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ACUTE CHANGES IN SPUTUM COLLECTED FROM EXPOSED HUMAN SUBJECTS IN MINING CONDITIONS

Simon S. Wong[1], **Nina N. Sun**[1], **Hugh B. Miller**[3], **Mark L. Witten**[1], and **Jefferey L. Burgess**[2]

[1]College of Medicine, University of Arizona, Tucson, Arizona, USA

[2]College of Public Health, University of Arizona, Tucson, Arizona, USA

[3]College of Engineering, University of Arizona, Tucson, Arizona, USA

Abstract

Neprilysin *(NEP)* is a key cell surface peptidase in the maintenance of airway homeostasis and the development of pulmonary disorders. However, little information is available about the effect of particulate matter (PM) on airway *NEP*. In this controlled human exposure study, changes in induced sputum were measured in eleven subjects at baseline, overshot (OS) mucking, and diesel exhaust (DE) exposure days. Neither OS condition nor DE exposure was found to induce significant changes in total protein, but DE induced significant increases in cell numbers of macrophages and epithelium. Moreover, significant increases in soluble *NEP* were observed following OS mining dust particulates (0.43 ± 0.06, $p = 0.023$) and DE exposure (0.30 ± 0.04, $p = 0.035$) when compared with the baseline control (0.40 ± 0.03), with 42% and 31% average net increase, respectively. Pearson's correlation analyses indicated that sputum *NEP* activity were significantly associated with personal exposure product [Elemental carbon concentration, mg/m^3 X time, min. (C X T)]. Data suggest that the changes in *NEP* activity may be an early, accurate endpoint for airway epithelial injury and provided a new insight into the mechanism of the airway effects in these exposure conditions.

Keywords

Particulate matter; Diesel exhaust; Mining dust; Sputum; Neprilysin; Airways; Inflammation

Introduction

Airborne particulate matter (PM) pollution is a major risk to human health. Acute and chronic adverse effects of PM, especially association between elevated levels of ambient PM and mobility or mortality, have been demonstrated epidemiologically (Dockery et al., 1994; Samet et al., 2000; US EPA., 2002; Health Effect Institute. 2003; Nel, 2005). Studies over a broad range of geographical areas indicate that with each 10 μg/m^3 ambient PM increase, the daily mortality is augmented by approximately 1-5 % (Pope et al., 2002; Schwartz et al., 2002). Small short-term increases in PM levels, especially diesel exhaust particles (DEP),

Corresponding Author: Simon S. Wong, MD, MPH, Department of Pediatrics, College of Medicine, 1501 N. Campbell Avenue, Tucson, AZ 85724-5073, shengjun@email.arizona.edu, Phone (520) 626-6572, Fax (520) 626-4993.

have been associated with increases in symptoms of respiratory illnesses, including asthma, bronchitis and airway hyper-responsiveness (Pandya et al, 2002; Gong et al.,2003). PM exposure also causes irritation (Rudell et al., 1999; Wong et al., 2003), inflammation (Diaz-Sanchez et al., 1994; Salvi et al., 1999; Nightingale 2000), and functional impairment in the lung (Brunekreef, 1997; McCreanor et al., 2007). However, the mechanisms underlying these health effects still need to be elucidated and biomarkers of effect should be adopted to better address these health risks.

Neprilysin *(NEP*, also known as neutral endopeptidase, enkephalinase, and CALLA*)* is a key cell surface peptidase which plays an important role in the maintenance of homeostasis and in the development of many disorders including asthma, chronic obstructive pulmonary disease (COPD), and lung cancer (Borson, 1991; Di Maria et al., 1998; D'Adamio et al., 1989; Djokic et al., 1989). *NEP* is abundantly expressed on airway epithelial cells, and is also presented in airway smooth muscle cells, submucosal gland cells, and fibroblasts in the lung (Baraniuk et al., 1995). *NEP* is also present on immune-inflammatory cells, such as macrophages and neutrophils. Its substrates include neurokinins, cytokines, endothelins, angiotensin-II, bombesin, gastrin-releasing peptide, atrial natriuretic peptide, enkephalins, insulin-B chain, and the chemotactic peptide N-formyl-Met-Leu-Phe. These substrates play important roles in numerous physiological and pathophysiological processes, including inflammatory processes (Bozic, 1996; Lotz et al.,1988), airway hyperresponsiveness (Wu & Lee, 1999; Lilly et al., 1994), and carcinogenesis (Papandreou et al., 1998; Usmani et al, 2000; Suzuki et al., 2001; Tomoda et al., 2003). When *NEP* activity is inhibited, its substrates are less rapidly inactivated and accumulate in the tissue, thus contributing to the exaggerated response or individual susceptibility to environmental stressors (Dusser et al., 1989). Moreover, *NEP* is a necessary modulator in the development of childhood asthma (Joos et al., 2000), not only because of the vulnerable nature of developmental processes, but also because their airway sensory innervations develop rapidly during early postnatal life in parallel with the developing lung (Hislop et al., 1990). These studies taken together suggest that loss or a decrease of *NEP* may possibly be involved in the mechanisms of PM-induced effects (Di Maria et al., 1998; Joos et al., 2000).

