ORIGINAL ARTICLE

Molecular targets of the natural antioxidant pterostilbene: effect on protein kinase C, caspase-3 and apoptosis in human neutrophils *in vitro*

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Submitted: 2010-09-19 Accepted: 2010-11-24 Published online: 2010-12-28

Key words: **pterostilbene; reactive oxygen species; neutrophils; protein kinase C; caspase-3; apoptosis**

Neuroendocrinol Lett 2010; **31**(Suppl.2):84–90 **PMID:** 21187824 NEL31S210A14

Abstract

OBJECTIVE: Pterostilbene, a naturally occurring phenolic derivative, exhibits various pharmacological effects, e.g. anti-cancerous, antioxidant, anti-inflammatory and anti-diabetic. Based on our previous study, we assessed the cellular and molecular effects of pterostilbene on human neutrophils and in cell free systems. Experimental and theoretical molecular descriptors of stilbene derivatives were also determined.

METHODS: We assessed the antioxidant properties of pterostilbene using cell free system and computational methods. The effect of pterostilbene on protein kinase C activation/phosphorylation was detected by special anti-phospho protein kinase C antibodies. Membrane associated changes determining the life span of neutrophils and human recombinant caspase-3 assay were examined.

RESULTS: Pterostilbene possessed comparable antioxidant properties as resveratrol in cell free system. Computational methods were used to establish the molecular characteristics of stilbene derivatives. The values of electronic parameters suggest a slight enhancement of electron donor properties of pterostilbene compared to resveratrol. Phosphorylation and thus activation of protein kinase C alpha/beta II in activated neutrophils was not decreased by pterostilbene. Pterostilbene in concentrations of 10–100 μM was found to inhibit the activity of human caspase-3 purified enzyme and did not influence cell viability significantly.

CONCLUSION: Pterostilbene, an analog of resveratrol, was identified as a good natural antioxidant compound. However, reducing the oxidative burst of human neutrophils during their activation *in vitro* with pterostilbene does not include protein kinase C phosphorylation pathway. Pterostilbene showed dose dependent activation/inhibition of caspase-3 enzyme activity.

INTRODUCTION

Knowledge that consumption of red wine leads to lower cardiovascular risk opened the scientific scene for resveratrol from stilbene type polyphenols (Fauconneau *et al.* 1997; Šmidrkal *et al.* 2001). Chemical similarity in the stilbene group made us switch from resveratrol (trans-3,4´,5–trihydroxy-stilbene) to pterostilbene, i.e. trans-3,5-dimethoxy-4´-hydroxy-stilbene (Figure 1.A). Both chemical entities exist in cis/trans conformation, but naturally they occur mostly in the trans form, which is also more effective as to antioxidant properties than the cis form (Šmidrkal *et al.* 2001).

Pterostilbene is of natural origin, contained in leaves and grapes of Vitis vinifera, berries of Vaccinium spp., Pterocarpus marsupianum, etc. (Grover *et al.* 2005; Paul *et al.* 1999). Pterostilbene was reported to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and to inhibit lipid peroxidation in rat liver microsomes and there are reports about growth inhibition, antiproliferative effects and induction of apoptosis by cell cycle arrest induced by derivatives of stilbene in a number of normal and cancer cell lines (Pan *et al.* 2008; Rimando and Suh 2008; Stivala *et al.* 2001). Pterostilbene is assumed to possess anti-inflammatory, antioxidant, anti-diabetic, antifungal and anti-cancerous effects (Remsberg *et al.* 2008; Roupe *et al.* 2006).

The physiological activity of human neutrophils plays an important role in fighting intruding microorganisms or foreign particles (Bissonnette *et al.* 2008). Neutrophils may be called "first line defense system". In the process of oxidative burst, neutrophils by using the enzyme NADPH-oxidase produce a variety of cytotoxic products, e.g. superoxide, hydrogen peroxide and hypochloric acid (El-Benna *et al.* 2008). All three substances are known as reactive oxygen species (ROS) and are effective in killing microorganisms. The whole process of NADPH-oxidase activation is strictly controlled by various regulating/phosphorylating enzymes, such as p21-activated kinase, phosphatidyl inositol 3-kinase and nuclear factor κB (El-Benna *et al.* 1996; Myhre *et al.* 2009; Sheppard *et al.* 2001). A key enzyme in NADPH-oxidase activation is protein kinase C (PKC) (Dekker *et al.* 2000). Increased production of ROS during inflammation could be harmful to surrounding tissues. This has been observed in chronic inflammatory diseases, e.g. rheumatoid arthritis (Cross *et al.* 2006). The importance of the regulation of activated human neutrophils is the subject of our study. Activated neutrophils represent a good model for testing inflammation.

