ROS-mediated TNF-α and MIP-2 gene expression in alveolar macrophages exposed to pine dust

Huayan Long¹, Tingming Shi², Paul J Borm², Juha Määttä³, Kirsti Husgafvel-Pursiainen³, Kai Savolainen³ and Fritz Krombach*¹

Address: ¹Institute for Surgical Research, University of Munich, Munich, Germany, ²Institut für Umweltmedizinische Forschung, University of Düsseldorf, Düsseldorf, Germany and ³Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Helsinki, Finland

Email: Huayan Long - huayan_long@hotmail.com; Tingming Shi - Tmingshi@yahoo.com; Paul J Borm - P.Borm@HSzuyd.nl; Juha Määttä - juha.maatta@ttl.fi; Kirsti Husgafvel-Pursiainen - Kirsti.Husgafvel-Pursiainen@ttl.fi; Kai Savolainen - Kai.Savolainen@ttl.fi; Fritz Krombach* - krombach@med.uni-muenchen.de

* Corresponding author

Published: 13 December 2004

This article is available from: http://www.particleandfibretoxicology.com/content/1/1/3

© 2004 Long et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Respiratory symptoms, impaired lung function, and asthma have been reported in workers exposed to wood dust in a number of epidemiological studies. The underlying pathomechanisms, however, are not well understood. Here, we studied the effects of dust from pine (PD) and heat-treated pine (HPD) on the release of reactive oxygen species (ROS) and inflammatory mediators in rat alveolar macrophages.

Methods: Tumour necrosis factor-alpha (TNF-α) and macrophage inflammatory protein-2 (MIP-2) protein release, TNF-α and MIP-2 mRNA expression, and generation of ROS were studied as end points after treatment of rat alveolar macrophages with PD or HPD. In a separate series of experiments, the antioxidants glutathione and N-acetyl-L-cysteine were included in combination with wood dust. To determine the endogenous oxidative and antioxidant capacity of wood dusts, electron spin resonance (ESR) spectroscopy was used.

Results: After 4 h incubation, both PD and HPD elicited a significantly (p < 0.05) increased mRNA expression of TNF-α and MIP-2 as well as a concentration-dependent release of TNF-α and MIP-2 protein. Interestingly, PD induced a significantly higher TNF-α and MIP-2 production than HPD. Moreover, a significantly increased ROS production was observed in alveolar macrophages exposed to both PD and HPD. In the presence of the antioxidants glutathione and N-acetyl-L-cysteine, the PD- and HPD-induced release of ROS, TNF-α, and MIP-2 was significantly reduced. Finally, electron spin resonance analyses demonstrated a higher endogenous antioxidant capacity of HPD compared to PD. Endotoxin was not present in either dust sample.

Conclusion: These results indicate that pine dust is able to induce expression of TNF-α and MIP-2 in rat alveolar macrophages by a mechanism that is, at least in part, mediated by ROS.
Background
In addition to sino-nasal cancer [1], exposure to wood dust has been shown to be associated with a wide variety of acute and chronic non-malignant respiratory health effects as well as eye irritation and dermatitis [2,3]. However, the underlying mechanisms involved are not well understood and subject of controversial discussion. Although inflammatory markers were found in nasal and bronchoalveolar lavage fluid from wood-dust exposed individuals [4-6], other studies do not corroborate the hypothesis that inflammation plays a part in wood dust-induced airway obstruction [7]. Moreover, recent studies do not support the assumption that the complaints related to exposure to wood dust are IgE-mediated [8,9].

In wood processing facilities, the proportion of respirable wood dust ranges from 6% to 75% of the total wood aerosol [2]. Respirable wood dust particles may deposit in the pulmonary alveoli and interact with alveolar macrophages, a cell type that plays an important role in phagocytosis and clearance of inhaled particulates. Upon interaction with noxious particles, alveolar macrophages can produce a broad spectrum of pro-inflammatory mediators, such as tumour necrosis factor-alpha (TNF-α) and macrophage inflammatory protein-2 (MIP-2) as well as reactive oxygen (ROS) and nitrogen species [10-13]. TNF-α is one of the pre-eminent cytokines that acts as an initiator of inflammatory processes in the lung [14]. The chemokine MIP-2 is known to mediate neutrophilic inflammatory responses in the lung [10,15]. ROS have been shown not only to damage cells by peroxidizing lipids and disrupting DNA and proteins, but also to exert signaling functions and modulate gene transcription [16,17]. Moreover, ROS are suggested to mediate the release of TNF-α and MIP-2 in alveolar macrophages exposed to noxious particles [18]. Interestingly enough, a recent study demonstrated that exposure to pine dust induced increased ROS production and caused cell death in both murine RAW 264.7 macrophages and human polymorphonuclear leukocytes [19].

