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Risk assessment of Nitrogen Dioxide exposure on Welders

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This study evaluated the hazard caused by NO_2, an oxidant generated in the process of welding. We compared hematological and biochemical parameters in workers who chronically inhale NO_2 and office workers not exposed to NO_2. NO_2 exposure affected the hematological and biochemical parameters. Increasing NO_2 concentrationincreased the number of leukocytes, while decreasing the number of erythrocytes. Blood urea nitrogen, creatinine, uric acid, and lactate dehydrogenase were increased, while total protein and triglycerides were decreased. The mean concentration of NO_x(NO_2^-/ NO_3^-) in the serum of welders and the control group was 35.97±2.85 and 55.40±5.81 μmol/L, respectively. The difference was significant (p<0.05), although NO_2^- was not detected in the serum.

Key Words : Nitrogen dioxide, Hematological factors, Biochemical factors, Welders

1. Introduction

Nitrogen dioxide is a red-brown, strong-smelling, oxidative gas that produces free radicals[1]. It is generated during combustion in power plants and industrial facilities, which are fixed sources of contamination, and from internal combustion engines of vehicles using fossil fuels, which emit NO. NO is rapidly oxidized to NO_2, a highly toxic gas[2~4]. NO_2 is an indoor air pollutant generated by smoking, cooking, and heating appliances, which are the main sources of human exposure[5,6].

In the work environment, exposure to nitrogen oxides, including NO_2, occurs with the use of nitric acid and during welding using an oxy-acetylene flame. The main pathway into the body is inhalation via the respiratory organs[2]. Exposure to NO_2 affects the immune system, decreasing the number of lymphocytes.

Welding, which is widely used in industry, causes many hazards to the health of welders[7~9].

Welding generates NO_2, which is inhaled. Ninety percent of the inhaled NO_2 is absorbed in the tracheobronchial tree and respiratory region. Acute exposure to NO_2 causes a cough, headache, fatigue, eye irritation, respiratory difficulty, pneumonia, asthma, and vesicular emphysema[10]. According to Matthew *et al.* (1996)[11], abnormal immune function also results.

This study examined hematological and biochemical parameters in welders chronically exposed to NO_2 in the work environment and the serum NO_x concentration in order to evaluate the hazard of vocational exposure to NO_2.

2. Materials and Methods

2.1. Materials

Thirty-six male arc welders and 40 male office workers (no occupational exposure), between the ages of 45 and 50, who worked in a shipyard and the assembly of metal accessories, participated in this study.

2.2. NO_2 in workplace air and serum

NO_2 samples were collected from the respiratory region of workers using a triethanolamine coated passive tube (SKC 226-40, USA) with a personal air sampler (MSA, Pittsburgh, PA, USA) at a flow rate of 0.2 L/min. The absorbance at 540 nm was measured with a UV/VIS spectrophotometer (UV-2201, Shimadzu, Japan) and quantified.

NO_x in the serum was analyzed using an NO-analyzing system (ENO-20, Eicom Corp., Kyoto, Japan) and the analytical conditions are shown in Table 1.

Methanol (Sigma, HPLC grade) was added to a

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serum sample, which was deproteinized, and centrifuged at 12,000 *g* for 15 min. The supernatant was analyzed. Nitrite (NO_2^-) and nitrate (NO_3^-) ions were separated in a column packed with polystyrene polymer. The NO_2^- was reacted form a purple diazo compound; the flow rate of the mobile phase was 0.33 L/min and that of the Griess reagent was 0.1 L/min. The absorbance was measured at 540 nm. NO_3^- was analyzed using a cadmium reduction column after reduction to nitrite[12].

2.3. Analysis of hematological parameters

Blood was collected from 36 welders and 40 office workers at the Industrial Health Center at Inje University. Vacutainer tubes containing EDTA (ethylenediaminetetraacetic acid) were used to collect samples under standard conditions and stored at room temperature. The numbers of erythrocytes and leukocytes were counted with an automatic cell counter (Micros 60, France).

2.4. Analysis of biochemical parameters

Blood was collected in Vacutainer tubes containing gel and clot activator, stored at room temperature for 30 min, centrifuged at 2,500 *g* to separate the serum, and stored at -70℃ until analysis. The concentrations of creatinine, total protein, albumin, uric acid, triglycerides, BUN (blood urea nitrogen), LDH (lactate dehydrogenase), and HDL (high density lipoprotein) were measured with a biochemical analyzer (LISA, France).

2.5. Statistical analysis

The analysis and statistical processing of data were performed with the program SPSS (ver. 10.0) and the mean and standard error were calculated. Student's *t*-test was used to compare the results and the level of significance was $p<0.05$.

3. Results

3.1. NO_2 in the workplace air and serum NO_x

The concentrations of NO_2 inhaled by the welders were analyzed. The mean concentration that welders in the shipyard and metal assembly company were exposed to was 0.025±0.002 and 0.020±0.001 mg/m^3, respectively. The difference was thought to result from the amount of welding and work intensity (Table 2).