NEP activity is reduced by mechanical removal of the epithelium, some virus infections, and cigarette smoke. Several of the stimuli known to induce bronchoconstrictor responses in asthmatic patients have been found to decrease airway NEP activity (Di Maria et al., 1998). Little information is available about the effect of PM on airway *NEP* and its relevancy to PM-induced health effects. Our *in vivo* study has first demonstrated that *NEP* activity in rat lung was significantly reduced by the ambient level of diesel exhaust for three weeks (DE, Wong et al., 2003; 2007). Because of its high density of *NEP* expression in airway epithelium and important regulatory role, it is not surprising that reduction in *NEP* activity is accompanied with increases in bronchopulmonary plasma extravasation, vascular permeability, cytokine expression, as well as inflammatory/mast cell infiltration, possibly evoked by endogenous peptides after DE exposure. In this human investigation, we evaluated acute changes in airway *NEP* activity in human subjects following exposure either to mining dust particulates or to DE, a major source of ultrafine particles. It is hypothesized that airway tissue *NEP* activity is decreased in these mining exposure conditions, as

indicated by increases of soluble *NEP* activity in induced sputum. This hypothesis was generated based on the following evidence: 1) soluble forms of *NEP* activity have been detected in body fluids, including BAL fluid (Van Der Velden et al., 1999). These soluble counterparts may either be derived from shedding of the entire membrane-bound enzyme or may be formed by post-translational cleavage of membrane-bound form. Therefore, induced sputum could provide an ideal, simple method of testing soluble and cellular *NEP*; 2) Expression of *NEP* varies widely in 'normal human lung' tissue from different individuals (Cohen et al., 1996), which could, at least in large part if not all, be attributed to environmental factors including PM exposure. 3) Furthermore, a significant decrease in *NEP* activity in lung tissue has been demonstrated after repeat exposure of rats to the ambient and occupational levels of DE (Wong et al., 2003).

In an effort to test this hypothesis, we found that soluble *NEP* activity in sputum of subjects significantly increased, indicating loss of airway *NEP* activity following acute mining dust particulate or DE exposure. Changes in *NEP* activity may be through the epthelial membrane injury, possiblly being independent of pre-inflammary response of cytokins reported in our previous publication (Burgess et al., 2007).

Methods

Experimental design

The current study was approved by the University of Arizona (UA) Institutional Review Board. The study methods are described in greater detail in a previous publication (Burgess et al., 2007). Informed consent was obtained from all subjects volunteering to participate in the study. Mining students undergoing undergraduate and graduate training in mining engineering at UA were eligible for participation if they were 18 year old or greater. Students, who were current or previous smokers, had existing lung diseases, and who were taking inhaled steroids, were excluded. Eleven subjects, 10 males and one female, ranging in age from 19-33 (mean 23.7 ± 4.3 years), completed the study (Table 1). Seven (64%) of the subjects described themselves as White, 2 (18%) as Hispanic, and one each as Asian and other. None of the subjects were current asthmatics, and no subjects reported taking anti-inflammatory medications. At baseline and before exposure experiments, none of the subjects reported having had a cold, flu, allergies or respiratory symptoms within the previous six days. These subjects had no records having exposed to significant high emission exhaust or room dust, drilling mist, and other particulate sources within one month. The study was carried out at the San Xavier Mining Laboratory, a research and training faculty devoted to occupational health and safety in the mining and underground construction industries, operated under the auspices of the UA College of Engineering, in collaboration with the UA College of Public Health. Mine access and ventilation are designed to simulate underground conditions found in an actual production facility. Respiratory protection was not worn during the experimental process. Changes of cell numbers by type, protein, *NEP* activity in collected sputum of subjects were evaluated following mining dust particulate and DE exposure that characteristics two major health risks during mucking (removal of ore) operations.