Pine is one of the most extensively used wood species in the wood processing industry and several studies have shown that exposure to pine dust induced respiratory symptoms, reduced lung function, and asthma [3,20-22]. Moreover, pine is one of the most common wood species used for heat treatment, one of the treatment processes for stabilization and preservation of wood. After heat-treatment, both physical and chemical properties of wood are changed [23].

This study aimed to investigate the effect of dust from untreated as well as from heat-treated pine on the production of TNF-α, MIP-2, and ROS by primary rat alveolar macrophages and to elucidate the role of oxidative stress in pine dust-induced cytokine production.

Methods
Wood dust
Dust from untreated pine (PD) and heat-treated pine (HPD) was obtained from the Kuopio Regional Institute of Occupational Health (Kuopio, Finland). Dusts were produced using a dust collecting face-grinding machine with 400-grit sanding paper. For particle size distribution analyses, wood dust specimens, gold-coated for 170 seconds with BAL-TEC SCD 005 Sputter Coater (BAL-TEC AG, Liechtenstein), were examined on a JEOL JSM-6400 scanning electron microscope (JEOL Inc., Peabody, MA) at an acceleration voltage of 20 kV. More than 1700 particles for each dust were analyzed from electron micrographs. More than 95% of wood dust particles from both pine and heat-treated pine had a diameter less than 5 μm (Table 1). The endotoxin content in PD and HPD as analyzed with a LAL gel-clot assay (Charles River, Germany) was below the detection limit of 0.06 EU/ml. For experiments, pine dust was suspended in RPMI-1640 medium with 10% fetal calf serum, ultrasonicated, and vortexed.

Collection of Alveolar Macrophages
Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) were anesthetized by an intraperitoneal injection of sodium pentobarbital (30 mg/KG body weight) and killed by exsanguination from the abdominal aorta. The lungs were lavaged ten times with 10 ml of sterile, non-pyrogenic phosphate-buffered saline solution (PBS; Serva, Heidelberg, Germany). The pooled samples were centrifuged at 300 g for 10 min, and the cell pellet was washed twice and re-suspended in RPMI 1640 (Seromed, Munich, Germany) supplemented with L-glutamine, gentamycin (0.16 mg/ml), and 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, Egggenstein, Germany). Total cell counts were assessed with a standard hemocytometer (Coulter Electronics, Krefeld, Germany). Air-dried cytocentrifuged smears served to identify the cellular populations after staining with May-Grünwald-Giemsa. The preparation of bronchoalveolar cells contained about 97–
100% alveolar macrophages. Cell viability as determined by trypan blue exclusion was greater than 90%.

Treatment of cells
Alveolar macrophages were adjusted according to the differential cell counts to $2 \times 10^6$ cells/ml. Then, 100 µl-samples of cell suspension were plated to 96-well flat-bottomed cell culture plates (Nunclon Delta, Roskilde, Denmark), and incubated at 37°C in 5% CO₂ and 21% O₂. After 2 h, non-adherent cells were removed by washing twice with RPMI 1640, and the adherent alveolar macrophages were covered with 100 µl of pine dust suspension at concentrations ranging from 5 to 200 µg/ml. As a negative control, 3-µm polystyrene microspheres (Polysciences, Eppelheim, Germany) were used at a concentration of 100 µg/ml. As a positive control, Escherichia coli LPS serotype 055:B5 purchased from Sigma Chemie (Taufkirchen, Germany) was used at a concentration of 100 ng/ml. In a separate series of experiments, alveolar macrophages were treated with 6 mM glutathione (GSH; Polysciences, Eppelheim, Germany) or 20 mM N-acetyl-dimethyl-1-pyrroline-N-oxide (DMPO, 0.05 M in PBS) (Sigma, Steinheim, Germany) for 30 min. Subsequently, the culture medium was replaced with 100 µl of pine dust suspension at a final concentration of 200 µg/ml. After 4 h incubation in the absence or presence of GSH (6 mM) or NAC (20 mM), supernatants were removed and stored at -20°C. There was no effect of either treatment on cell viability as measured by a LDH assay kit (Merck, Germany).

