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Inhibition of ozone-induced SP-A oxidation by plant polyphenols

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Abstract

Surfactant protein-A (SP-A) is the best studied and most abundant of the protein components of lung surfactant and plays an important role in host defense of the lung. It has been shown that ozone-induced oxidation of SP-A protein changes its functional and biochemical properties. In the present study, eight plant polyphenols (three flavonoids, three hydroxycinnamic acids, and two hydroxybenzoic acids) known as strong antioxidants, were tested for their ability to inhibit ozone-induced SP-A oxidation as a mechanism for chemoprevention against lung damage. SP-A isolated from alveolar proteinosis patients was exposed to ozone (1 ppm) for 4 h. The flavonoids protected SP-A from oxidation in a dose dependent manner. (−)-Epicatechin was the most potent flavonoid and exhibited inhibition of ozone-induced formation of carbonyls by 35% at a concentration as low as 5 μM. Hydroxybenzoic acids inhibited SP-A oxidation in a dose-dependent manner although they were less potent than flavonoids. On the other hand, hydroxycinnamic acids exhibited a different inhibitory pattern. Inhibition was observed only at medium concentrations. The results indicate that inhibition of SP-A oxidation by plant polyphenols may be a mechanism accounting for the protective activity of natural antioxidants against the effects of ozone exposure on lungs.

Keywords: *SP-A, ozone, lungs, plant polyphenols, protein oxidation*

Abbreviations: *AP, alveolar proteinosis; BAL, bronchoalveolar lavage; Cys, cysteine; DNP, 2,4-dinitriphenylhydrazone; DNPH, 2,4-dinitriphenylhydrazine; His, histidine; HRP, horseradish peroxidase; LDL, low density lipoprotein; LPS, lipopolysaccharide; Met, methionine; NF-κB, nuclear factor κB; PC, phosphatidylcholine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SP-A, surfactant protein; TNF-α, tumor necrosis factor α; Trp, tryptophane; Tyr, tyrosine*

Introduction

Pulmonary surfactant is a complex and highly regulated mixture of lipids and proteins. A key function of surfactant is to lower surface tension at the air-liquid interface and prevent alveolar collapse at low lung volumes in order to assure continuation of optimal O_2/CO_2 exchange as well as the regulation of pulmonary host defense [1]. The most abundant and extensively characterized of its protein components

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is surfactant protein A (SP-A), that is secreted by alveolar type II cells and by cells of the tracheal and bronchial submucosal glands and in some species by nonciliated bronchiolar epithelial cells [2–5]. SP-A plays an important role in surfactant physiology including surfactant structure and metabolism, as well as in host defense and the modulation of inflammatory and anti-inflammatory processes in the lung [6–8].

Ozone (O_3) is a major component of photochemical air pollution or smog. It is produced by the sunlight-catalyzed reaction between nitrogen oxides and volatile organic compounds (Figure 1) [9]. It has been estimated that in the USA nearly 113 million people live in areas that have ozone levels above the National Ambient Air Quality Standards daily exposure limit of 0.08 ppm for an 8 h period [10]. Exposure to ozone has been associated with impaired lung function [11–12], as ozone is a strong oxidizing agent and is rapidly converted to other reactive oxidant species which are capable of oxidizing biological macromolecules such as lipids and proteins [13–14]. A number of studies have shown that oxidation of SP-A by ozone impairs many aspects of SP-A function including decreased ability to interact with alveolar macrophages [15], to stimulate cytokine production by THP-1 cells [16–17] to inhibit phosphatidylcholine (PC) and surfactant secretion by type II cells [18] and to stimulate TNF-α production by THP-1 cells in the presence of bleomycin [19]. In general, it has been found that SP-A was one of the most frequently oxidized lung proteins in children with chronic pulmonary diseases [20] as well as in patients with cystic fibrosis [21].

It has been shown that supplementation of antioxidant compounds present in plant foods, such as carotenoids, protected from the detrimental effects of ozone on lung function [22–23]. Another major class of dietary antioxidants exhibiting beneficial health effects on human health is the plant polyphenols. Over the last several years a number of *in vivo* and *in vitro* studies have shown that, due to their strong antioxidant properties

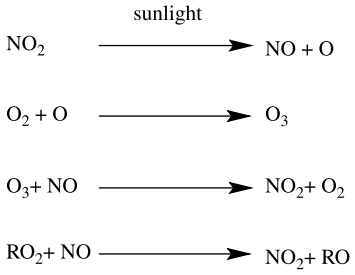

Figure 1. (i) Photodecomposition of NO_2 by sunlight results in the formation of NO and O; (ii) reaction of atomic oxygen with molecular oxygen produces ozone; (iii) then ozone reacts spontaneously with NO and regenerates NO_2 and also produces O_2; (iv) organic compounds such as alkoperoxyls (RO_2) react with NO resulting in an increase of NO_2/NO ratio and O_3 concentration.