Exposure condition

Two different exposure conditions were used in this study:

1) Mining dust particulate exposure was characterized following overshot (OS) mucking process with a pneumatic (no emission) Eimco 12B OS Mucker. Experiments were conducted in 3 m × 3 m drifts and a 15 hp axial auxiliary fan provided ventilation for the heading. Personal particulate exposure was performed during OS mucking, using SKC aluminum cyclones in the subjects' breathing zone attached to sampling pumps (SKC AirChek 2000, Eighty Four, PA). The pumps were calibrated at 2.5 SL/min, with an expected 50% cut size of 4.0 μm. Pre- and post-sampling flow rates of the pumps were within 95%. Particulate sample measurements were collected on preweighed PVC filters and analyzed by gravimetric analysis (Cahn 21 Automatic Electrobalance, Ventron Corporation, CA). Gravimetric sampling results were corrected for changes in weight of field blanks collected each sampling day. Particles concentration during OS mucking indicated a mean of 500 ± 770 $\mu g/m^3$ and a range of 0 to 1,520 $\mu g/m^3$. Sampling times averaged 110 minutes (range 81-199 minutes).

2) DE exposure was characterized from a diesel-powered 1984 Jarvis Clark JS-220 load-haul-dump (LHD) vehicle with a two cubic yard bucket and an 82 HP Deutz F6L-912W diesel engine fitted with a catalytic converter. Experiments conducted using the LHD were employed in a conventional 4 m × 4 m tunnel decline and employed the same type of ventilation as that of OS mucking. During baseline and DE exposure, two samples, as particulate background, collected over a period of 66-68 minutes demonstrated concentrations of less than10 $\mu g/m^3$. Diesel exhaust particles (DEP) were collected on precleaned 37mm open-face quartz fiber filters (SKC, Eighty Four, PA) with MSA personal sampling pumps (Escort Elf, Pittsburgh, PA) and analyzed for elemental carbon according to NIOSH method 5040 by the Wisconsin State Hygiene Laboratory (Madison, WI). Personal exposure to DE, as measured by elemental carbon (N = 11) averaged 538 ± 512 (range 91-1800) $\mu g/m^3$ (Table 1). Exposure times averaged 89 (range 56-134) minutes. Nitrogen dioxide and carbon monoxide concentrations were assessed with a MSA multi-gas detector (Mine Safety Appliance Company, Pittsburgh, PA) during a single mucking shift using the diesel powered LHD. For a single experimental shift monitored for 60 minutes, peak concentrations for NO_2 and CO respectively were 1.5 ppm and 22 ppm.

All subjects underwent three evaluations, including a baseline non-exposure day, an OS (non-DE) mucking day, and an LHD (DE exposure) mucking day. These exposure days for all but two subjects per exposure (three day for OS exposure, four days for DE) were at least one week apart. First sputum induction and a health history and exposure questionnaire were completed on non-exposure days. On mucking days, groups of 1-3 subjects first completed an interim health history and mucked for a 1-2 hour period, depending on individual available non-class period, using either OS or an LHD unit. One hour following cessation of mucking, the subjects completed sputum induction. One hour post-exposure test time was chosen to consider the timelines of both the acute response of airway and diffusion of soluble *NEP* activity. Mucking using the LHD and OS mucker involve low levels of physical activity, since the energy required for moving rock is provided by the piece of

equipment used, not the operation. However, the contribution of low level physical exertion to the changes seen in airway NEP activity could not be determined in the current study design and is therefore a limitation that should be further examined in future studies

Sputum induction and treatment

Induced sputum was collected using DeVilbiss Ultra-Neb 99HD ultrasonic nebulizers (Somerset, PA) filled with 3% saline set on maximum output Sputum samples were diluted with 10% Sputolysin (Calbiochem, San Diego, CA) in phosphate buffered saline with penicillin-streptomycin and 0.5% bovine serum albumin. Supernatant was removed by centrifugation and frozen to −80 °C for later analysis of *NEP* activity. The cellular pellet was reconstituted in 1 ml of the phosphate buffered saline in order to perform total cell counts with the use of a hemocytometer and Trypan Blue stain (Sigma Chemical CO, St. Louis). A portion of the cell pellet was cytocentrifuged (Shandon Cytospin, ThermoShandon, Pittsburgh, PA) onto a microscope slide and stained with Diff-Quik® (Dade Behring AG, Switzerland) for cell number analysis. Protein concentration was determined using a Coomassie Plus Protein Assay (Pierce, Rockford, IL) using BSA as a standard.

