

Proteomics analysis revealed changes in rat bronchoalveolar lavage fluid proteins associated with oil mist exposure

Yung-Shan Lee¹, Pang-Wei Chen¹, Perng-Jy Tsai¹, Shu-Hui Su², Pao-Chi Liao^{1,*}

¹Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan

²Department of Physiology Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

E-mail: liaopc@mail.ncku.edu.tw

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The association between oil mist exposure and adverse health effects has been of increased concern.

Previous studies indicated that exposure to fumes emitted from cooking oils appeared to be an important risk factor for lung cancer in Taiwanese women [1]. Occupational exposure to oil mist derived from metalworking fluids through inhalation may cause a variety of chronic and acute respiratory diseases or symptoms, such as chronic bronchitis, hypersensitivity pneumonitis, asthma, acute airway irritation, and impaired lung function [2]. The technique of bronchoalveolar lavage (BAL) has been used to collect the cellular and soluble components of the lower respiratory tract for more than two decades. Centrifugation of BAL samples allows separating cells from the supernatant bronchoalveolar lavage fluid (BALF) which contains a large number of soluble proteins comprising a potential resource to study the respiratory disorders. Several studies have investigated the changes in BALF protein patterns using two-dimensional gel electrophoresis (2-DE) combined with mass spectrometry.



The aim of this study was to investigate changes in rat BALF proteins following exposure to oil mist generated from cutting oil, a metalworking fluid used to improve cutting performance and extend tool life, in a fastener-manufacturing factory using nano-high performance liquid chromatography electrospray ionization tandem mass spectrometry (nano-HPLC-ESI-MS/MS). Due to the invasive characteristics of BAL sampling, a rat model was used to study the effect of oil mist exposure. Rats were exposed to cutting oil mist generated from the thread rolling process at a fastener-manufacturing factory for 21 days. The rats in the control group exposed little oil mist at the inventory area of the same fastener-manufacturing factory. BALF samples from both exposed and control rats were collected and subjected to nano-HPLC-ESI-MS/MS and nano-HPLC-ESI/MS for qualitative and quantitative analysis, respectively.

The quantification of protein levels remains an elusive but a challenging goal for proteomic research. Traditional 2-DE suffers from high degree of gel-to-gel variation as well as variable linear dynamic range of spot intensity limited by different staining methods, resulting in problematic detection and quantification of differences in protein expression. To achieve absolute quantification a way to employ

isotopically labelled “internal standards” that are added to the sample before digestion has been frequently used. However, this method is laborious and limited by the availability of the isotopically labelled internal standards. We proposed here an alternative “label-free” strategy for relative quantification without the use of isotope labeling at low analytical costs. A simple strategy for relative quantification of protein expression was developed and its experimental scheme is shown in Figure 1. Using transforming growth factor alpha (TGF- α), a significantly up-regulated protein associated with oil mist exposure found in this study, as an example, the strategy for protein quantification is exemplified here. In the first phase of the experimental scheme, TGF- α was identified with two unique peptide sequences from the pooled BALF sample using nano-HPLC-ESI-MS/MS and database searching. The MS/MS spectra for the peptide sequences matched to TGF- α by database searching are shown in Figure 2. In the second phase of the experimental scheme, the LC-MS signals of these two peptides from individual BALF sample at the retention time and mass to charge ratio given in the first phase were detected and subjected to integration of peak intensity. Concerning the potential problem of signal drifts resulting in problematic signal quantification, the variation of peptide intensities were examined before performing further data analyses. The coefficients of variation of the two TGF- α peptides for both control and exposure group were between 29 and 47%, suggesting that the problem of signal drifts cannot be negligible and signal normalization is necessary for quantification purpose.