The present study is based on our previous research on the generation of ROS by activated neutrophils by using chemiluminescence assay (Perečko *et al.* 2008). To evaluate the antioxidant capacity of pterostilbene, we used oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) assays (Číž *et al.* 2010). Moreover, we examined the effect of pterostilbene on the life-span of isolated human neutrophils. Delayed apoptosis of neutrophils may play a role in the injury of surrounding tissue in chronic inflammatory diseases, e.g. rheumatoid arthritis (Wong *et al.* 2009). Finally, we investigated the effect of pterostilbene on phosphorylation of PKC of neutrophils pre-activated with phorbol-myristate-acetate, which activates cells via PKC (Klink *et al.* 2009). For better understanding the biological effects of pterostilbene, its molecular descriptors were estimated.

MATERIALS AND METHODS

Chemicals

Pterostilbene and resveratrol were prepared by targeted regioselective synthesis, both compounds purely as trans isomers (Šmidrkal *et al.* 2010). Phorbol-myristate-acetate (PMA) was purchased from Sigma (Steinheim, Germany). Polyclonal antibody against phosphorylated-PKC-alpha/betaII (Thr638/641) was purchased from Cell Signaling (Danvers, MA, USA), secondary anti-rabbit antibody and Lumigen Detection Reagent were supplied by GE Healthcare Life Sciences (former Amersham), Little Chalfont, UK. Human AnnexinV/

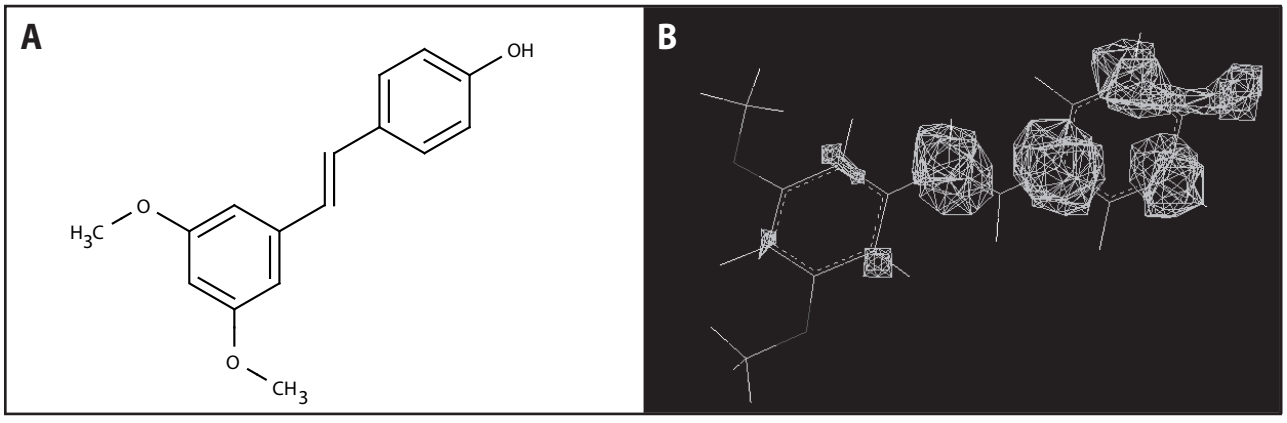

Fig. 1. Pterostilbene. (**A**) Structure of pterostilbene. (**B**) Spin density isosurface map with a contour value of 0.002 of the optimal conformer of pterostilbene in the form of radical derived from the only position (ΔH = 37.2 kcal/mol).

FITC Kit was purchased from Bender MedSystems, Vienna, Austria. Caspase-Glo 3/7 Assay was from Promega (Madison, WI, USA), human purified caspase-3 was from Enzo Life Sciences, Lausen, Switzerland. All other products are available commercially or their origin is mentioned in the text.