NEP enzyme activity measurement

Cell-free *NEP* activity in sputum was measured spectrophotometrically by a coupled assay as described previously (Wong et al., 2004). Briefly, five μl of cell-free extract were incubated with 1 mM succinyl-Ala-Ala-Phe-p-nitroanilide (Suc-Ala-Ala-Phe-pNA) (Bachem Bioscience Inc., King of Prussia, PA) as a substrate in 0.1 M Tris-HCl (pH 7.6) and 1 μl (0.14 units/μl) of porcine kidney aminopeptidase N (Sigma, St. Louis, MO). The reaction (total volume: 250 μl) was measured in duplicate in a 96-well microtiter plate. In this coupled activity assay, *NEP* cleaves Suc-Ala-Ala-Phe-pNA between Ala and Phe, yielding Phe-pNA. AP-N subsequently cleaves Phe-pNA, generating pNA as the final product. The increase in specific absorbance at 405 nm (as a result of the accumulation of free p-nitroaniline) was determined after 30 min incubated at 37 °C using a plate reader (BIO-TEK Instruments, Winooski, Vermont). Cell-free (substrate alone or substrate with AP-N) and substrate-free blanks were run in parallel. Protein concentration was determined by using a Coomassie Plus Protein Assay (Pierce, Rockford, IL) with BSA as a standard.

Statistical Analysis

Statistical analyses were performed using SPSS version 15 (Chicago, Illinois). ANOVA and/or *t*-test was used to compare means of sputum *NEP* activity, total protein, and cell profile among groups which were normalized as appropriate if not following a Gaussian distribution. Additionally, Pearson's correlation coefficient was calculated to determine whether there is a linear relationship either between personal exposure and *NEP* activity or betwwen NEP activity and cell types. Data were expressed as mean ± standard error of the mean (SEM) and $p < 0.05$ will be considered to be significant (2-tailed).

Results

Cell profile

Exposure of subjects to OS or DE induced an increased trend of inflammatory cells (Table 2). For DE exposure, there was a significant increase in total cell number in sputum. The increase in cell type was macrophages, but not neutrophils and lymphocytes following DE exposure. However, the changes in all of these measurements did not reach a statistical significant level after OS exposure, possibly due to limited sample size. Data suggests that DE exposure resulted in macrophage-related pre-inflammatory or inflammatory response in airways.

Moreover, sputum epithelia were significantly increased by 3.34-fold and 4.44-fold following exposure of subjects to OS and DE, respectively (Table 2). Obviously both exposure conditions resulted in acute airway epithelial shedding.

Total Protein and NEP activity

Neither OS exposure (0.89 ± 0.07, mean ± SEM) nor DE exposure (0.88 ± 0.08) was found to induce a significant change in total sputum protein when compared with baseline control (0.82 ± 0.09) (Table 3). Analysis of soluble *NEP* activity in sputum was done for all 11 healthy nonsmoking volunteers before exposure as baseline. The result showed a range of values from 0.115 to 0.445, averaging 0.303 ± 0.036 (mean ± SEM) nmol/μg protein/minute. Individual increases in *NEP* activity were observed to be 72.7% (8/11) following OS exposure and 81.8% (9/11) following DE exposure (Table 3). When compared with baseline control, the average net increases in OS and DE exposure groups were 42% and 31%, respectively, with maximum changes 0.53 and 0.26 nmol/μg protein/minute. The changes indicated that a decrease or loss of airway *NEP* activity occurred after acute exposure of subjects to either ~500 $\mu g/m^3$ concentrations of $\mu g/m^3$ mining dust particulates (OS) or ~538 $\mu g/m^3$ elemental carbon of DE (including particles background <10 $\mu g/m^3$). To further determine the effects of OS and DE on soluble *NEP* activity in sputum, an effort were made with Pearson's correlation analyses. The result indicated that individual sputum *NEP* activity after DE exposure, but not baseline exposure, were significantly associated with products of exposure concentration X exposure time (Figure 1). However, the association degree between OS exposure and *NEP* activity was not intensify enough to induce a significant change statistically in current sample size.

