caused cytotoxic effects on cultured rat neurons at levels of 20.5 and 24.3 %, respectively. On the contrary, none of both SA concentrations induced neurotoxicity. Moreover, SA applications into the CIS-treated cells provided significant protection levels against CIS-induced neurotoxicity in a dose independent manner. No significant differences were observed between the 10 μM SA plus CIS and 100 μM SA plus CIS applied groups. In addition, the observed viabilities were still lower than the control values after both SA applications against neurotoxicity by CIS.

Tables 3 and 4 show TAC and TOS levels in SA, PAC and CIS treated neuron cultures. The TAC and TOS levels were modified by the treatments with different concentrations of PAC (10^{-1} and 10^{-2} μM) and of CIS (50 and 100 μM) due to oxidative stress induction. On the other hand, SA supported antioxidant capacity without changing TOS levels. After the treatments with different concentrations of SA in combination with PAC it was observed that both SA concentrations exhibited different levels of protection against PAC induced oxidative stress. Likewise, significant ($p < 0.05$) protection levels were observed when 10 and 100 μM of SA were applied to the culture plates in combination with CIS. But significant differences were not observed between the 10 μM SA plus CIS or PAC and 100 μM SA plus CIS or PAC applied groups.

Table 1 The effects of SA applications on cell viability against PAC-induced cell death

Group	Cell viability (%)
Control (ISS)	100[c]
10^{-1} μM PAC	63.4 ± 5.6[a]
10^{-2} μM PAC	71.5 ± 6.3[b]
10 μM SA	99.2 ± 7.1[c]
100 μM SA	98.3 ± 8.3[c]
10^{-1} μM PAC + 100 μM SA	70.2 ± 5.8[b]
10^{-1} μM PAC + 10 μM SA	63.5 ± 6.1[a]
10^{-2} μM PAC + 100 μM SA	58.1 ± 5.2[a]
10^{-2} μM PAC + 10 μM SA	70.4 ± 6.3[b]

The results are given as the mean ± SD from six independent experiments. The groups in the same column with different letters are statistically significant at the level of $p < 0.05$

PAC paclitaxel, *SA* salicylic acid, *ISS* isotonic saline solution

Table 2 The effects of SA applications on cell viability against CIS-induced cell death

Group	Cell viability (%)
Control (ISS)	100[c]
50 μM CIS	79.5 ± 7.5[b]
100 μM CIS	75.7 ± 7.1[a]
10 μM SA	99.2 ± 7.1[c]
100 μM SA	98.3 ± 8.3[c]
50 μM CIS +10 μM SA	92.5 ± 8.3[c]
100 μM CIS +10 μM SA	82.1 ± 7.8[b]
50 μM CIS +100 μM SA	95.6 ± 8.6[c]
100 μM CIS + 100 μM SA	82.7 ± 7.3[b]

The results are given as the mean ± SD from six independent experiments. The groups in the same column with different letters are statistically significant at the level of $p < 0.05$

CIS cisplatin, *SA* salicylic acid, *ISS* isotonic saline solution

Discussion

In the present investigation, we assessed the protective role of SA against PAC and CIS-induced neurotoxicity on rat neuron cell cultures. Cytotoxic effect was evaluated using MTT assay. According to the MTT assay, the cytotoxic effects of CIS and PAC were demonstrated by their strong inhibition on cell viability on neuron cells (Tables 1, 2). In accordance with our results, PAC exposure caused a decrease of cell viability and an increase in the ratio of apoptotic cells in dorsal root ganglion (DRG) neurons in vitro (Chen et al. 2015). Likewise, it was reported that CIS caused significant neurotoxicity via induction of lipid peroxidation and reduction in the potency of the antioxidant

Table 3 Antioxidant capacity and oxidative status of PAC alone and in combination with SA on neuron cell lines