[24], polyphenols may prevent diseases associated with oxidative stress, such as cancer [25–26], cardiovascular [27], and neurodegenerative diseases [28]. In addition, it has been reported that polyphenols modulate the activity of proteins [29] and enzymes [30–31] by forming complexes with them. In the present study, we investigated the effects of eight common plant polyphenols on ozone-induced SP-A oxidation. These polyphenols were three flavonoids (rutin hydrate, (+)-catechin, and (−)-epicatechin), three hydroxycinnamic acids (caffeic acid, ferulic acid, and *p*-coumaric acid) and two hydroxybenzoic acids (gallic acid, and protocatechuic acid) (Figure 2). A potential inhibitory activity of the tested compounds against ozone-induced oxidation of SP-A may indicate a protective activity of dietary antioxidant products from air-pollutants.

Materials and methods

Chemicals and reagents

The plant polyphenols, caffeic acid, ferulic acid, *p*-coumaric acid, (+)-catechin, (−)-epicatechin, rutin hydrate, gallic acid, and protocatechuic acid were purchased from Sigma (St Louis, MO, USA). All other reagents used were of analytical grade.

Preparation of native human SP-A

The native human SP-A was purified from BAL fluid obtained from six alveolar proteinosis patients (AP1-AP6) using a butanol-extraction method as described [32] with slight modifications. In brief, after extraction with butanol, the pellet was completely dried with a flux of nitrogen gas and then homogenized twice in the buffer (20 mM *n*-octyl β-D-glucopyranoside, 10 mM Hepes, 150 mM NaCl, pH 7.4). After pelleting of the sample, insoluble protein was dissolved in 5 mM Tris/HCl, pH 7.4 and dialyzed for 48 h against the same buffer. The dialyzed solution was centrifuged at 155,000*g* at 4°C for 30 min, and the supernatant containing SP-A was collected, aliquoted and kept at − 80°C. The SP-A samples were checked routinely and there was not any auto-oxidation during the storage period. All procedures were performed at 4°C or on ice. Protein concentration was determined using the Bio-Rad Microassay kit (Bio-Rad, CA, USA). Moreover, protein samples were subjected to nonreducing SDS-PAGE electrophoresis in order to test the degree of SP-A purification. In particular, the SP-A samples were mixed with a loading buffer containing 2% w/v SDS, 0.1 M Tris/HCl (pH 6.8), and 10% glycerol and heated at 95°C for 10 min and then electrophoresis was made on 4–15% acrylamide gradient gels at 90 V for 1 h followed by silver staining (Figure 3(B)).

Furthermore, the oxidation status of the SP-A samples from proteinosis patients was tested using the Oxyblot Oxidized Protein Detection Kit (Intergen, NY, USA). This kit uses a method similar to that