The NOxconcentration in serum was analyzed with an NO-analyzing system (ENO-20, Eicom Corp., Kyoto, Japan) and standard solutions ($NaNO_2$ and $NaNO_3$) were purchased from Sigma Co. Chromatograms of standard NO_2^- and NO_3^- (10 μmol/L) and that for the serum of a welder are shown in Figs. 1(A) and 1(B), respectively. The standard NO_2^- / NO_3^- curve is shown in Fig. 2.

The total NO_x concentration, which is the product of metabolism, was calculated as the sum of the nitrite (NO_2^-) and nitrate (NO_3^-) concentrations. The serum NO_x concentration is shown in Table 3.

The NO_x concentration in welders and the control group was 35.972.85 and 55.40±5.81 μmol/L, respectively, and the difference was significant ($p<0.05$). Tsujii *et al.*[13] and Giroux *et al.*[14] reported increased nitrates in the serum of animals and humans and the NO_2^- and NO_3^- concentrations were increased in patients with generalized infections[15]. We did not detect

Table 1. The analytical condition of NO_x

Column	Eicom, ENO-20
Column	NO-PAK(packed with polystyrene polymer, 4.6×50 ㎜) NO-RED(cadmium reduction column)
Mobile phase	10% methanol (containing 0.15 M NaCl-NH4Cl,0.5 g/L of EDTA · 4Na)
Flow rate	0.33 ㎖/min
Injection volume	5 ㎕
Detection wavelength	540 nm

Table 2. The mean concentration of NO_x to inhalation exposure in welding workplace

Type of industry	NO_x (mg/m^3)
Manufacture of shipment part company	0.025±0.002
Manufacture of assembly metal component	0.020±0.001

Values are mean±standard error

NO_2^- in serum (Table 3). Therefore, we postulate that NO_2^- reacted with oxy-hemoglobin in whole blood and was oxidized rapidly to NO_3^-, as reported in Moshage[16]. Many researchers have concluded that NO_3^- is stable in the synthesis of NO, while NO_2^- is difficult to detect.

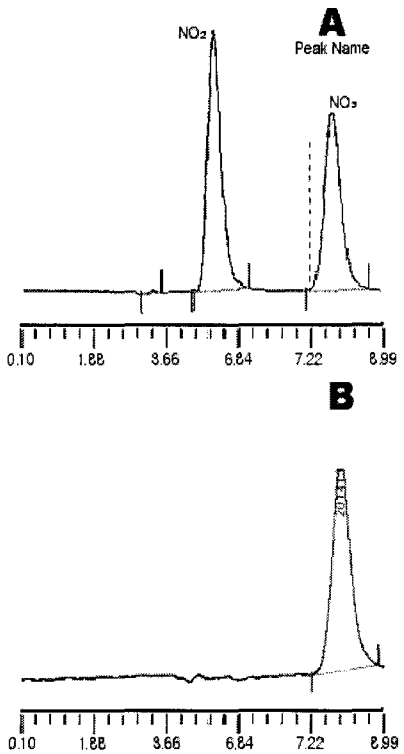

Fig. 1. Chromatogram of the standard NO_2^- and NO_3^- solution(A) and NO_x analysis in serum prepared from welder and control persons(B).

3.2. Hematological parameters

The results of the blood analysis are shown in Table 4. The number of leukocytes in the exposure and control groups was 7.78±0.37 and 6.69±0.21×10^9 cells/L, respectively, and the difference was significant (p<0.05). A previous study reported a decrease in immune function in the respiratory organs and inflammation, with increased erythrocytes in the peripheral blood and bronchoalveolar lavage (BAL) fluids[17]. Rutowski[18] reported that NO_2 and NO exposure increased lymphocytes and leukocytes in smokers and non-smokers. We saw a similar trend in the welders. The number of erythrocytes in the welders and office

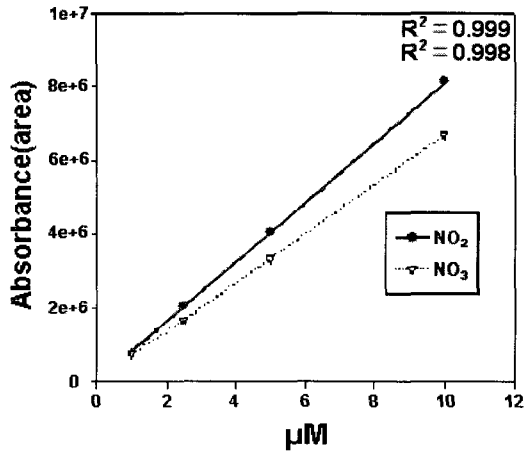

Fig. 2. Standard curve of NO_2^- and NO_3^-.