Group	TAC (mmol Trolox Equiv./L)	TOS (mmol H_2O_2 Equiv./L)
Control (ISS)	1.23 ± 0.32^{c}	1.44 ± 0.51^{b}
10 μM SA	1.96 ± 0.35^{d}	1.38 ± 0.44^{a}
100 μM SA	1.89 ± 0.29^{d}	1.35 ± 0.38^{a}
10^{-1} μM PAC	1.12 ± 0.27^{a}	1.68 ± 0.48^{c}
10^{-2} μM PAC	1.11 ± 0.19^{a}	1.71 ± 0.62^{c}
100 μM SA +10^{-1} μM PAC	1.21 ± 0.37^{bc}	1.54 ± 0.47^{b}
10 μM SA +10^{-1} μM PAC	1.18 ± 0.25^{b}	1.50 ± 0.53^{b}
100 μM SA +10^{-2} μM PAC	1.18 ± 0.29^{b}	1.43 ± 0.39^{b}
10 μM SA + 10^{-2} μM PAC	1.19 ± 0.18^{b}	1.43 ± 0.44^{b}

The abbreviations are as in Table 1

Table 4 Antioxidant capacity and oxidative status of CIS alone and in combination with SA on neuron cell lines

Group	TAC (mmol Trolox Equiv./L)	TOS (mmol H_2O_2 Equiv./L)
Control (ISS)	1.23 ± 0.32^{c}	1.44 ± 0.51^{b}
10 μM SA	1.96 ± 0.35^{d}	1.38 ± 0.44^{a}
100 μM SA	1.89 ± 0.29^{d}	1.35 ± 0.38^{a}
50 μM CIS	1.11 ± 0.17^{b}	1.66 ± 0.54^{c}
100 μM CIS	1.06 ± 0.23^{a}	1.78 ± 0.47^{d}
50 μM CIS +10 μM SA	1.21 ± 0.19^{c}	1.47 ± 0.38^{b}
100 μM CIS +10 μM SA	1.13 ± 0.26^{b}	1.56 ± 0.61^{bc}
50 μM CIS +100 μM SA	1.24 ± 0.31^{c}	1.46 ± 0.52^{b}
100 μM CIS + 100 μM SA	1.14 ± 0.29^{b}	1.70 ± 0.58^{c}

The abbreviations are as in Table 2

defense system (Kamisli et al. 2014). Besides, Colak et al. (2011) suggested natural antioxidant compounds could prevent neurotoxicity in experimental animal models. At this point, our findings indicated that 100 μM SA but not 10 μM SA provided protection against the 10^{-1} μM PAC-induced neurotoxicity. Again, both SA applications to the CIS-treated cells provided protection against CIS-induced neurotoxicity in a clear dose independent manner. Similar to our findings, SA showed protective effect against CIS-induced ototoxicity (Minami et al. 2004). Besides, Pisano et al. (2003) demonstrated that acetyl-L-carnitine (ALC) co-treatment was able to significantly reduce the neurotoxicity of both CIS and PAC in rat models. It was demonstrated that olesoxime, a small cholesterol-like compound, has shown marked neuroprotective properties in animals treated with PAC and vincristine (Rovini et al. 2010). Again, Openshaw et al. (2004) found that amifostine, a thiophosphate cytoprotectant, was ineffective in preventing or reducing the neurotoxicity of high doses of PAC. Conversely, in another study, two randomized trials with PAC and CIS have shown no significant neuroprotective effect of amifostine as determined by clinical assessment (Rick et al. 2001).

Oxidative stress has been known as imbalance between the free radicals and antioxidant defense system. Neurons are more sensitive to oxidative stress because of low activity of antioxidant enzymes (Karpińska and Gromadzka 2013). Experimental studies support that there are evidences about PAC toxicity related with reactive oxygen and nitrogen species (Ramanathan et al. 2005). Likewise, it was suggested that reactive oxygen species were related with CIS cytotoxicity (Feghali et al. 2001; Van den Berg et al. 2006; Zhang et al. 2007). In the present study, we investigated protective effect of SA against PAC and CIS-induced oxidative effects by determining TAC and TOS levels. We reported that SA was more effective in decreasing CIS-induced oxidative stress than PAC-induced oxidative stress due to its antioxidant effect. In addition, significant differences between the 10 μM SA plus CIS or PAC and 100 μM SA plus CIS or PAC applied groups were not observed in the present study. As a matter of fact, Mohanakumar et al. (2000) suggested that SA acts as a free radical

scavenger in the brain that indicates its effectiveness as a valuable neuro-protector in mice in vivo. In addition, Ferger et al. (1999) suggested that the neuroprotective properties of SA were based on its effect as a hydroxyl radical scavenger rather than other mechanisms.