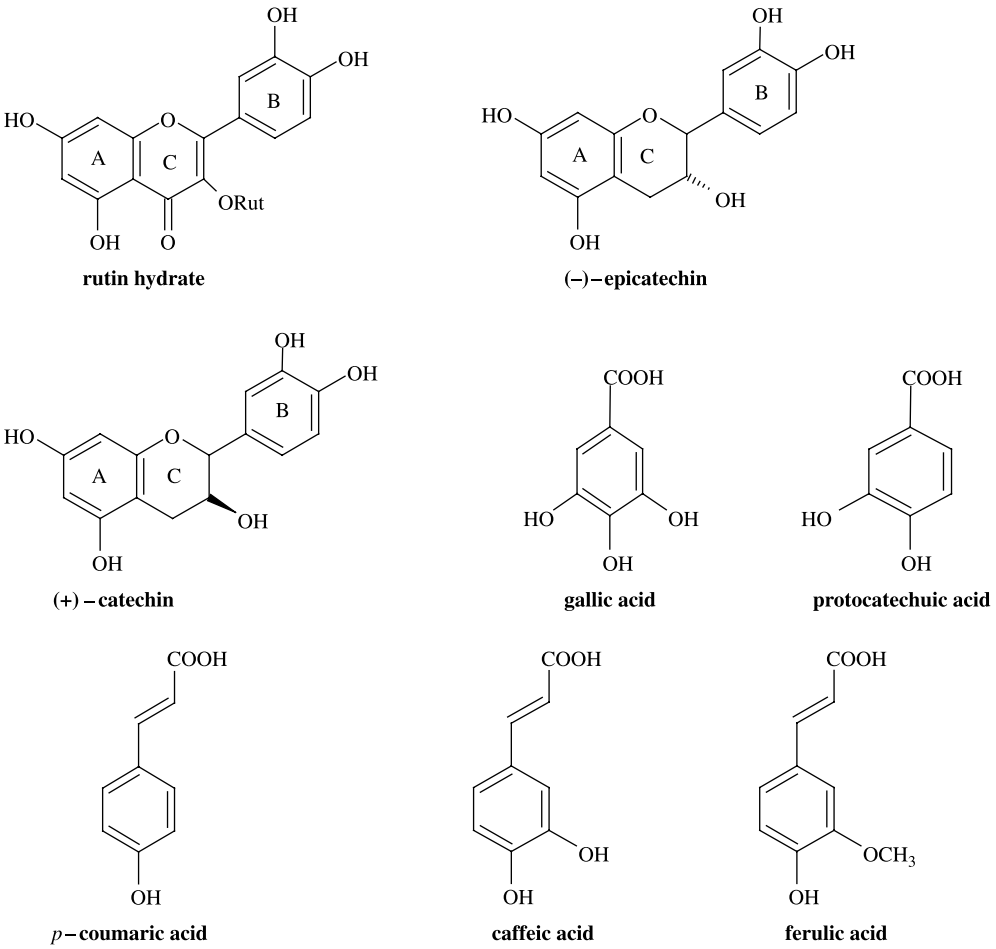

Figure 2. Chemical structure of polyphenols under study.

described by Levine and colleagues [33] and can detect carbonyl groups that have been introduced into proteins through oxidation. Briefly, 5 μl of protein were denatured by adding an equal volume of 12% SDS. Samples were then derivatized with 5 μl of 0.5 × 2,4-dinitriphenylhydrazine (DNPH) solution and incubated for 10 min at room temperature. Derivatization was stopped by the addition of 7 μl of neutralizing solution and then the samples were analyzed by dot blot. For protein dot blots, aliquots containing 250 ng of DNPH-derivatized proteins were brought up to a volume of 250 μl with 0.1 M phosphate buffered saline (pH 7.5), and 100 ng of protein were blotted onto nitrocellulose membrane in duplicate using a 96-well dot-blot apparatus (Bio-Rad, CA, USA). Immunodetection of oxidized proteins was performed according to the instructions included in the Oxyblot kit, and used the rabbit anti-DNP (1:600) and goat anti-rabbit IgG (HRP-congugated) (1:1200) antibodies supplied by the vendor. Blots were exposed to XAR film (Eastman Kodak, NY, USA) after detection by enhanced chemiluminescence. Afterwards, the oxidation of proteins was expressed as the product of the optical density of dots times their area using the Quantity One software (Bio-Rad, CA, USA). The corresponding dot blot and densitometric values are shown in Figure 3(A). SP-A proteins from proteinosis patients are usually more oxidized than those from normal subjects and thus only the samples with low oxidation (i.e. AP1, AP2, and AP4) were used after pooling for the present experiments (Figure 3(A)).

Exposure of SP-A to ozone and detection of protein oxidation

The system for *in vitro* ozone exposure was described previously [34] and has been used in several of our previous studies regarding the effects of ozone exposure on proteins (especially SP-A) and cells [16–19,35]. This system delivers precisely controlled flow rates of gases (filtered air with 5% carbon dioxide saturated with water vapor at 37°C) to the exposure vessels with precise ozone concentrations. A dose-respone curve was designed in order to select the appropriate SP-A concentration for use in the experiments (Figure 4). Namely, 25, 50, 100, and 200 μg/ml of SP-A were exposed to ozone (1 ppm) for 4 h. In the presence of filtered air, SP-A oxidation was