Computational methods for pterostilbene and resveratrol

The lowest energy molecular conformations of the compounds tested were calculated using Conformational Search module in HyperChem molecular modeling software (Račková *et al.* 2005) using Austin model 1, and Polak-Ribiere conjugate gradient algorithm with 0.01 convergence limit in vacuum. For optimal conformers of antioxidants, the heat of formation was calculated. The ΔH values were calculated as described previously (Račková *et al.* 2005). Further calculated parameters of the compounds tested included energy values of the highest occupied molecular orbital (HOMO), energy of the lowest unoccupied molecular orbital (LUMO), HOMO–LUMO energy gap (Δε) and spin density (S_D) belonging to the oxygen radical derived from OH groups, which characterizes the distribution of the electron spin and therefore decides the stability of radicals (Cao *et al.* 2003).

Determination of R_m values

The lipophilicity parameters represented by R_m values were measured by reversed-phase thin layer chromatography. The mobile phase consisted of diluted acetic acid (pH 2.5) mixture with acetonitril (20:80, v:v) (Király-Véghely *et al.* 2004). The stationary phase was obtained by impregnation of the layer of Silica gel G F254 plates with 5% solution of liquid paraffin in ether. The compounds were dissolved in methanol and about 1 μg of the compound was spotted onto the plates. A migration of 10 cm was obtained by spotting the compound on a line 2 cm from the lower edge of the plate. The developed plates were dried and the compounds were detected in UV light at 254 nm. The R_m values were calculated by the formula: $R_m=\log(1/R_F-1)$.

Antioxidant assays

The protective effects of pterostilbene and resveratrol (0.01–100 μM) were measured by comparing the area under curve of the sample to that of a known antioxidant, trolox for ORAC assay and gallic acid for HORAC assay. The loss of fluorescein fluorescence indicated the formation of peroxyl radicals (ORAC) or hydroxyl radicals (HORAC) in the sample. Production of peroxyl radicals was induced by 2, 2´-azobis-(2-amidino-propane)-dihydrochloride (AAPH) at 37 °C. The HORAC assay reveals the metal-chelating activity of polyphenols, and thus the protective action against hydroxyl radical formation. Complex Co (II) was used as an initiator. High ORAC and HORAC values indicated that the sample tested possessed a high potency of antioxidant activity (Číž *et al.* 2010). The values of ORAC are expressed as relative trolox equivalent (TE), HORAC as relative gallic acid equivalent (GAE). Results are expressed as means of triplicates in three independent measurements.

PKC blotting

Human neutrophils were isolated as described previously (Jančinová *et al.* 2009). Neutrophil suspension (100 μl) containing 5×10^6 cells was preincubated at 37°C for 60 seconds with different concentrations of pterostilbene (10 or 100 μM) prior to addition of PMA (0.15 μM). Incubation with PMA (60 s) was stopped by using a lysing medium containing protease and phosphatase inhibitors (Na_3VO_4 and NaF). The suspension was sonicated at 4°C for 20 minutes and centrifuged at 18 625×g at 4°C for 5 min. Finally, the supernatant was taken for blotting assay. Total protein was measured using Bradford Dye Reagent detection kit from Bio-Rad.

For the assay, the supernatant was boiled in sample buffer (0.05 M Tris, 2% SDS, 2.5% mercaptoethanol) containing 0.01% bromphenol-blue. The samples (20 μl with 20 μg of protein fraction) were loaded on 10% polyacrylamide gel. Separated proteins were transferred onto a polyvinylidene difluoride membrane (Millipore). The blot containing the transferred proteins was blocked in 1% BSA (Sigma-Aldrich) buffer followed by incubation with primary anti-phospho-PKC-alpha/beta II (Thr638/641) antibody (1:8 000) and secondary anti-rabbit antibody (1:10 000). After washing in TBS, the proteins were detected with Lumigen Detection-Reagent kit, scanned and measured densitometrically using free ImageJ program.

Analysis of apoptosis

Citrated whole human blood was collected from healthy male volunteers. Dextrane (3%) was added (blood 2:1 dextrane) and centrifugated at 10×g at room temperature. One ml of buffy coat containing mainly leukocytes was collected and stored on ice before use. The cells were counted on hemocytometer (Coulter Counter), focusing on granulocytes. The cell suspension was then dissolved to reach 200 000 neutrophils per sample. Three different concentrations of pterostilbene (1, 10 and 100 μM) and a control sample were incubated at 37°C for 10 min. The cells were stained with Annexin-V conjugated with FITC (fluorescein isothiocyanate) (BenderMedSystems) in dark at 4°C for 10 min, followed by staining with propidium iodide (1μg/ml) and analyzed immediately by Beckman Coulter Cytomics FC500 cytometer. All samples were analyzed under the same conditions (gains, volts). From the granulocytic area, 5 000 cells were gated and analyzed.