In conclusion, both PAC and CIS show severe neurotoxicity in cultured rat primary neuronal cells. These negative effects could be prevented by SA pretreatments in vitro. SA applications seemed to be more effective against neurotoxicity by CIS than toxicity by PAC. Therefore, we suggest that co-application of SA could be a useful way to decrease the cytotoxic effects of CIS and PAC on the nervous system. The in vivo interactions of SA and CIS are still unknown. Therefore, further studies are needed to fully elucidate the neuroprotective action of SA.

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Conflict of interest The authors declared that there are no conflicts of interest.

References

Aydın E, Türkez H, Keleş MS (2014) The effect of carvacrol on healthy neurons and N2a cancer cells: some biochemical, anticancerogenicity and genotoxicity studies. Cytotechnology 66:149–157

Barabas K, Milner R, Lurie D, Adin C (2008) Cisplatin: a review of toxicities and therapeutic applications. Vet Comp Oncol 6:1–18

Cacciatore I, Caccuri AM, Di Stefano A, Luisi G, Nalli M, Pinnen F, Ricci G, Sozio P (2003) Synthesis and activity of novel glutathione analogues containing an urethane backbone linkage. Farmaco 58:787–793

Cacciatore I, Caccuri AM, Cocco A, De Maria F, Di Stefano A, Luisi G, Pinnen F, Ricci G, Sozio P, Turella P (2005) Potent isozyme-selective inhibition of human glutathione S-transferase A1-1 by a novel glutathione S-conjugate. Amino Acids 29:255–261

Chen C, Bai X, Bi Y, Liu G, Li H, Liu Z, Liu H (2015) Insulin-like growth factor-1 attenuates apoptosis and protects neurochemical phenotypes of dorsal root ganglion neurons with paclitaxel-induced neurotoxicity in vitro. Nutr Neurosci. doi:10.1179/1476830514Y.0000000147

Chentanez V, Thanomsridejchai N, Duangmardphon N, Agthong S, Kaewsema A, Huanmanop T, Maneesri S (2009) Ganglioside GM1 (porcine) ameliorates paclitaxel-induced neuropathy in rats. J Med Assoc Thai 92:50–57

Cingolani GM, Di Stefano A, Mosciatti B, Napolitani F, Giorgioni G, Ricciutelli M, Claudi F (2000) Synthesis of L-(+)-3-(3-hydroxy-4-pivaloyloxybenzyl)-2,5-diketomorpholine as potential prodrug of L-dopa. Bioorg Med Chem Lett 10:1385–1388

Colak S, Geyikoğlu F, Keles ON, Turkez H, Topal A, Unal B (2011) The neuroprotective role of boric acid on aluminum chloride-induced neurotoxicity. Toxicol Ind Health 27:700–710

Daikhin Y, Yudkoff M (2000) Compartmentation of brain glutamate metabolism in neurons and glia. J Nutr 130:1026–1031

Dirican E, Turkez H (2014) In vitro studies on protective effect of *Glycyrrhiza glabra* root extracts against cadmium-induced genetic and oxidative damage in human lymphocytes. Cytotechnology 66:9–16

Di Stefano A, D'Aurizio E, Trubiani O, Grande R, Di Campli E, Di Giulio M, Di Bartolomeo S, Sozio P, Iannitelli A, Nostro A, Cellini L (2009) Viscoelastic properties of *Staphylococcus aureus* and *Staphylococcus epidermidis* mono-microbial biofilms. Microb Biotechnol 2:634–641

Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37:277–285

Feghali JG, Liu W, Van De Water TR (2001) L-n-acetyl-cysteine protection against cisplatin-induced auditory neuronal and hair cell toxicity. Laryngoscope 111:1147–1155

Ferger B, Teismann P, Earl CD, Kuschinsky K, Oertel WH (1999) Salicylate protects against MPTP-induced impairments in dopaminergic neurotransmission at the striatal and nigral level in mice. Naunyn Schmiedebergs Arch Pharmacol 360:256–261