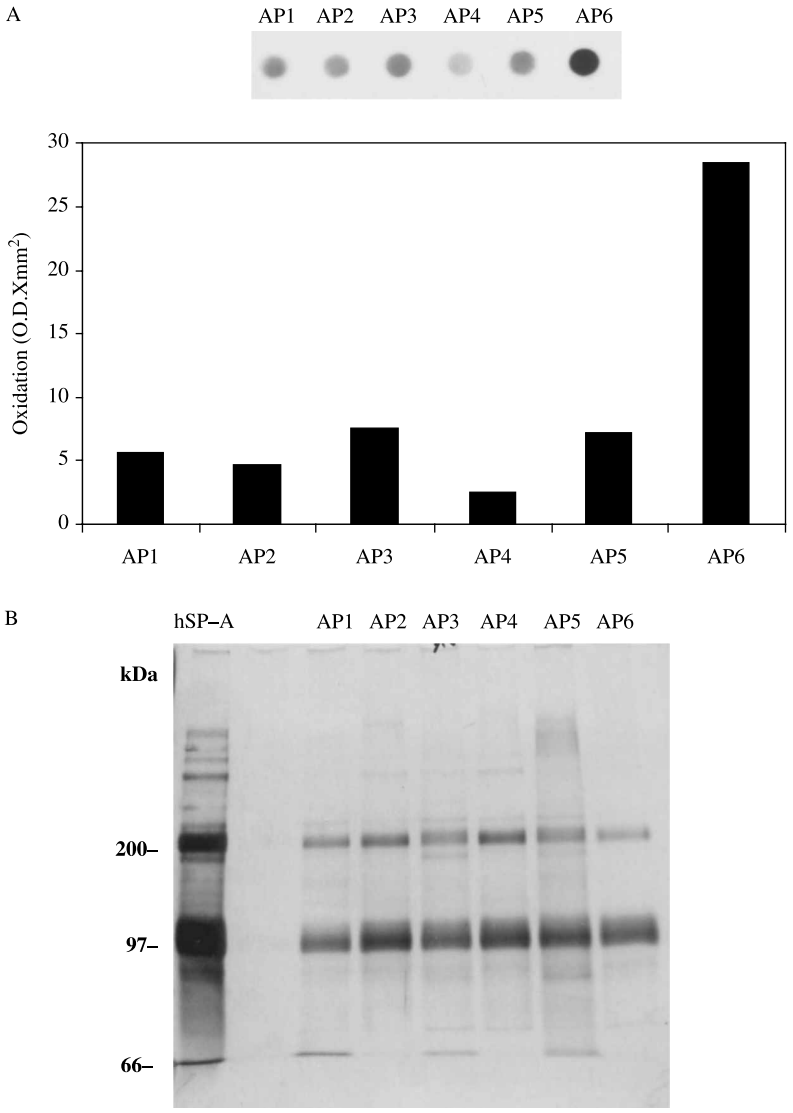

Figure 3. Oxidation and purity of SP-A. SP-A samples were obtained from six alveolar proteinosis patients (AP1, AP2, AP3, AP4, AP5, and AP6) and subjected to 4–15% SDS-PAGE electrophoresis under non-reducing conditions followed by silver staining. Their oxidation status was tested using the Oxyblot Oxidized Protein Detection Kit. (A) Oxidation status of SP-As by protein dot blot (100 ng/dot) and densitometric measurement of dots. Samples with low oxidation (i.e. AP1, AP2, and AP4) were used for subsequent experimentation. (B) SDS-PAGE electrophoresis of 1 μg of each SP-A sample was performed in a 4–15% gradient polyacrylamide gel after heating at 95°C for 10 min. hSP-A, human SP-A from a healthy volunteer served as positive control.

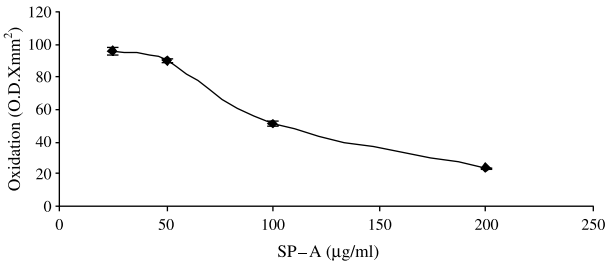

Figure 4. Dose response experiments for ozone-induced SP-A oxidation. Concentrations of 25, 50, 100, and 200 μg/ml of SP-A were exposed to ozone (1 ppm) for 4 h. Oxidation was expressed as the product of optical density (OD) by the area (mm^2) of dots. Values are the mean ± SE from two independent experiments carried out in triplicate.

low while after exposure to ozone there was a sharp increase in its oxidation. Additionally, it was observed that as the concentration of SP-A decreases, its oxidation increases. Optimal concentration was considered to be the one at 50 μg/ml because at this concentration there was both a sufficient increase and a plateau in the oxidation of SP-A.

Thus, in the present study, SP-A protein solution (100 μl) at a concentration of 50 μg/ml were exposed to ozone (1 ppm) with or without polyphenols for 4 h in 24-well plates. In total eight polyphenols were tested to determine whether they could inhibit ozone-induced SP-A oxidation. These were five polyphenolic acids (i.e. caffeic acid, ferulic acid, *p*-coumaric acid, gallic acid, and protocatechuic acid) and three flavonoids (i.e. (+)-catechin, (−)-epicatechin, and rutin

hydrate). Each polyphenol was tested at four different concentrations (5, 25, 50, and 100 μM) and at least three independent experiments done in triplicate were performed. After exposure, SP-A proteins were transferred to sterile microcentrifuge tubes and their concentration and oxidation was determined immediately or stored at −80°C until further analysis.

Proper controls were also included in each experiment to avoid possible artifact during the assay: (a) SP-A was exposed to filtered air for 4 h at 37°C (negative control). (b) SP-A alone was exposed to ozone (1 ppm) for 4 h at 37°C (positive control). (c) SP-A protein was incubated with polyphenols at maximum concentration used in the study for 4 h at 37°C in the presence of filtered air. (d) The polyphenols at maximum concentration used in the study were added to oxidized SP-A just before the DNPH reaction.