Associations of sputum NEP activity with cell types

To further analyze the cellular sources effect of *NEP* activity, Pearson's correlation analyses was carried out. Data showed that sputum *NEP* activity was not significantly associated with changes in total cells number and cell types in sputum, including macrophages, neutrophils, and epithelial cells (Data not shown).

Discussion

Mining workers in underground frequently face two major occupational health risks, one from mining dust particulates and another from diesel exhaust. To characterize their acute

respiratory effects, we conducted the current controlled human exposure study in the San Xavier Mining Laboratory, a research and training faculty devoted to occupational health and safety in the mining and underground construction industries. Our previous publication has been shown that acute changes in sputum IL-10 following exposure of human volunteers to DE, while other sputum measurements including IL-1β, IL-4, IL-6, IL-8, TNF-a, 8-OHdG did not significantly change under OS and DE exposure conditions due to sampling time and limited sample size (Burgess et al., 2007). Also, there were no significant changes in cross-shift spirometry, as measured by FEV1 (3.81 ± 0.90 vs. 3.75 ± 0.90, $p = 0.367$ for OS exposure; 4.03 ± 0.99 vs. 3.89 ± 1.01, $p = 0.08$ for DE exposure) and FVC(L) (4.93 ± 1.16 vs. 4.84 ± 1.25, $p = 0.307$ for OS exposure; 5.16 ± 1.25 vs. 5.00 ± 1.34, $p = 0.178$ for DE exposure).

In this paper, we report that there is acute loss of *NEP* activity from airway tissue after exposure of these subjects to either mining dust particulates or DE, as indicated by increases of soluble *NEP* activity in sputum. This finding was similar to the results of our previous animal study, showing that *NEP* activities were consistently reduced in rat lungs exposed to the ambient (35.3 μg/ m^3 particles) and occupational (669.3 μg/ m^3 particles) levels of DE for three weeks (Wong et al., 2003). Although the relevancy and the mechanism underlying this effect remain to be determined, a decrease or loss of *NEP* activity in airways may be an early and important endpoint for these exposures. Obviously, changes in *NEP* activity occurred before current known preinflammatory cytokine biomarkers (such as IL-6 and IL-8), oxidative indicator (8-OHdG), and spirometry measurements. More importantly, its change may be mechanistically linked to many adverse effects in the lung, being observed in our previous animal studies (Wong et al., 2003: Witten et al., 2005).

The decrease or loss of *NEP* activity in airways could change its substrates-induced response pattern and degree, consequently resulting in inflammatory response. It is well known that *NEP* effectively controls the bioavailability of peptide mediators released via sensory nerve terminals or immuno-inflammatory cells. A critical factor that governs tissue response for *NEP* is its distribution density and co-location with the peptide receptors on cell membrane. When a decrease or loss of *NEP* activity from airway tissue, accumulating peptides rapidly diffuse into tissue, leading to an abnormal neuron-immune communication (Dusser et al., 1989; Wu & Lee, 1999). The resulting effects of these peptides, especially from sensory fibers, are characterized by obvious vasodilatation, increased postcapillary venule permeability, inflammatory cell influx, and mucus secretion, collectively termed as neurogenic inflammation. Therefore, it is possible that the exposure-induced change in *NEP* activity in this study may indirectly be associated with an increase in inflammatory cells through its substrate mechanisms above (Lilly et al., 1994; Lu et al., 1996; 1997).

Moreover, soluble *NEP* (especially extracellular domain) might possibly enter into the blood system with loss of airway epithelial barrier integrity, leading to systemic effects. This speculation is supported by several previous studies that increased *NEP* activities in serum were observed from underground miners exposed to coal dust particles (Soleilhac et al., 1996) or patients with pulmonary inflammatory diseases, such as sarcoidosis and adult respiratory distress syndrome (Johnson et al., 1985; Almenoff et al.,1986). Increased *NEP* activities in serum may reflect local tissue damage with subsequent shedding of membrane-

bound enzymes and an abnormal release from the airways in response to lung injury (Soleilhac et al., 1996). In this study, significant increases of soluble *NEP* activity following exposures suggest that membrane-bound *NEP* in airways was modified or impaired structurally and functionally. Therefore, it is reasonable to consider serum *NEP* as a potential biomarker in future studies if the systemic contribution of *NEP* was characterized in future studies.