RT-PCR
Total cellular RNA was extracted from pine dust-exposed alveolar macrophages using a ribonuclease protection kit (Rneasy Kit, QIAGEN, Hilden, Germany). RT-PCR was performed as described previously [24]. The oligonucleotide primers (MWG-Biotech, Ebersberg, Germany) used were 5′-TGC CTC AGC CTC TTC TCA TT-3′ and 5′-TGT GGG TGA GGA GCA CAT AG-3′ (EMBL: RNNTPAA, AC: X66539) for TNF, 5′-CAA TGC TGT AGC ACC CTA C-3′ and 5′-CAG TTA GCC TTG CCT TTG TTC-3′ [25] for MIP-2, and 5′-TCC CTC TCT AGG ATT GTC AGC AA-3′ and 5′AGA TCC ACA ACG GAT ACA TT-3′ [26] for the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The sizes of the PCR products were 376 bp for TNF, 194 bp for MIP-2, and 309 bp for GAPDH. PCR products were visualized in 2% agarose gels containing 1% ethidium bromide. For densitometric analyses, BIO-1D V 96 software (Vilber Lourmat, Marne La Vallee, France) was used.

TNF-α and MIP-2 ELISA
Concentrations of TNF-α and MIP-2 in culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Bio-source, Solingen, Germany).

Detection of intracellular ROS
To detect intracellular ROS, 2′,7′-dichlorofluorescin diacetate (DCFH-DA) (MoBiTec, Göttingen, Germany) was used. DCFH-DA diffuses into the cell and is hydrolyzed by intracellular esterases to polar 2′,7′-dichlorofluorescin. This non-fluorescent fluororescin analogue can be oxidized to highly fluorescent 2′,7′-dichlorofluorescin by intracellular oxidants [27]. Alveolar macrophages were cultured to adhere and incubated with 10 µM DCFH-DA for 30 min. The cultures were washed twice with RPMI 1640 and subsequently treated as described before. Baseline fluorescence was measured with a fluorometer (FLUOstar, BMG LabTechnologies, Offenburg, Germany) immediately after wood dusts were added. After 4 h of incubation under 37°C in 5% CO₂ and 21% O₂, fluorescence was measured again. The results are shown as percentage change from baseline values. The addition of 1 µM H₂O₂ served as an internal positive control.

Electron spin resonance spectroscopy
Hydroxyl radical formation by wood dusts was assessed by electron spin resonance (ESR) spectroscopy, as described previously [28]. Briefly, wood dust suspensions (20 mg/ml) were prepared in pure water. 100 µl of this suspension was mixed with 200 µl of the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, 0.05 M in PBS) (Sigma, St. Louis, MO) and 100 µl H₂O₂ (0.5 M in PBS) (Fluka, Seelze, Germany). The suspension was incubated for 15 min at 37°C in a shaking water bath, and filtered through a 0.2 µm filter (15 mm syringe filter, Satorius AG, Goettingen, Germany) to remove particles from the suspension. The filtrate was immediately transferred to a capillary and measured with a Miniscope ESR spectrometer (Magnetech, Berlin, Germany). The antioxidant activity of wood dust suspensions was measured by using the stable spin label TEMPO (Sigma, Steinheim, Germany). TEMPO was added to wood dust suspensions (10 mg/ml) at a final concentration of 5 µM, mixed and incubated at 37°C for 1 h in a shaking water bath. After filtering the suspension through a 0.2 µm filter, the filtrate was measured as mentioned above. ESR spectra were recorded at room temperature using the following instrumental conditions: Magnetic field: 3360 G, sweep width: 100 G, scan time: 30 sec, number of scans: 3, modulation amplitude: 1.8 G, receiver gain: 1000. Quantification was carried out as the sum of total amplitude on first derivation of ESR signal, and outcomes are expressed as the total amplitude in arbitrary units.