The inhibition percentage of ozone-induced SP-A oxidation was calculated by the following formula:

$$\% \text{ Inhibition} = [1 - S/(S_{PC} - S_{FA})] \times 100$$

where S: the oxidation of samples; S_{PC}: the oxidation of positive control; S_{FA}: the oxidation of SP-A exposed to filtered air.

Statisitical analysis

For statistical analysis the one-way ANOVA was applied followed by Dunnett's test for multiple pair wise comparisons. Correlations between variables were examined by Spearman's correlation analysis.

Results

Eight common plant polyphenols were tested for their effects on SP-A oxidation induced by ozone. These polyphenols were three flavonoids (rutin hydrate, (+)-catechin, and (−)-epicatechin), three hydroxycinnamic acids (caffeic acid, ferulic, acid and *p*-coumaric acid) and two hydroxybenzoic acids (gallic acid and protocatechuic acid). The three flavonoids inhibited the ozone-induced SP-A oxidation in a dose-dependent manner. (+)-Catechin demonstrated statistically significant inhibition by 25, 47, and 62% ($p < 0.05$) at concentrations of 25, 50, and 100 μM, respectively (Figures 5(B) and 6(A)) in a dose-dependent manner ($r = -0.75$, $p < 0.01$). (−)-Epicatechin significantly reduced protein carbonyl formation by 35, 43, 48, and 60% ($p < 0.01$) at concentrations of 5, 25, 50, and 100 μM respectively, in a dose-dependent manner ($r = -0.84$, $p < 0.01$) as shown in Figures 5(A) and 6(A). Moreover, rutin hydrate protected significantly SP-A from oxidation by 38, 40, and 51% ($p < 0.05$) at concentrations of 25, 50, and 100 μM, respectively (Figures 5(C) and 6(A)) in a dose-dependent manner ($r = -0.73$, $p < 0.01$).

The two hydroxybenzoic acids also inhibited oxidation of SP-A exposed to ozone. Dot-blot analyses and subsequently measurement of density of dots indicated that protocatechuic acid prevented significantly SP-A oxidation at all tested concentrations (Figure 5(E)). Specifically, the inhibition was 25% at 5 μM ($p < 0.05$) but reached a plateau at 50 μM (43% inhibition, $p < 0.01$) (Figure 6(B)). Gallic acid had no effect on ozone-induced SP-A oxidation at 5 μM, but like protocatechuic acid, there was a plateau in inhibitory activity at 50 μM (33% inhibition, $p < 0.05$) (Figures 5(D) and 6(B)).

Furthermore, the hydroxycinnamic acids inhibited ozone-induced SP-A oxidation but exhibited a different inhibitory pattern in comparison with the flavonoids and hydroxybenzoic acids. In particular, there was an optimum medium concentration at which the inhibition was maximum while at the lowest and highest concentrations there was not statistically significant inhibition. Caffeic acid exerted significant inhibition ($p < 0.05$) by 34 and 38% at 25 and 50 μM respectively while at 5 and 100 μM there was not any significant effect (Figures 5(F) and 6(C)). In addition, *p*-coumaric acid showed a maximum significant inhibition by 39% at 25 μM ($p < 0.05$) while at 50 and 100 μM there was only an inhibitory trend and at 5 μM there was not any effect (Figures 5(G) and 6(C)). Ferulic acid did not affect ozone-induced SP-A oxidation at 5, 50, and 100 μM while at 25 μM there was a trend of inhibition by 32% ($p < 0.1$) as shown in Figures 5(H) and 6(C).

None of the tested polyphenols affected the oxidation of SP-A in the presence of filtered air (Figure 5).

Discussion

The lung is one of the first physical interfaces between the outside environment and the body. As such, the lung is vulnerable to environmental infectious agents as well as to air pollutants; oxidative stress appears to play a role in the pathophysiological action of all these toxicants [20,36]. Ozone, a major air pollutant and reactive molecule, is converted to ROS capable of oxidizing lipids and proteins [37]. For example, ozone-induced oxidation of SP-A protein, an innate host defense molecule of lung surfactant [6], changes its functional and biochemical properties [15–19]. Epidemiologic studies have shown that dietary intake of natural antioxidants (carotenoids) was associated with improved lung function [38], and supplementation with a mixture of ascorbate, α-tocopherol, and carotenoids in healthy subjects exposed to ozone protected pulmonary function [22]. One of the most important class of natural antioxidants is plant polyphenols [26]. It is believed that the lung (along with the gastrointestinal tract, liver, skin, prostate, and breast) may be one of the most likely organs to benefit from the chemopreventive activity of plant