The cellular origin and mechanism of soluble *NEP* activity in sputum of exposed people are multiple. In this case, airway epithelial cells could be major source of soluble *NEP* activity found in sputum due to exposure-induced membrane damage. The reasons for this speculation are 1) Epithelial cells not only abundantly express *NEP* that was directly targeted by inhaled particles and/or gases; *2*) *NEP* structurally is a cell-surface metalloprotease with a large extracellular domain (700 amino acids), which contains six potential N-glycosylation sites and the pentapeptide consensus sequence (His-Glu-[Ile, Leu, Met]-X-His) of zinc-binding metalloproteases, in which the two histidines coordinate zinc and the glutamic acid. Obviously, its large body of extracellular domain with the catalytic sequence not only illustrates its critical structural basis to rapidly cleave substrates, but also to possibly be highly susceptible to toxic insults. Therefore, a most likely mechanism is the shedding process of affected epithelial cells in airways after exposure, with membrane-bound proteins being released with portions of plasmic membrane or as proteolipid aggregates. In addition, inflammatory cells, especially affected neutrophils and macrophages during degranulation after exposure, might be another possible resource of soluble *NEP* activity in sputum. However, we did not find in this study that the increase in sputum *NEP* activity was correlated with changes of either neutrophils or macrophages numbers following both exposure conditions. Increases in cell numbers of total, macrophages, and epithelium just occurred in DE exposure, but not OS exposure, suggesting the changes in *NEP* activity seems to be independent of increased inflammatory or epithelial cells. Taken together, we speculate that increase in sputum *NEP* activities of subject may be attributable more to non-shedding epithelial cells after airway injury induced by exposure conditions.

This study provides a novel endpoint and insight into a mechanism for the airway adverse effects in mining conditions. We realize that it dose not suffice to actually determine if lower exposures at realistic enviromental concentrations have an impact on *NEP* activity. Also, it unlikely that this human study alone will suffice to experimentally test whether exposure to realistic levels of PM cause a decrease in *NEP* activity that has functional consequences for airway biology due to limited sample size. In this regard, we are conducting the animal study to establish how the expression pattern of *NEP* is changed in tissues and BAL fluid with *NEP* null mice. We hope that soluble *NEP* activity in sputum could serve as potential early biomarker to identify population risk if these results are further confirmed by *in vivo* animal and larger human population of investigations at the ambient exposure level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

NEP	Neprilysin
DE	diesel exhaust
DEP	Diesel exhaust particle
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
IL	Interleukin
PM	particulate matter
LHD	load-haul-dump
OS	Overshot
SEM	standard error of the mean
COPD	chronic obstructive pulmonary disease
UA	University of Arizona
8-OHdG	8-hydroxy-2′-deoxyguanosine

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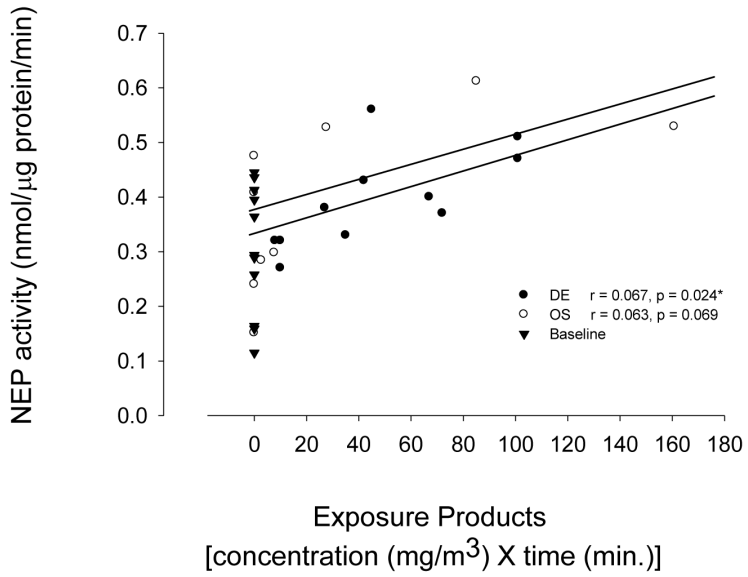

Figure 1.