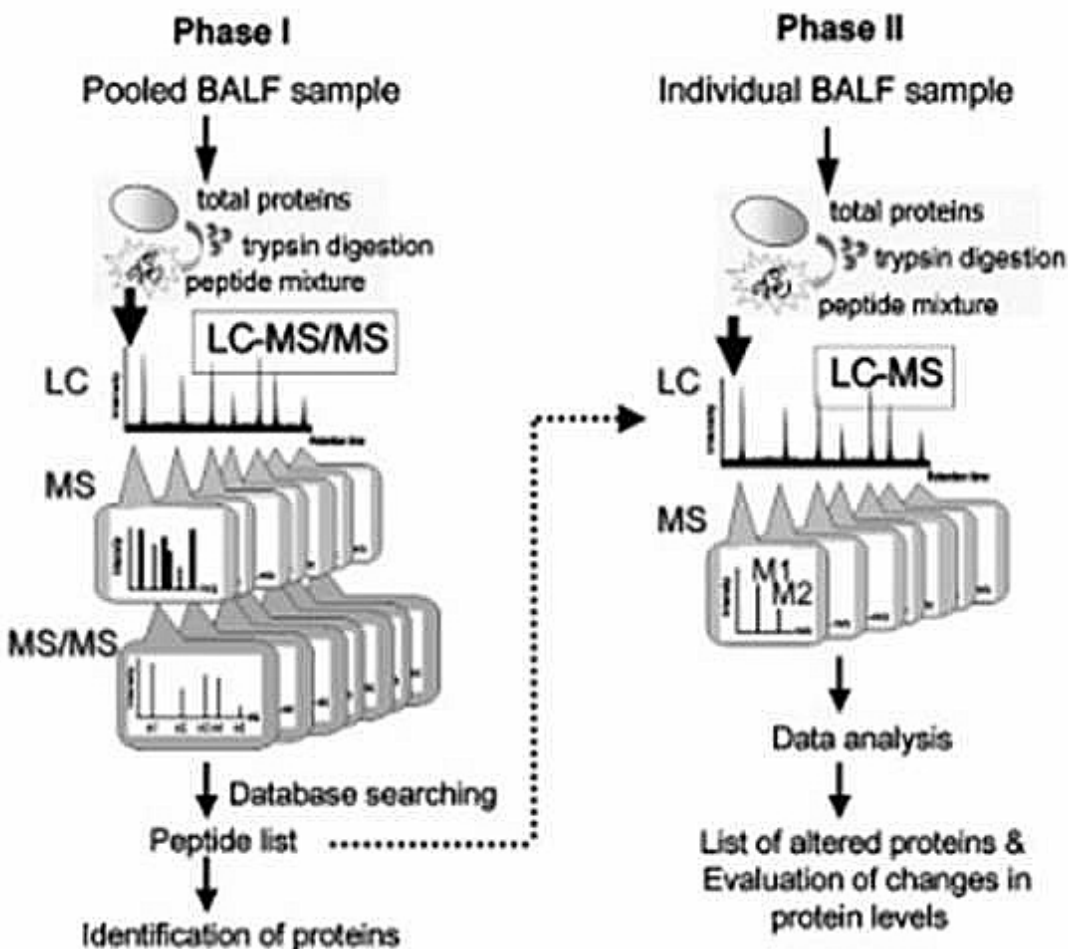


Fig 1. Experimental schemes of evaluation of changes in BALF protein levels used in this study.