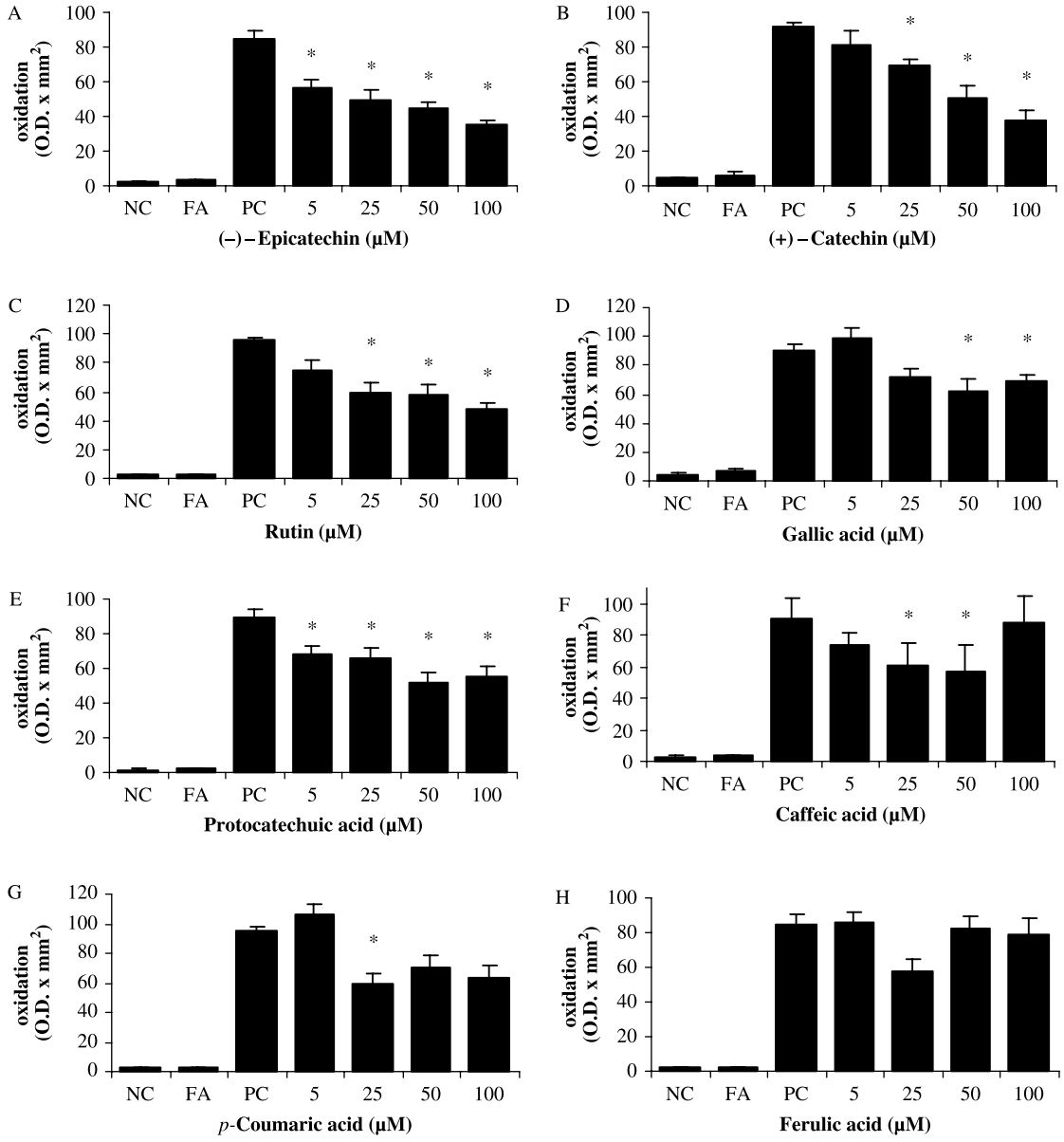

Figure 5. Effects of tested polyphenols on ozone-induced SP-A oxidation. SP-A was exposed to ozone (1 ppm) for 4 h. Oxidation was expressed as the product of optical density (OD) by the area (mm^2) of dots. Values are the mean ± SE from three independent experiments carried out in triplicate. NC: Negative control (SP-A alone exposed to filtered air); PC: Positive control (SP-A alone exposed to ozone); FA: SP-A plus polyphenol at a concentration of 100 μM exposed to filtered air. ANOVA was used for the statistical analysis. $^{\star}p < 0.05$ when compared with positive control.

polyphenols [39]. For example, a study using an animal model of acute inflammation caused by injection of carrageenan has found that green tea polyphenol extract attenuated lung injury and infiltration of lung with neutrophils [40]. Moreover, black tea infusion was shown to reduce oxidation of microsomal proteins in the lung of cigarette smoke-exposed guinea pigs and this protective activity was attributed to the catechins present in black tea [41].