Caspase-3 activity

Following caspase cleavage of the Z-DEVD-amino-luciferin substrate, resulting in the reaction of luciferase with amino-luciferin, the measurement of light production was made on the Luminometer Immunotech

Tab. 1. Electronic and physicochemical characteristics of pterostilbene and resveratrol.

	Pterostilbene	Resveratrol
ΔH (kcal/mol)	37.17	37.39
εHOMO (eV)	–8.45	–8.53
εLUMO (eV)	–0.417	–0.517
Δe (eV)	8.033	8.013
R_m	1.45	0.21

ΔH – parameter of 4´-OH bond strength (corresponding to minimum value of DH with regard to values of 3 and 5 –OH bonds in resveratrol), εHOMO – energy of highest occupied molecular orbital, εLUMO – energy of lowest unoccupied molecular orbital, Δe - energy difference between the HOMO and LUMO, R_m – lipophilicity index.

Tab. 2. Antioxidant profile of pterostilbene and resveratrol.

Pterostilbene (μM)	ORAC μM TE	HORAC μM GAE
100	14.75 ± 2.58	85.48 ± 15.65
10	5.45 ± 2.16	20.58 ± 1.30
1	0.94 ± 0.62	n.d.
Resveratrol (μM)	**ORAC μM TE**	**HORAC μM GAE**
100	17.98 ± 0.36	113.92 ± 23.18
10	10.71 ± 2.63	88.37 ± 13.66
1	2.84 ± 1.36	2.41 ± 1.50

ORAC (peroxyl radical quenching) is measured as trolox equivalent (TE), HORAC (hydroxyl radical quenching) as gallic acid equivalent (GAE). Results are expressed as Mean±SEM of triplicates in three independent measurements. n.d. for not detectable.

LM-01T. According to the manufacturers´ instructions, 10 μl of 0.1 IU caspase was added to 20 μl aliquots of pterostilbene and buffer solution, finally 50 μl of Caspase-Glo 3/7 Reagent was added, the mixture was measured for 60 minutes and the activity of caspase-3 (EC 3.4.22.56) was identified. The vehiculum solution for pterostilbene i.e. competent concentrations of NaOH, were also tested.

Statistics

Data are expressed as mean±SEM, unless stated otherwise. Statistical analysis was performed using Student´s *t*-test to examine differences between treatments and control. Differences were considered to be statistically significant when $p<0.05$ (*) or $p<0.01$ (**).

RESULTS

Computer assisted evaluation of pterostilbene and resveratrol properties and determination of R_m

With regard to its relation to the potential of radical scavenging effect, we computed the parameter energy of HOMO for pterostilbene and resveratrol. The εHOMO expresses the easiness of abstraction of an electron from the neutral molecule, which may be involved in the mechanism of reaction with free radicals (Cao *et al.* 2003; Velkov *et al.* 2007). The value of εHOMO for pterostilbene was –8.45 eV. Compared to resveratrol, a typical representative of natural stilbene derivatives (εHOMO = –8.53 eV), pterostilbene is more effective in electron-donating processes. Furthermore, the value of parameter of 4´–OH bond strength, ΔH (Korzekwa *et al.* 1990), was slightly lower for pterostilbene than for resveratrol molecule (37.17 kcal/mol vs 37.39 kcal/mol, respectively) (Table 1).

However, the values of HOMO-LUMO energy difference (a characteristic of the molecule excitability) for both molecules differed just by 0.02 eV (8.033 eV for pterostilbene vs 8.013 eV for resveratrol), indicating similar chemical reactivity of both compounds (Table 1).