Statistical analysis
Results are presented as mean ± SEM. Statistical comparisons were performed by using RM ANOVA with Student-Newman-Keuls method for multiple comparison procedures. A p value < 0.05 was considered significant.
Results

**TNF-α and MIP-2 mRNA expression**

After 4 h exposure of alveolar macrophages to PD and HPD, mRNA was extracted and the supernatants were collected for cytokine and chemokine measurement. A low, basal level of TNF-α and MIP-2 mRNA expression was observed in control macrophages. Compared to control, TNF-α and MIP-2 mRNA expression in alveolar macrophages exposed to PD and HPD was significantly increased. Interestingly, PD induced significantly ($p < 0.05$) higher levels of TNF-α and MIP-2 mRNA expression than HPD (Figure 1).
ROS generation
To detect ROS production in PD- and HPD-stimulated alveolar macrophages, the oxidant-sensitive dye DCFH-DA was used. After 4 h incubation, wood dusts at a concentration of 200 µg/ml induced a significantly (p < 0.05) increased ROS generation when compared to untreated control cells (Figure 3). However, the level of PD-induced ROS generation in alveolar macrophages was not statistically different from the level of HPD-induced ROS generation. Treatment of the cells with GSH (6 mM) or NAC (20 mM) caused significant suppression of both PD- and HPD-induced ROS generation.

Effect of antioxidants on cytokine and chemokine expression
To elucidate whether oxidative stress participates in the up-regulation of inflammatory cytokine expression, TNF-α and MIP-2 release was examined in PD- and HPD-exposed alveolar macrophages in the presence or absence of the antioxidants GSH and NAC. Treatment with both GSH and NAC significantly (p < 0.05) reduced the TNF-α and MIP-2 release elicited by the exposure of alveolar macrophages to PD and HPD (Figure 4).

Endogenous oxidant and antioxidant activity of pine dust
ESR spectroscopy showed that suspensions of both PD and HPD caused formation of •OH in the presence of H₂O₂. However, the ability of PD and HPD to generate •OH was not statistically different (Figure 5). The antioxidant capacity of pine dust suspensions was measured by the use of the stable spin label TEMPOL. Interestingly, HPD caused a significantly greater reduction of TEMPOL than PD, indicating that HPD has greater antioxidant capacity than PD (Figure 5).

Discussion
A higher prevalence of non-malignant respiratory diseases, such as bronchitis, chronic obstructive pulmonary disease, cryptogenic fibrosing alveolitis, and asthma has been reported in workers exposed to a variety of wood dusts [2]. Sensitization to wood dust from some wood species such as red cedar has been shown to be involved in mechanisms generating a work-related asthmatic response [29]. However, more recent studies have shown that sensitization to wood dust from pine, oak, beech and other wood species may not be the only or even the most
important mechanism involved in wood dust-induced respiratory symptoms [8,9]. Therefore, our study aimed to investigate the non-specific inflammatory response of primary lung macrophages to wood dust from pine, one of the most extensively used wood species in the wood processing industry.

Here we show that pine dust induces TNF-α and MIP-2 mRNA expression as well as TNF-α and MIP-2 protein release in rat alveolar macrophages. Alveolar macrophages are important in processing airborne particles and play a key role in mediating inflammatory responses of the lung through the release of various proteolytic enzymes, reactive oxygen and nitrogen species, arachidonic acid metabolites, cytokines such as TNF-α, and chemokines such as MIP-2 [11]. TNF-α plays an important role as a mediator of the respiratory tract’s response to particles. Studies have shown that a variety of agents which elicit marked lung inflammation can activate alveolar macrophages to release TNF-α, while agents with limited inflammatory activity do not stimulate macrophage TNF-α production. MIP-2 plays a major role in mediating the neutrophilic inflammatory response of the rodent lung to particles such as quartz and crocidolite asbestos [10]. TNF-α and MIP-2 gene expression is under the control of redox-sensitive inflammation-related transcription factors such as NF-κB. Activation of NF-κB is regulated via a number of second messengers, including calcium and ROS [16]. In addition to providing evidence that pine dust stimulates both TNF-α and MIP-2 mRNA production and protein release from rat alveolar macrophages, our study clearly demonstrates that pine dust stimulates the generation of ROS in alveolar macrophages, as previously shown in mouse macrophages and human leukocytes by Naarala et al. [19].