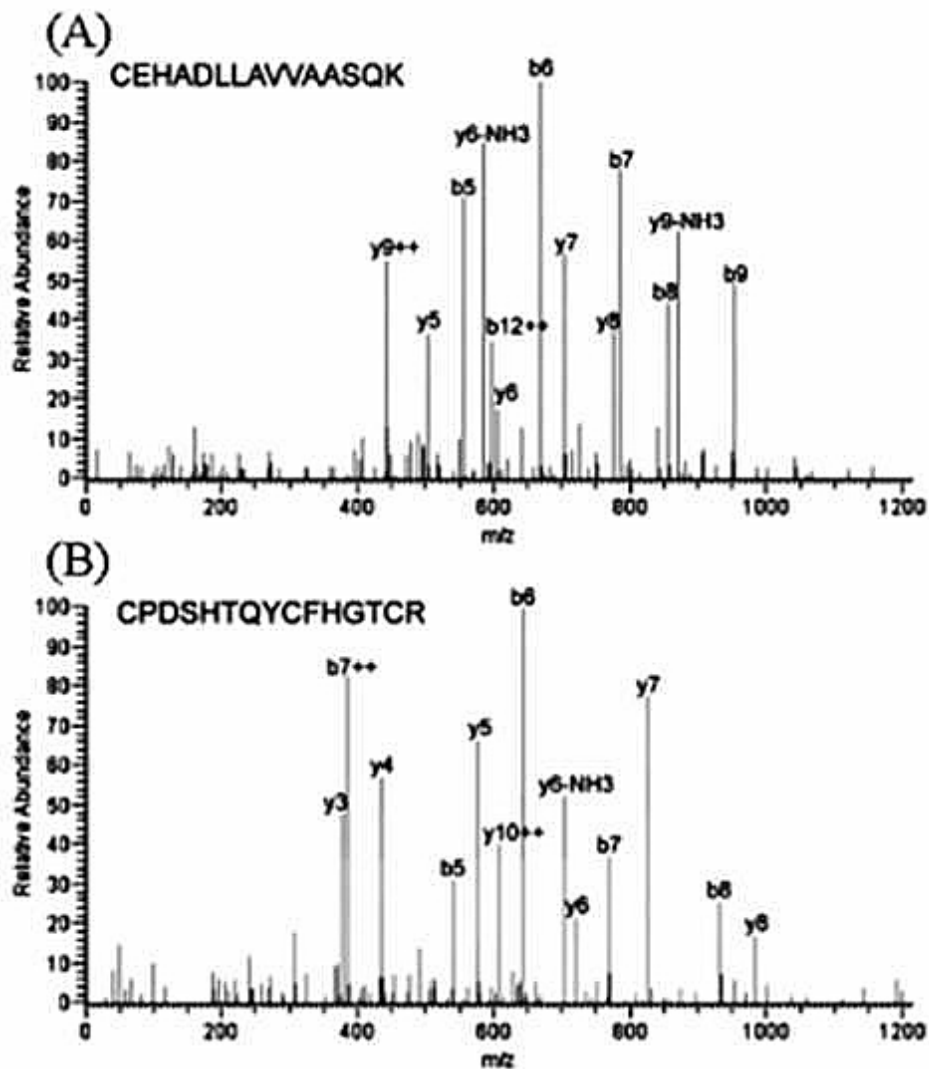


Fig 2. MS/MS spectra for the peptide sequences matched to TGF- α in pooled BALF sample. Sequence of the peptides as deduced from spectrum and database searching is shown on each panel. (A) MS/MS spectrum for the matched peptide sequence of CEHADLLAVVAASQK. (B) MS/MS spectrum for the matched peptide sequence of CPDSHTQYCFHGTCR. Peaks matched as b and y ions of the peptide sequence are labeled.

A frequently used normalization method is that the intensity of each peptide is presented in terms of percent intensity by using total peptide intensities as the normalization factor. However, this normalization factor also includes signals from peptides with significant changes due to exposure effect. This implies that using this method may reduce signal variability to some extent but under- or over-estimate the effect of exposure. To avoid this problem, the normalization method used in this study was based on a statistical method to select signals that did not contain signals from peptides with significant changes due to exposure effect. Therefore, a nonparametric Wilcoxon rank-sum test was performed on the median values of the triplicate measurements of each BALF sample to test the differences between exposure and control group for each peptide ($p < 0.05$ was considered significant). The summation of intensities from peptides without significant changes ($p > 0.05$) between exposed and control animals was used as the normalization factor in this study. Thus the intensity of each peptide was divided by this normalization factor to obtain the normalized peptide intensities. The coefficients of variation of the two TGF- α peptides for both control and exposure group after normalization, for example, were between 6 and 14%. In general, the coefficients of variation for peptide intensities in exposure and control group

were significantly reduced from 20-60% to within 20% by conducting the process of signal normalization (Figure 3). This suggested that the normalization method used in this study could effectively reduce the quantification interferences from between-run signal drifts.