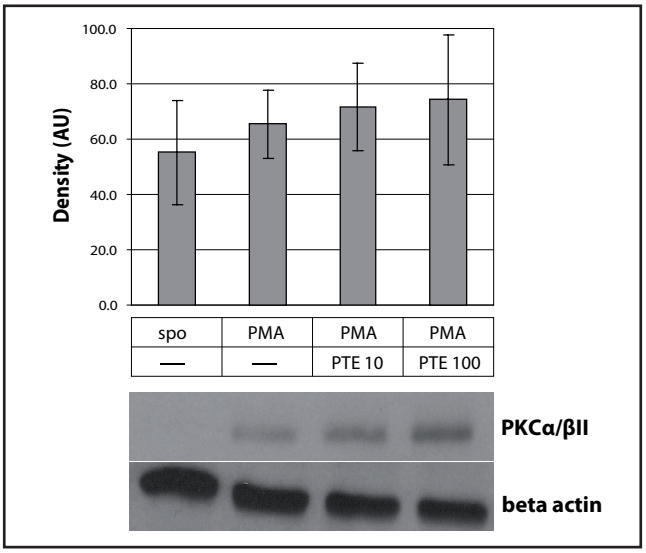

Fig. 2. Effect of pterostilbene on PKC phosphorylation. Densitometric evaluation of protein kinase C phosphorylation from control, PMA (0.15 μM) and pterostilbene (10 and 100 μM) treated isolated human neutrophils. (Mean±SEM, n=4) (spo – control, PTE – pterostilbene, PMA – phorbol-myristate-acetate, AU – Arbitrary Units).

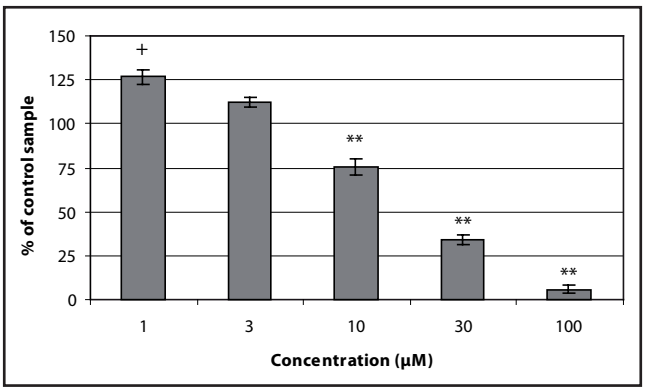

Fig. 3. Effect of pterostilbene on human recombinant caspase-3 activity. Effect of different concentrations of pterostilbene (1–100 μM) on human recombinant caspase-3 activity shown as percentage of control sample. (Mean±SEM, n=5). +$p<0.05$ for activation, *$p<0.05$ and ** $p<0.01$ for inhibition (versus vehiculum).

The spin density isosurface map shows the efficient delocalization of the radical resulting from the only 4´-OH group through the extended conjugation system of the stilbenoid structure (Figure 1.B). Correspondingly spin density (S_D) belonging to the oxygen radical derived from 4`-OH groups showed the same values for the molecules of both resveratrol and pterostilbene (0.101).

Due to substitution by two methyl-groups, pterostilbene showed the higher value of lipophilicity parameter (R_m = 1.45) than resveratrol (R_m = 0.21) (Table 1).

Antioxidant activity assays

The ORAC assay provides data to measure the quenching of peroxyl radicals expressed as trolox equivalent. HORAC measures quenching of hydroxyl radicals (due to metal-chelating activity) expressed as gallic acid equivalent. Results on pterostilbene and resveratrol antioxidant properties are expressed in Table 2. The antioxidant capacity of 10 μM pterostilbene is equal to that of 5.45 μM trolox and 20.58 μM gallic acid standards. Pterostilbene of 100 μM concentration possessed less antioxidant capacity in comparisson with trolox (ORAC), but using HORAC method pterostilbene was as equal as gallic acid standard (85.48 μM). However, resveratrol in concentration of 1, 10 and 100 μM is better antioxidant than gallic acid (HORAC), using ORAC, the antioxidant effect diminished.

PKC response to incubation with pterostilbene

To get further insight into molecular mechanisms underlying inhibition of ROS generation in whole human blood and isolated neutrophils by pterostilbene, we examined the effect of pterostilbene on the phosphorylation of PKC α/β II. No significant changes in PKC α/β II (Thr638/641) phosphorylation after PMA stimulus were achieved by pterostilbene in either concentration (10 and 100 μM) (Figure 2).

Effect of pterostilbene on life-span of human neutrophils

The percentage of viable and apoptotic cells was calculated by flow cytometer measurement after staining the cells with FITC conjugated Annexin-V plus propidium iodide (PI). Only Annexin positive cells were considered for apoptotic (also mentioned as pre-apoptotic) cells and double positive cells (Annexin+/PI+) were considered for late-apoptotic or dead cells. Pterostilbene in any concentration tested (1, 10 and 100 μM) did not change neutrophil viability significantly (data not shown).