To investigated the role of oxidative stress in pine dust-induced cytokine and chemokine response we treated pine dust-exposed alveolar macrophages with the antioxidants GSH and NAC. GSH plays a major role in the antioxidant system by working as a substrate for glutathione peroxidase, and it has been previously shown that extracellular GSH can elevate intracellular GSH levels and protect phagocytes against oxidant damage [30]. NAC is a thiol compound that can act as a cysteine source for the repletion of intracellular glutathione and act as a direct scavenger of ROS. NAC has been shown to attenuate oxidant-mediated toxicity induced by chrysotile fibres in rats [31] and to down-regulate the nitric oxide pathway in alveolar macrophages [22]. We found that treatment with GSH or NAC attenuated pine dust-induced ROS generation as well as TNF-α and MIP-2 protein release. These findings are concordant with previous studies on silica and ultrafine particles [18,33,34] and indicate that pine dust-induced oxidative stress mediates, at least in part, the expression of TNF-α and MIP-2 in alveolar macrophages. Interestingly enough, dust from untreated pine (PD) induced a significantly stronger inflammatory response in alveolar macrophages than dust from heat-treated pine (HPD). Consequently, we used ESR spectroscopy to assess the endogenous oxidative and antioxidant capacity of the wood dusts under study. Whereas the ability to generate hydroxyl radical did not differ among PD and HPD, HPD exhibited greater antioxidant capacity than PD. As we have demonstrated in this study that oxidative stress may play a role in mediating the expression of TNF-α and MIP-2, we suggest that the greater antioxidant capacity of HPD may neutralize oxidative stress and thus attenuate expression of TNF-α and MIP-2. As mentioned before, both physical and chemical properties of wood are changed when heat-treated for several hours with temperatures up to 230°C. In particular, the pine resin is easily volatilized and almost completely removed from the wood [23]. One of the components of resin, δ-3-carene, has been reported to decrease the viability of alveolar macrophages and affect the engulfment of particles in vitro [35]. Another component of pine resin, abietic acid, has been shown to produce lytic damage to alveolar, tracheal, and bronchial epithelial cells [36]. Further studies are warranted to confirm our results and to determine the specific chemical and physical properties of dust from heat-treated pine that might be responsible for the effects seen in this study. Recently, metabolites of pine bark extract have been shown to have antioxidant activity and to inhibit matrix metalloproteinases (37).

In summary, our findings indicate that non-specific inflammatory reactions, mediated via ROS production, may play a role in pulmonary effects of wood dust. However, it is not clear from this in vitro study whether the...
oxidative stress driving TNF-α and MIP-2 protein release is due to ROS derived directly from the dust particles or from cell-generated ROS.

Conclusions

Here, we demonstrate that pine dust is able to induce inflammatory responses in vitro. Oxidative stress seems to play an important role in the pine dust-induced cytokine and chemokine response, suggesting that wood dust particles may exert pro-inflammatory effects by a mechanism that is, at least in part, mediated by ROS.

Authors’ contributions

HI performed the isolation of alveolar macrophages, subsequent cytological and biochemical analyses, and writing and preparation of the manuscript. TS and PJB carried out ESR spectroscopy. JM analysed the size distribution of wood dust particles. KHP, KS, and FK participated in the direction of the study as well as in writing and preparation of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We gratefully acknowledge the excellent technical assistance of Anne-Marie Almeling, University of Munich, and Jaakko Santti, Finnish Institute of Occupational Health. We thank Irma Welling, Lappeenranta Regional Institute of Occupational Health, Finland, for help in obtaining the two types of pine for production of wood dust. This study was supported by the EU 5th Framework Programme, Key Action 4, Environment and Health, Quality of Life and Management of Living Resources, Project No. QLK4-2000-00573.