Therefore, the aim of the present study was to investigate the potential inhibitory activity of three groups of plant polyphenols against ozone-induced oxidation of SP-A as a possible protective mechanism against air pollutants by dietary antioxidants. Although it was used an *in vitro* experimental system, the concentration of SP-A (50 μg/ml) was within the physiological ranges according to the assumptions made by various laboratories. Thus, it has been estimated that the BAL levels of SP-A in normal individuals are in the range of 2–10 μg/ml and are likely to have been diluted 10–100 fold by the lavage procedure [42–43]. Moreover, ozone concentration (1 ppm) used in the present study can be achieved in urban air in high pollution conditions [44]. As regards polyphenols, the concentrations used were in part similar to those found in human plasma, since the maximum plasma polyphenol concentrations attained after a polyphenol-rich meal are in the range

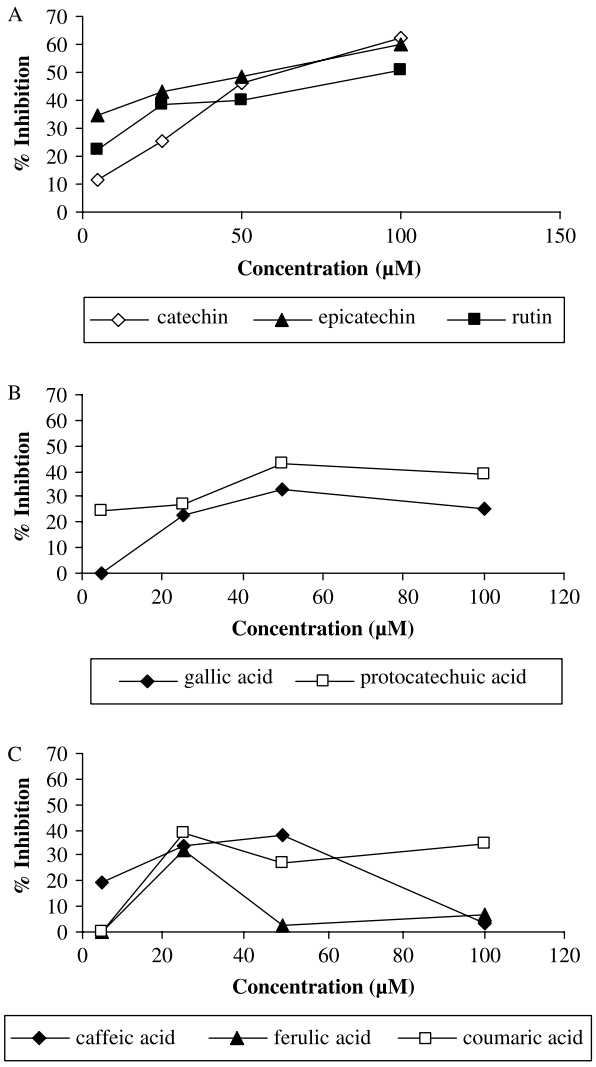

Figure 6. Percentage inhibition of ozone-induced SP-A oxidation by plant polyphenols at 5, 25, 50 and 100 μM. Each value represents the mean of three experiments carried out in triplicate.

of 0.1–10 μM [45–47]. Data concerning the bioavailability of polyphenols in human tissues other than plasma are too limited [47]. It should also be noted that aglycone polyphenol forms were used in the present study, while polyphenols reaching the blood are usually conjugates of glucuronate or sulfate which may exhibit different activity than their corresponding aglycone forms [47].

The results showed that the polyphenols variably inhibited oxidation of SP-A by ozone, and exhibited distinctive inhibitory patterns based on the group and/or type of polyphenol, as assessed by measurements of the formation of protein carbonyls, which is one of the most reliable markers of protein oxidation [48]. The flavonoids protected SP-A from oxidation in a dose dependent manner (Figure 6(A)), with (−)-epicatechin being the most potent flavonoid. It is noteworthy that a concentration as low as 5 μM exhibited inhibition of ozone-induced formation of carbonyls by 35%. At 5 and 25 μM (−)-epicatechin was more potent than (+)-catechin, while at 50 and 100 μM their inhibitory activities were almost the same (Figure 6(A)). (−)-Epicatechin and (+)-catechin are stereoisomeres, that is the chiral carbon (position-3) in the former is R, while in the latter is S (Figure 2). Thus, the present results suggested that the R form of these flavonoids was more effective inhibitor of ozone-induced SP-A oxidation than the S one. Moreover, in another study [49], (−)-epicatechin was more effective scavenger of the stable radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) than (+)-catechin. Rutin hydrate was less potent than (−)-epicatechin at all concentrations, while it was more potent than (+)-catechin at 5 and 25 μM and less potent at 50 and 100 μM (Figure 6(A)). The difference in the chemical structure between rutin hydrate and the two tested catechins is that the former has a 2,3 double bond, a 4-keto group and a glycosidic moiety (rutinose) in the 3- position in the C ring (Figure 2). Although it is considered that the 2,3 double bond and the 4-keto group in the C ring enhance the antioxidant activity of flavonoids, the blocking of the 3-OH in the C ring weakens the antioxidant effect of flavonoids because diminishes the coplanarity of the B ring relative to the rest of the molecule [24]. Planarity increases phenoxyl radical stability and consequently the antioxidant effectiveness of flavonoids [50].