The role of pterostilbene on caspase-3 activity

Figure 3 shows biphasic concentration-dependent effect of pterostilbene on the human recombinant caspase-3 activity: in the concentration of 1 μM, pterostilbene induces the activity of caspase-3 significantly, in concentrations of 10, 30 and 100 μM, pterostilbene inhibits the activity of the enzyme. The effect of appropriate vehiculum concentrations (4–400 μM NaOH) did not significantly influence the activity of caspase-3.

DISCUSSION

The antioxidant properties of pterostilbene have, over the years, attracted the interest of many researcher groups however the effect of pterostilbene on human neutrophils has not yet been fully investigated. Pterostilbene is a dimethylether analogue of the well-known stilbene derivative resveratrol. These compounds are mainly synthetized in plants after stress stimuli (e.g. irradiation, microorganism infection) (Keller *et al.* 2003) and act as phytoalexins (Breuil *et al.* 1999). But there are few reports on pterostilbene effects on human cells. Pterostilbene has been widely discussed because of its anti-cancer and chemopreventive properties in different *in vivo* and *in vitro* cancer models and cultured cell lines (Cichocki *et al.* 2008; Rimando and Suh 2008; Tolomeo *et al.* 2005). Pterostilbene exhibits also immunomodulatory (Šmidrkal *et al.* 2010) and anti-inflammatory effects (Cichocki *et al.* 2008). Based on this information and our previous studies, we investigated antioxidant effects of pterostilbene in cell free systems and focused also on the effects of pterostilbene on activated human neutrophils *in vitro*. Electronic parameters for the minimum energy conformers of pterostilbene in comparison to resveratrol were calculated. Lipophilicity parameters for the compounds tested were evaluated as well.

The polyphenolic structure of stilbenes, and thus of pterostilbene, justifies its antioxidant testing. Pterostilbene has been suggested to be a good antioxidant in different cell free system methods, e.g. based on formation of DPPH. In the study of Stivala *et al.* (2001) pterostilbene gave a value comparable to trans-resveratrol in antioxidant testing, using DPPH assay. Our experiments with the ORAC and HORAC method describe the antioxidant effects against peroxyl (ORAC) and hydroxyl (HORAC) radicals in cell free system. In the concentrations of 1, 10 and 100 μM, pterostilbene was less effective in comparison to trolox standard. Remsberg *et al.* (2008) showed better prevention of oxidation in the lower concentration of pterostilbene. This may be due to the pro-oxidant activity in higher concentrations that is in agreement with its higher value of εLUMO (–0.417 eV) compared to resveratrol (–0.517 eV) (Table 1). Our study suggests that substitution of both 3 and 5 hydroxy-groups with methoxy-groups does not decrease the antioxidant properties of pterostilbene in cell free system dramatically (compared to resveratrol), though resveratrol is showing better antioxidant properties in HORAC assay (Table 2). The 4´-hydroxy group in pterostilbene is able to assure sufficient antioxidant activity. However 4´-methoxy derivative was less effective in the study of Stivala *et al.* (2001). We may hypothesize that the 4´-hydroxy group may play a crucial role in antioxidant effects.

For better understanding of the antioxidant effects of pterostilbene, we calculated the molecule parameters for the minimum energy conformers of this molecule

in comparison to the well-known stilbene representative resveratrol. The structure of pterostilbene (Figure 1.A) enables only one proton subtraction in 4´-position, thus forming a phenoxy-radical. The distribution of spin density can have a decisive effect on the stability of the phenoxyl radicals derived from stilbenoid structure (Cao *et al.* 2003). Comparably to resveratrol, the abstraction of proton from OH in para-position to the double-bond of ethene group allows efficient electron delocalization of the generated radical through the pterostilbene molecule, as shown in the spin density map (Figure 1.B). Moreover, the spin densities (S_D) belonging to the oxygen radical derived from 4´-OH group for both stilbene analogues showed the same value. Thus, the methylation of the free OH groups at meta-position of ring A in pterostilbene can not significantly reduce the scavenging activity compared to resveratrol. In general, both pterostilbene and resveratrol showed comparable values of parameters εHOMO, ΔH and Δε (HOMO-LUMO energy gap), suggesting similar free radical scavenging properties of both stilbene derivatives. This is in accordance with the results of Stivala *et al.* (2001), showing comparable DPPH radical scavenging activity of both compounds in a chemical system.