References

1. IARC Working Group: Wood dust and formaldehyde. In IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Volume 62. Lyon France, 1995.
2. Demers PA, Teschke K, Kennedy SM: What to do about softwood? A review of respiratory effects and recommendations regarding exposure limits. Am J Ind Med 1997; 31:385-398.
3. Douwes J, McLean D, Slater T, Pearce N: Asthma and other respiratory symptoms in New Zealand pine processing sawmill workers. Am J Ind Med 2001; 39:608-615.
4. Johard U, Elbod A, Dahlqvist M, Ahlander A, Alexandersson R, Ekholm U, Tornling G, Ulfvarsson U: Signs of alveolar inflammation in non-smoking Swedish wood trimmers. Br J Ind Med 1992; 49:428-434.
5. Dahlqvist M, Palmberg L, Malmberg P, Sundblad BM, Ulfvarson U, Zhiping W: Acute effects of exposure to air contaminants in a sawmill on healthy volunteers. Occup Environ Med 1996, 53:586-590.
6. Ahman M, Holmstrom M, Ingelman-Sundberg H: Inflammatory markers in nasal lavage fluid from Industrial Arts teachers. Am J Ind Med 1995, 28:541-550.
7. Borm PJ, Jetten M, Hidayat S, van de Burgh N, van de Burgh N, Leunissen P, Kant I, Houba R, Soeprapto H: Respiratory symptoms, lung function, and nasal cellularity in Indonesian wood workers: a dose-response analysis. Occup Environ Med 2002, 59:338-344.
8. Ahman M, van Hage-Hamsten M, Johansson SG: IgE-mediated allergy to wood dust probably does not explain the high prevalence of respiratory symptoms among Swedish woodwork teachers. Allergy 1995, 50:559-562.
9. Skovsted TA, Schlunsen V, Schaumburg I, Wang P, Staan-Olsen P, Skov PS: Only few workers exposed to wood dust are detected with specific IgE against pine wood. Allergy 2003, 58:772-779.
10. Driscoll KE: TNFα and MIP-2: role in particle-induced inflammation and regulation by oxidative stress. Toxicol Lett 2000, 112-113:177-183.
11. Dorger M, Krombach F: Interaction of alveolar macrophages with inhaled mineral particulates. J Aerosol Med 2000, 13:369-380.
12. Donaldson K, Tran CL: Inflammation caused by particles and fibres. Inhal Toxicol 2002, 14:5-27.
13. Donaldson K, Stone V, Borm PJ, Jimenez LA, Gilmour PS, Schins RP, Knaapen AM, Rahman I, Faint SP, Brown DM, MacNee W: Oxidative stress and calcium signaling in the adverse effects of environmental particles (PM10). Free Radic Biol Med 2003, 34:1369-1182.
14. Jimenez LA, Drost EM, Gilmour PS, Rahman I, Antonicelli F, Ritchie H, MacNee W, Donaldson K: PM(10)-exposed macrophages stimulate a proinflammatory response in lung epithelial cells via TNF-alpha. Am J Physiol Lung Cell Mol Physiol 2002, 282L237-L248.
15. Nakanishi CJ, Driscoll KE, Finkelstein JN, Bags R, O’Reilly MA, Carter J, Geleijn R, Oberdorster G: Pulmonary chemokine and mutagenic responses in rats after subchronic inhalation of amorphous and crystalline silica. Toxicol Sci 2000, 56:405-413.
16. Forman HJ, Torres M: Redox signaling in macrophages. Mol Aspects Med 2001, 22:189-208.
17. Tao F, Gonzalez-Flecha B, Kobzik L: Reactive oxygen species in pulmonary inflammation by ambient particles. Free Radic Biol Med 2003, 35:327-340.
18. Barrett EG, Johnston C, Oberdorster G, Finkelstein JN: Antioxidant treatment attenuates cytokine and chemokine levels in murine macrophages following silica exposure. Toxicol Appl Pharmacol 1999, 158:211-220.