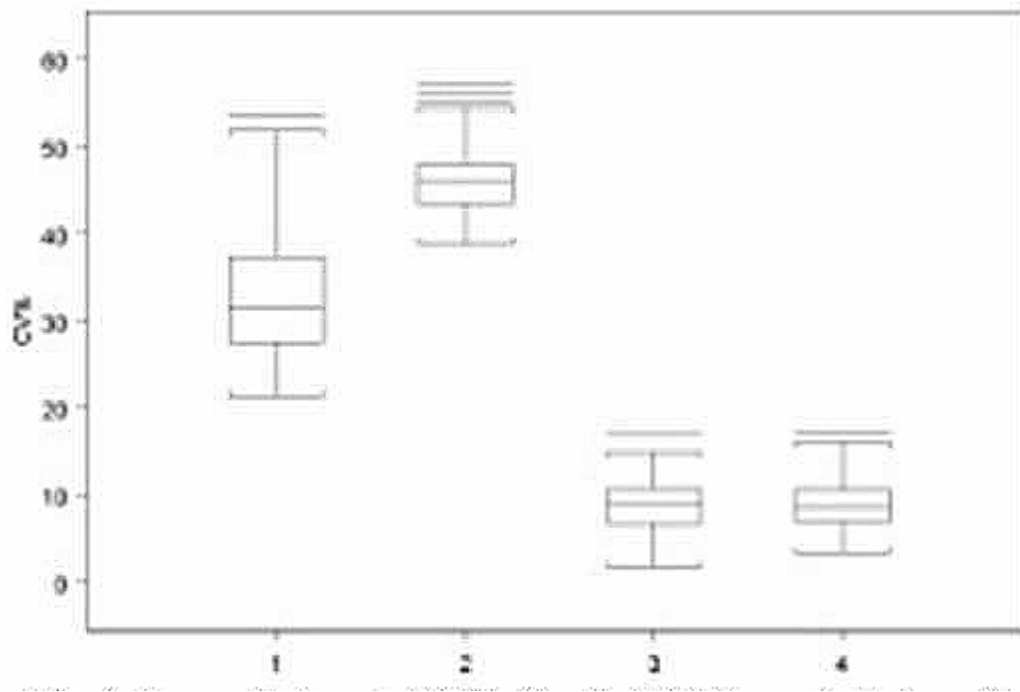


Fig 3.Box plots of CV% for LC-MS peak intensities in both control and exposure group before and after signal normalization. (1)Control group, original peak intensities; (2) exposure group, original peak intensities; (3) control group, normalized peak intensities; (4) exposure group, normalized peak intensities. CV% was calculated for original or normalized LC-MS peak intensities from six exposed or six control rats, each with tripli-cate LC-MS measurements. For each measurement, the LC-MS signals for 294 peptides were detected. Box plots display the distribution of CV% for 294 peptides.

In the last step of data analyses, the Wilcoxon rank-sum test was performed again on the normalized peptide intensities to test the differences between exposure and control group ($p < 0.05$ was considered significant). For the two identified TGF- α peptides, the changes in peptide levels following oil mist exposure were 5.05 and 3.93, respectively. Then the geometric mean of the exposure/control ratio for each peptide of the protein was calculated to represent the relative changes in protein levels after exposure. Following this method, the changes in protein level of TGF- α after oil mist exposure was 4.46 (the geometric mean of 5.05 and 3.93), which means that the level of TGF- α in exposed rats was 4.46-fold higher than that in controls.

The result obtained from the first phase of analysis using LC-MS/MS and database searching (phase I, in Figure 1) revealed that 69 proteins from pooled rat BALF samples. Using the “label-free” relative quantification strategy (phase II, in Figure 1), 29 proteins exhibited significantly altered levels after exposure to cutting oil mist. Among these proteins, 22 were up regulated and 7 were down regulated. These altered proteins can be roughly classified as surfactant-associated proteins, inflammatory proteins, growth factors, calcium-binding proteins, and others. To our knowledge, this is the first report to investigate changes in BALF proteins following oil mist exposure. The results obtained from this study are of considerable interest for understanding the mechanisms involved in the oil mist-induced lung effects.

Reference

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