As discussed in the two paragraphs above, the physicochemical characteristics of both derivatives tested are conformable, but according to our results with chemiluminescence assay (Perečko *et al.* 2008), the stilbenes tested vary in biological medium. Substitution of two hydroxyl groups with methoxy-groups increased lipophilicity parameter R_m of resveratrol from 0.21 to 1.45 for pterostilbene. This may enhance the bioavailability of pterostilbene in contrast to the low bioavailability of resveratrol (Cichocki *et al.* 2008). On the other side lipophilicity may influence the transition of substances through cell membrane into cytosol. Also the number and position of hydroxyl groups play a role in the antioxidant effects of polyphenols in different cell assays. Taking into consideration the effects of different resveratrol derivatives on the production of thiobarbituric acid reactive substances (TBARS) in normal human fibroblasts (Stivala *et al.* 2001), pterostilbene was as good as resveratrol. Its 4´-methoxy derivative as well as 3, 4´, 5-trimethoxystilbene did not exert a significant inhibition of TBARS production (Stivala *et al.* 2001). These results support our findings with chemiluminescence assay in whole blood. The 3, 5-methoxy groups increased the antioxidant properties of pterostilbene compared to resveratrol in whole human blood. However, inhibition of extracellular and intracellular chemiluminescence with pterostilbene was lower than with resveratrol and the difference was more significant for intracellular compartment (Perečko *et al.* 2008). In agreement with the biological activity-lipophilicity parabolic findings (Hansch and Clayton 1973) during transportation through the membrane system, the entrapment of more hydrophobic pterostilbene in the lipid phase of outer membrane could diminish its inhibitory effect on intracellular ROS production. Thus, free hydroxy-groups of resveratrol may be appropriate for antioxidant activity in respect of its intracellular availability directed by lipophilicity parameter.

In the attempt to elucidate the molecular mechanism of reducing the production of reactive oxygen species, we examined the effect of pterostilbene on activation/phosphorylation of PKC. Phosphorylation of PKC is an activation marker and antecedent step of phosphorylation of NADPH-oxidase subunits. Pterostilbene is known to affect e.g. the p38-phosphorylation pathway in breast cancer cell line (Pan *et al.* 2009), but the effect on human neutrophil PKC phosphorylation has not been established. In our study, pterostilbene did not change the phosphorylation of PKC α/β II of isolated human neutrophils activated by PMA, which is known to activate cells just via activation of PKC (Klink *et al.* 2009). We are suggesting that other mechanisms in reducing of ROS production may be involved, e. g. inhibition of NADPH-oxidase.

The *in vitro* Annexin-V/propidium iodide staining assay revealed no significant effects of pterostilbene in concentrations tested on human neutrophil cell death. This is in agreement with other studies (Stivala *et al.* 2001). Remsberg *et al.* (2008) showed apoptotic effect of pterostilbene on different cancer cell lines, suggested also by other studies (Tolomeo *et al.* 2005), yet pointing out that pterostilbene, and thus other stilbene derivatives, was not cytotoxic against e.g. normal human umbilical vein epithelial cells. Generally, pterostilbene, comparable to resveratrol, was found to have antiproliferative, cytostatic activity on different cell lines, but it did not exhibit cytotoxic activity in the concentrations tested (Stivala *et al.* 2001). This was acknowledged by our study using ATP-cytotoxicity test (Perečko *et al.* 2008). According to caspase-3 activity assay, Pan *et al.* (2007) showed activation of caspase-2,-3,-8 and -9 by pterostilbene in human gastric carcinoma AGC cells. In our experiment, pterostilbene in concentration of 1 μM showed significant increase of activity of caspase-3, while in concentrations of 10, 30 and 100 μM it inhibited caspase-3 activity. This result is in agreement with no apoptotic changes caused by pterostilbene in the concentrations tested on human neutrophil viability studied by flow cytometry. Nevertheless caspase transduction is not the only apoptotic signaling pathway.

Taking into account the above presented results and discussion, pterostilbene represents a derivative with significant antioxidant effect. The structure-activity relationship analysis may be used to advantage in rational design and synthesis of derivatives with desired antioxidant, anti-inflammatory and anti-proliferative properties.

ACKNOWLEDGEMENTS

This study was supported by grants VEGA 2/0003/10, APVV-0315-07, GAČR 203/07/1227, OC08058 (MEYS, CR) and APVV-SK-CZ-0034-09. Authors are thankful to Prof. Magda Kouřilová for language corrections.

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