19. Naarala J, Kasanen JP, Pasanen P, Pasanen AL, Liitamanen A, Pennanen S, Liesivuori J: The effects of wood dusts on the redox status and cell death in mouse macrophages (RAW 264.7) and human leukocytes in vitro. J Toxicol Environ Health A 2003, 66:1221-1235.
20. Whitehead LW, Ashikaga T, Vacek P: Pulmonary function status of workers exposed to hardwood or pine dust. Am Ind Hyg Assoc J 1981, 42:78-186.
21. Shamsaas MH: Pulmonary function and symptoms in workers exposed to wood dust. Thorax 1992, 47:84-87.
22. Hessel PA, Herbert FA, Melenka LS, Yoshida K, Michaelchuk D, Nukaza M: Lung health in sawmill workers exposed to pine and spruce. Chest 1995, 108:642-646.
23. Finnish Thermowood Association: Thermowood Handbook,. 2003 [http://www.thermowood.fi/data.php/200312/7954602003/1211_156/to_handbook.pdf].
24. Dorger M, Joch NK, Rieger G, Hirvonen MR, Savolainen K, Krombach F, Messmer K: Species differences in NO formation by rat and hamster alveolar macrophages in vitro. Am J Respir Cell Mol Biol 1997, 16:413-420.
25. Lee PT, Holt PG, McWilliam AS: Role of alveolar macrophages in innate immunity in neonates: evidence for selective lipopolysaccharide binding protein production by rat neonatal alveolar macrophages. Am J Respir Cell Mol Biol 2000, 23:652-661.
26. Liu S, Adcock IM, Old RW, Barnes PJ, Evans TW: Lipopolysaccharide treatment in vivo induces widespread tissue expression of inducible nitric oxide synthase mRNA. Biochem Biophys Res Commun 1993, 196:1208-1213.
27. Imrich A, Kobzik L: Flow cytometric analysis of macrophage oxidative metabolism using DCFH. Methods Mol Biol 1998, 91:97-108.
28. Shi T, Schins RP, Knaapen AM, Kuhlbusch T, Pitz M, Heinrich J, Borm PJ: Hydroxyl radical generation by electron paramagnetic resonance as a new method to monitor ambient particulate matter composition. J Environ Monit 2003, 5:350-356.
29. Frew A, Chan H, Dryden P, Salar H, Lam S, Chan-Yeung M: Immunologic studies of the mechanisms of occupational asthma caused by western red cedar. J Allergy Clin Immunol 1993, 92:466-478.
30. Seres T, Knickelbein RG, Warshaw JB, Johnston RB Jr: The phagocytosis-associated respiratory burst in human monocytes is associated with increased uptake of glutathione. J Immunol 2000, 165:3333-3340.
31. Afag F, Abidi P, Rahman Q: N-acetyl L-cysteine attenuates oxidant-mediated toxicity induced by chrysotile fibers. Toxicol Lett 2000, 117:53-60.
32. Pepperl S, Dorger M, Ringel F, Kupatt C, Krombach F: Hyperoxia upregulates the NO pathway in alveolar macrophages in vitro: role of AP-1 and NF-kappaB. Am J Physiol Lung Cell Mol Physiol 2001, 280:L905-L913.
33. Dick CA, Brown DM, Donaldson K, Stone V: The role of free radicals in the toxic and inflammatory effects of four different ultrafine particle types. Inhal Toxicol 2003, 15:39-52.
34. Brown DM, Donaldson K, Borm Pj, Schins R P, Deehnhardt M, Gilmour P, Jimenez LA, Stone V: Calcium and ROS-mediated activation of transcription factors and TNF-alpha cytokine gene expression in macrophages exposed to ultrafine particles. Am J Physiol Lung Cell Mol Physiol 2004, 286:L344-L353.
35. Johansson A, Lundborg M: Effects of low concentrations of 3-carene on alveolar macrophages in vitro. Toxicology 1997, 27(120):99-104.
36. Ayars GH, Altman LC, Frazier CE, Chi EY: The toxicity of constituents of cedar and pine woods to pulmonary epithelium. J Allergy Clin Immunol 1989, 83:610-618.
37. Grimm T, Schafer A, Hogger P: Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (pycnogenol). Free Radic Biol Med 2004, 36:811-822.