Table 3. The concentration of NO_2^- and NO_3^- in serum of welder and control group(unit : ㎛ol/L)

	NO_2^-	NO_3^-	Total NO_x	p value
Exposure	ND	35.97±2.85	35.97±2.85	0.002*
Control	ND	55.40±5.81	55.40±5.81	0.002*

Values are mean±standard error
Significant difference from controls *(p <0.05)
ND : Not detected

Table 4. The effect of NO_2 inhalation exposure on the hematological factor in exposure and control group

Factor	Exposure	Control	p value
Leukocyte (10^9 cells/L)	7.78±0.37	6.69±0.21	0.009*
Erythrocytes (10^{12} cells/L)	4.57±0.06	4.77±0.06	0.019*

Values are mean±standard error,
Significant difference from controls *(p<0.05)

workers was 4.57±0.06 and 4.77±0.06×10^{12} cells/L, and the decrease was significant (p<0.05).

Posin *et al.*[19] reported that exposure to 1 mg/L NO_2 significantly decreased the acetylcholinesterase activity in erythrocyte cell membranes and exposure to 2 mg/L NO_2 increased the super oxidation of erythrocytes.

3.3. Biochemical parameters

The biochemical parameters of the welders and office workers are shown in Table 5. The total serum protein in the welders and office workers was 8.10± 0.11 and 8.42±0.11 g/dl, respectively, and the decrease was significant (p<0.05). This counters the result of William *et al.*[20], and may be due to the response to long-term exposure in welders. This needs to be studied further. The albumin concentration in welders and office workers was 4.30±0.02 and 4.25±0.01 g/dl, respectively. A study by Frampton *et al.*[21] found no change in the total protein and albumin concentrations in BAL fluid with NO_2 exposure.

BUN is formed in the urea cycle with the deamination of amino acids. The BUN in welders and office workers was 13.55±0.59 and 9.53±0.45 mg/dl, respectively, and the increase was significant (p<0.05). The creatinine, an index of adrenal function, in welders and office workers was 0.79±0.02 and 0.75± 0.01 mg/dl, respectively (p<0.05). Giroux and Ferrieres[14] obtained a similar result.

The triglyceride concentration in welders and office workers was 104.90±6.86 and 134.90±10.61 mg/dl, respectively (p<0.05). The concentration of uric acid, the final product of purine metabolism, in welders and office workers was 5.45±0.14 and 4.95±0.19 mg/dl, respectively (p<0.05). By contrast, Kelly *et al.*[22] stated that uric acid recovered to normal levels 24 h after exposure. Our result was judged to be due to the long-term exposure of welders to NO_2, and further research is necessary.

The LDH in the respective groups was 175.90± 10.62 and 154.87±3.42 IU/L, and the increase was significant (p<0.05). William *et al.*[20] reported a similar result. The HDL in welders and office workers was 57.62±2.35 and 56.50±1.81 mg/L, respectively. The difference was not significant.

4. Discussion

NO_2 affected the hematological parameters, significantly(p<0.05) increasing the number of leukocytes in welders. This might be the result of a change in NO_2, which triggers inflammation, a bio-defense mechanism to produce oxidants. The effects of chronic exposure to low concentrations should be studied. The number of erythrocytes was significantly decreased in the welders (p< 0.05).

NO_2 decreased the protein and triglycerides in welders, while the BUN, generated in the urea cycle, and creatinine, an index of renal function, were significantly increased. This suggests that the high NO_2 concentrations in the exposure group break down tissue proteins and disrupt adrenal function.

The serum LDH was significantly increased in the

Table 5. The biochemical factor in welder and control group for NO_2 inhalation exposure

Factor	Exposure	Control	p value
Total protein (g/dℓ)	8.10±0.11	8.42±0.11	0.048*
Albumin (g/dℓ)	4.30±0.02	4.25±0.01	0.032*
BUN (㎎/dℓ)	13.55±0.59	9.53±0.45	0.000*
Creatinine (㎎/dℓ)	0.79±0.01	0.75±0.01	0.027*
Triglyceride (㎎/dℓ)	104.90±6.86	134.90±10.61	0.037*
Uric acid (㎎/dℓ)	5.45±0.14	4.95±0.19	0.040*
LDH (IU/L)	175.90±0.62	154.87±3.42	0.041*
HDL (㎎/L)	57.62±2.35	56.50±1.81	0.417

Values are mean±standard error,
Significant difference from controls *(p<0.05)
Abbreviations, BUN ; blood urea nitrogen,
LDH ; lactate dehydrogenase,
HDL ; high-density lipoprotein,
IU ; international unit.

welders ($p<0.05$), suggesting that NO_2 exposure affects the cells in the respiratory organs. The serum HDL was increased in welders, but the difference was not significant.

The mean serum NO_x concentration (NO_2^-/ NO_3^-) in welders and the control group was 35.97±2.85 and 55.40 ±5.81 μmol/L, respectively, and the decreased was significant ($p<0.05$). Nevertheless, NO_2^- was not detected in the serum, since NO_2^- reacts with oxy-hemoglobin and is oxidized rapidly. The exposure to NO_2 varied with welding time and work intensity. Welders at the shipyard inhaled 0.0250.002 mg/m^3 versus 0.020±0.001 mg/m^3 at the metal component manufacturer.

In summary, the increased leukocyte number in serum, but decreased the erythrocyte count in blood and NO_x concentration in serum. Moreover, total protein and triglycerides decreased, while BUN, creatinine, uric acid, and LDH were increased.

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