Geyikoglu F, Turkez H (2008) Boron compounds reduce vanadium tetraoxide genotoxicity in human lymphocytes. Environ Toxicol Pharmacol 26:342–347

Gören B, Mimbay Z, Bilici N, Zarifoğlu M, Oğul E, Korfali E (2009) Investigation of neuroprotective effects of cyclooxygenase inhibitors in the 6-hydroxydopamine induced rat Parkinson model. Turk Neurosurg 19:230–236

Gornstein E, Schwarz TL (2014) The paradox of paclitaxel neurotoxicity: mechanisms and unanswered questions. Neuropharmacology 76:175–183

Hassan HA, Edrees GM, El-Gamel EM, El-Sayed EA (2014) Amelioration of cisplatin-induced nephrotoxicity by grape seed extract and fish oil is mediated by lowering oxidative stress and DNA damage. Cytotechnology 66:419–429

Heuking S, Iannitelli A, Di Stefano A, Borchard G (2009) Toll-like receptor-2 agonist functionalized biopolymer for mucosal vaccination. Inter J Pharmaceut 381:97–105

Hospers GA, Eisenhauer EA, de Vries EG (1999) The sulfhydryl containing compounds WR-2721 and glutathione as radio- and chemoprotective agents. A review, indications for use and prospects. Br J Cancer 80:629–638

Huang GC, Liu SY, Lin MH, Kuo YY, Liu YC (2004) The synergistic cytotoxicity of cisplatin and taxol in killing oral squamous cell carcinoma. Jpn J Clin Oncol 34:499–504

Jekunen AP, Christen RD, Shalinsky DR, Howell SB (1994) Synergistic interaction between cisplatin and taxol in human ovarian-carcinoma cells in-vitro. Br J Cancer 69:299–306

Kamisli S, Ciftci O, Cetin A, Kaya K, Kamisli O, Celik H (2014) Fish oil protects the peripheral and central nervous systems

against cisplatin-induced neurotoxicity. Nutr Neurosci 17:116–126

Karpińska A, Gromadzka G (2013) Oxidative stress and natural antioxidant mechanisms: the role in neurodegeneration. From molecular mechanisms to therapeutic strategies. Postepy Hig Med Dosw 6 67:43–53

Kusano C, Ferrari B (2008) Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J Cell Mol Biol 7:1–15

Mao-Ying QL, Kavelaars A, Krukowski K, Huo XJ, Zhou W, Price TJ, Cleeland C, Heijnen CJ (2014) The anti-diabetic drug metformin protects against chemotherapy-induced peripheral neuropathy in a mouse model. PLoS One 9:e100701

Marupudi NI, Han JE, Li KW, Renard VM, Tyler BM, Brem H (2007) Paclitaxel: a review of adverse toxicities and novel delivery strategies. Expert Opin Drug Saf 6:609–621

Minami SB, Sha SH, Schacht J (2004) Antioxidant protection in a new animal model of cisplatin-induced ototoxicity. Hear Res 198:137–143

Mohanakumar KP, Muralikrishnan D, Thomas B (2000) Neuroprotection by sodium salicylate against 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine-induced neurotoxicity. Brain Res 864:281–290

Openshaw H, Beamon K, Synold TW, Longmate J, Slatkin NE, Doroshow JH, Forman S, Margolin K, Morgan R, Shibata S, Somlo G (2004) Neurophysiological study of peripheral neuropathy after high-dose Paclitaxel: lack of neuroprotective effect of amifostine. Clin Cancer Res 10:461–467

Pisano C, Pratesi G, Laccabue D, Zunino F, Lo Giudice P, Bellucci A, Pacifici L, Camerini B, Vesci L, Castorina M, Cicuzza S, Tredici G, Marmiroli P, Nicolini G, Galbiati S, Calvani M, Carminati P, Cavaletti G (2003) Paclitaxel and Cisplatin-induced neurotoxicity: a protective role of acetyl-L-carnitine. Clin Cancer Res 9:5756–5767

Ramanathan B, Jan KY, Chen CH, Hour TC, Yu HJ, Pu YS (2005) Resistance to paclitaxel is proportional to cellular total antioxidant capacity. Cancer Res 65:8455–8460