Correlations of soluble neprilysin *(NEP)* activity in subject sputum (N =11) with mining dust particulates (OS) and diesel exhaust (DE) exposure [concentrations, mg/m^3 X time, min. (C X T)]. DE exposure was not corrected for pre-exposure levels in *NEP* activity (Data not shown). Cell-free *NEP* activity in induced sputum was measured spectrophotometrically. Personal particulate exposure was performed during OS mucking, using SKC aluminum cyclones in the subjects' breathing zone attached to sampling pumps (SKC AirChek 2000, Eighty Four, PA). DE was collected on precleaned 37mm open-face quartz fiber filters (SKC, Eighty Four, PA) with MSA personal sampling pumps (Escort Elf, Pittsburgh, PA) and analyzed for elemental carbon according to NIOSH method 5040 by the Wisconsin State Hygiene Laboratory (Madison, WI). *Statistically significant.

Table 1

Characteristics of Subjects and Exposure Conditions

Subjects	Age (years)	Gender	Race Codes[a]	Exposure Conditions (mg/m^3)			
				Baseline[b]	OS	DE	Time (min.)
01	26	M	1	0.01	0.46	1.20	60
02	33	M	4	0.01	0.00	0.64	66
03	19	M	1	0.01	1.52	1.80	56
04	21	M	1	0.01	–	0.75	134
05	22	M	1	0.01	1.20	0.50	134
06	23	M	1	0.01	–	0.38	119
07	21	F	1	0.01	0.00	0.32	85
08	19	M	1	0.01	0.09	0.12	85
09	30	M	2	0.01	0.00	0.38	93
10	26	M	3	0.01	0.00	0.15	66
11	22	M	2	0.01	0.03	0.09	90

[a] Race codes: 1, White; 2, Hispanic; 3, Asian; 4, Others;

[b] Two samples collected over a period of 66-68 minutes as baseline particulate background for all of subjects;

-Subject had no personal particulate exposure data due to handling.

Table 2

Sputum cell profiles in subjects[a] exposed to mining dust or diesel exhaust

	Cell Numbers[b]		
Cell types	**Baseline**	**OS[c]**	**DE[d]**
Inflammatory cells ($X10^6$/ml)			
Total	7.98 ± 1.68	12.53 ± 3.28	20.29 ± 7.65*
Macrophages	6.53 ± 1.32	10.00 ± 2.61	16.10 ± 5.57*
Neutrophils	1.29 ± 0.49	2.23 ± 0.68	4.05 ± 2.32
Lymphocytes	0.15 ± 0.05	0.22 ± 0.07	0.21 ± 0.08
Epithelial cells ($X10^3$/ml)	2.80 ± 0.823	9.34 ± 5.27*	12.43 ± 7.98*

[a] Eleven healthy subjects underwent three evaluations, at least one week apart, at a non-exposure (baseline) day, a dust, non-DE, exposure (OS) day, and a DE exposure (DE) day;

[b] Cell number analysis was performed with Diff-Quik® (Dade Behring AG, Switzerland) on a microscope slide;

[c] Mining particles sample were collected on pre-weighed PVC filters and analyzed by gravimetric analysis;

[d] DE exposure levels were measured by elemental carbon according to NIOSH method 5040;

* $p < 0.05$ when compared to Baseline (N = 11).

Table 3

Induced sputum protein and Neprilysin (NEP)

	Total protein (μg/μl)[a]			NEP (nmol/μg protein/min)[b]		
	Baseline	**OS**	**DE**	**Baseline**	**OS**	**DE**
	1.05	0.95	1.07	0.364	0.527	0.369
	0.66	0.96	0.99	0.445	0.475	0.431
	1.00	0.90	0.97	0.436	0.612	0.507
	1.27	1.30	1.21	0.258	0.380	0.465
	0.41	0.63	0.84	0.395	0.529	0.399
	0.56	0.62	0.43	0.294	0.824	0.558
	0.82	1.08	1.13	0.413	0.408	0.272
	1.16	0.84	0.79	0.159	0.298	0.382
	0.74	0.95	0.92	0.289	0.240	0.332
	0.94	1.09	1.00	0.164	0.151	0.324
	0.41	0.47	0.32	0.115	0.284	0.320
Mean	0.82	0.89	0.88	0.303	0.430	0.396
SEM	0.09	0.07	0.08	0.036	0.058	0.026
p-values*		0.24	0.42		0.023	0.035

[a] Protein concentration was determined using a Coomassie Plus Protein Assay using BSA as a standard.

[b] Cell-free *NEP* activity in induced sputum was measured spectrophotometrically;

* *p*-values based on a two-tailed, paired sample t-test (N = 11).