Although hydroxybenzoic acids inhibited SP-A oxidation by ozone, their inhibitory activity was less potent than that observed for flavonoids and exhibited a plateau at 50 μM (Figure 6(B)). Protocatechuic acid showed inhibition at the lowest concentration of 5 μM and was more potent than gallic acid. The difference between their chemical structures is that gallic acid possesses three hydroxyl groups in the 3-, 4- and 5-positions, while protocatechuic acid has two hydroxyl groups in the 3- and 4- positions (Figure 2). Thus, the present results were intriguing, since gallic acid can donate more hydrogen atoms for scavenging free radicals and thus it is a more effective antioxidant than protocatechuic acid [51]. An explanation that could be given for this contradiction is that polyphenols may prevent ozone-induced SP-A oxidation not only by scavenging free radicals but also by binding to SP-A, thus protocatechuic acid may be more effective than gallic acid to interact with proteins. Moreover, in another report of our lab [31], protocatechuic acid inhibited topoisomerase I catalytic activity, while gallic acid did not.

Hydroxycinnamic acids showed a different inhibitory mode in comparison with flavonoid and hydroxybenzoic acids. The maximum inhibition of oxidation was observed at medium concentrations while at higher and lower concentrations there was not significant inhibition (Figures 5(F)–(H) and 6(C)). Among the three hydroxycinnamic acids, the most potent was caffeic acid, since it exerted stastistically

significant inhibition at both 25 and 50 μM, while *p*-coumaric acid only at 25 μM and ferulic acid did not show significant inhibition at any concentration (Figure 5(F)–(H)). The difference in their chemical structures is that caffeic acid has two hydroxyl groups in 3- and 4- positions, *p*-coumaric acid has only one hydroxyl group at 4- position and ferulic acid has one hydroxyl group at 4- position and one methoxy group at 3- position (Figure 2). The *ortho*-dihydroxy structure of caffeic acid is considered, in general, the most important factor for the antioxidant activity of polyphenols because it confers higher stability to the phenoxyl radical and participates in electron delocalization [24]. As regards the bell-shaped pattern of hydroxycinnamic acids, it is in agreement with results of other reports regarding the activity of antioxidative compounds, such as (−)-epigallocatechin-3-gallate [52], green tea extract [53], vitamin C [54], R-apomorphine and dopamine [55]. These antioxidants were protective at low concentrations while showing pro-oxidant activity at higher concentrations. Although there is not a definite explanation for this activity, it could be attributed to the fact that polyphenols could not inhibit oxidation at the lower concentrations, while at the higher ones there may be an auto-oxidation of polyphenols which abolishes their protective effects.

The mechanism via which the above polyphenols prevent ozone-induced SP-A oxidation is not known. However, it is likely that polyphenols prevent ozone-induced SP-A oxidation either by scavenging free radicals and/or by binding to SP-A. Polyphenols are multidentate ligands, whereby each phenolic ring is a potential binding site and thus are capable of binding at more than one point on the protein. Moreover, polyphenols appear to bind preferentially to proline-rich proteins that have either random coil or collagen-like helical conformations [56–57]. It is noteworthy, that SP-A has a collagen-like domain containing a series of 23 Gly-X-Y triplets with Y being proline or hydroxyproline and this region along with the hydrophobic "neck" and carboxyl-terminal domains play a role in the structure and function of the native protein [6]. The principal driving force for polyphenol-protein binding is multiple non-covalent bonds, mainly hydrophobic interactions complemented by hydrogen bonding [58–60]. However, it has also been reported that under oxidizing conditions polyphenols can form covalent bonds with proteins [61]. Interestingly, polyphenols seem to retain their antioxidant capacity within polyphenol-protein complexes formed after oxidation [29].

In conclusion, according to our knowledge, this is the first report regarding the protection of natural antioxidants against the oxidation of SP-A protein, a molecule that plays an important role in normal lung function and innate host defense. Although only two of the tested polyphenols, (−)-epicatechin and protocatechuic acid, exerted inhibition at a concentration achievable in the human organism through the diet, possible synergistic effects between polyphenols may result in exhibiting inhibition at even lower concentrations as has been shown by several studies [62–64]. Although there are obvious limitations in extrapolating data from *in vitro* experiments to whole organisms, our results along with relevant published literature support the possibility that plant polyphenols present in dietary sources, such as fruits and vegetables, may be used to prevent the detrimental effects of air pollutants on the lung.

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