Rick O, Beyer J, Schwella N, Schubart H, Schleicher J, Siegert W (2001) Assessment of amifostine as protection from chemotherapy-induced toxicities after conventional-dose and high-dose chemotherapy in patients with germ cell tumor. Ann Oncol 12:1151–1155

Rispoli V, Rotiroti D, Carelli V, Liberatore F, Scipione L, Marra R, Giorgioni G, Di Stefano A (2004) Choline pivaloyl esters improve in rats cognitive and memory performances impaired by scopolamine treatment or lesions of the nucleus basalis of Meynert. Neurosci Lett 356:199–202

Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant growth and development. J Exp Bot 62:3321–3338

Rovini A, Carré M, Bordet T, Pruss RM, Braguer D (2010) Olesoxime prevents microtubule-targeting drug neurotoxicity: selective preservation of EB comets in differentiated neuronal cells. Biochem Pharmacol 80:884–894

Shabana YM, Abdel-Fattah GM, Ismail AE, Rashad YM (2008) Control of brown spot pathogen of rice (*Bipolaris oryzae*) using some phenolic antioxidants. Braz J Microbiol 39:438–444

Song W, Tang Z, Li M, Lv S, Sun H, Deng M, Liu H, Chen X (2014) Polypeptide-based combination of paclitaxel and cisplatin for enhanced chemotherapy efficacy and reduced side-effects. Acta Biomater 10:1392–1402

Sozio P, D'Aurizio E, Iannitelli A, Cataldi A, Zara S, Cantalamessa F, Nasuti C, Di Stefano A (2010) Ibuprofen and lipoic acid diamides as potential codrugs with neuroprotective activity. Arch Pharm 343:133–142

Togar B, Turkez H, Tatar A, Hacimuftuoglu A, Geyikoglu F (2014) Cytotoxicity and genotoxicity of zingiberene on different neuron cell lines in vitro. Cytotechnology. doi: 10.1007/s10616-014-9729-9

Turkez H, Geyikoglu F (2011) The efficiacy of bismuth subnitrate against genotoxicity and oxidative stress induced by aluminum sulphate. Toxicol Ind Health 27:133–142

Turkez H, Tatar A, Hacimuftuoglu A, Ozdemir E (2010) Boric acid as a protector against paclitaxel genotoxicity. Acta Biochim Pol 57:95–97

Turkez H, Geyikoglu F, Yousef MI, Celik K, Bakir TO (2012a) Ameliorative effect of supplementation with L-glutamine on oxidative stress, DNA damage, cell viability and hepatotoxicity induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat hepatocyte cultures. Cytotechnology 64:687–699

Turkez H, Geyikoglu F, Mokhtar YI, Togar B (2012b) Eicosapentaenoic acid protects against 2,3,7,8-tetrachlorodibenzo-p-dioxin- induced hepatic toxicity in cultured rat hepatocytes. Cytotechnology 64:15–25

Turkez H, Celik K, Toğar B (2014) Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. Cytotechnology 66:597–603

Van den Berg JH, Beijnen JH, Balm AJ, Schellens JH (2006) Future opportunities in preventing cisplatin induced ototoxicity. Cancer Treat Rev 32:390–397

Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47:177–206

Wang Y, Wang M, Qi H, Pan P, Hou T, Li J, He G, Zhang H (2014) Pathway-dependent inhibition of paclitaxel hydroxylation by kinase inhibitors and assessment of drug-drug interaction potentials. Drug Metab Dispos 42:782–795

Xue LJ, Guo W, Yuan Y, Anino EO, Nyamdari B, Wilson MC, Frost CJ, Chen HY, Babst BA, Harding SA, Tsai CJ (2013) Constitutively elevated salicylic acid levels alter photosynthesis and oxidative state but not growth in transgenic populus. Plant Cell 25:2714–2730

Zhang H, Mizumachi T, Carcel-Trullols J, Li L, Naito A, Spencer HJ, Spring PM, Smoller BR, Watson AJ, Margison GP, Higuchi M, Fan CY (2007) Targeting human 8-oxoguanine DNA glycosylase (hOGG1) to mitochondria enhances cisplatin cytotoxicity in hepatoma cells. Carcinogenesis 28:1629–1637