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Disulfide stress in carbon monoxide poisoning

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ABSTRACT

Objectives: Carbon monoxide (CO) remains the most common cause of lethal poisoning around the world. The purpose of this study was to investigate the homeostasis between thiol-disulfide couples and to evaluate oxidative status comprehensively in acute CO poisoning, using new parameters along with other well-known oxidant-antioxidant molecules.

Design and methods: This case study consisted of 43 subjects who were diagnosed with carbon monoxide poisoning and 35 healthy individuals who were used as controls. Thiol-disulfide paired tests were examined in both groups using the method developed recently.

Results: Patients with CO poisoning had significantly higher levels of serum disulfide than the control patients (20.7 ± 5.03 versus 16.43 ± 3.97, $p = 0.001$). Native thiol and total thiol levels were lower in the CO patient group than in the control group ($p < 0.001$, for each variable). The disulfide/native thiol ratios and disulfide/total thiol ratios were significantly higher, while native thiol/total thiol ratios were significantly lower, in patients with acute CO poisoning than in the healthy controls ($p < 0.001$, for all ratios). The disulfide/native ratios were negatively correlated with both total antioxidant response and paraoxonase and arylesterase values and were positively correlated with total oxidant status and ceruloplasmin values ($p < 0.05$, for all correlations).

Conclusions: Excessive disulfide levels and their related ratios were found in CO poisoning patients. In particular, the disulfide/native thiol ratio was identified as an indicator for overall oxidative status. Among CO poisoning patients, the thiol-disulfide balance was found to be impaired. Therefore, the disruption of thiol-disulfide homeostasis might be involved in CO toxicity.

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1. Introduction

Carbon monoxide (CO) poisoning that results in injury or death is the cause of more than half of poisonings worldwide [1]. The recognition of CO poisoning proves to be challenging because of CO poisoning's nonspecific signs and symptoms [2]; therefore, the true morbidity and mortality rates are considered to be higher due to the misdiagnosis or non-recognition of CO poisoning [1]. CO poisoning is called the "silent killer," owing to its odorless, colorless, and non-irritating properties [3]. CO is produced by incomplete combustion of carbon-containing compounds. Motor vehicle exhaust gases, forest fires, gas line leakage, insufficient ventilation of flame-based heaters, and industrial paints are all common sources of CO [4].

The potential mechanisms of CO toxicity depend on the hemoproteins, cytochrome oxidase, and cytochrome p450 systems [5]. Formation of carboxyhemoglobin (COHb) results in tissue hypoxia and ischemia. Impaired perfusion and CO-related cellular damage generate CO toxicity [6]. CO has directly toxic effects in the electron transport chain of mitochondria via binding cytochrome oxidase. This disturbance in the respiratory chain triggers oxidative stress and decreases glutathione levels [7]. Inhibition of the mitochondrial enzymes leads to lipid peroxidation on membranes [6]. Moreover, CO also binds platelets' hemoproteins, inducing nitric oxide (NO) release. Enhanced NO generates peroxynitrites and results in nitrosative stress [5]. Intravascularly, CO induces leukocyte sequestration, platelet-neutrophil aggregation, and neutrophil degranulation. Therefore, CO causes free radical production, apoptosis, and lipid peroxidation that results in endothelial dysfunction [4].

The perturbation of the cell redox balance occurs when reactive oxygen species are generated excessively, and antioxidants are not able to counteract this overproduction [8]. In the normal oxidative metabolism, reactive oxygen species are produced by the mitochondrial electron transport chain, and oxygen generation increases by the time electron transport is inhibited [9]. Thiols are known to be the most important and essential antioxidant buffers, which interact with almost all physiological oxidants [8]. Low antioxidant capacity and accompanying

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oxidative stress results from the decreased glutathione and oxidized glutathione ratio (GSH/GSSG) in the intracellular environment [10].

Thiols exist in proteins, including albumin and other thiolated proteins, or in low molecular-weight molecules, such as glutathione and homocysteine [10]. Groups of thiol proteins go through oxidation reactions when oxygen molecules are present, and the proteins turn into reversible forms called disulfide bridges (–S–S–). This form can be reduced to thiols again [11]. Thus, thiol-disulfide homeostasis occurs. The thiol-disulfide couple maintains a homeostatic intracellular and tissue redox status [12]. Dynamic thiol-disulfide homeostasis acts in cellular signal mechanisms, antioxidant defense, detoxification, apoptosis, inflammation, and immune response [13]. In addition, impaired thiol-disulfide status is implicated in many diseases [14,15].

Until recently, just one side of the thiol-disulfide balance was measured. However, using the latest testing methods, both sides of the balance can be detected, and the thiol-disulfide status can be completely evaluated [12]. The primary aim of this study was to examine thiol-disulfide homeostasis, which performs a crucial role in CO toxicity, through thiol-disulfide paired tests. A secondary purpose of this study was evaluating the oxidative status of patients with CO poisoning through new parameters along with well-known oxidant-antioxidant parameters, including total antioxidant response (TAR), total oxidant status (TOS), paraoxonase (PON), arylesterase (ARES), and ceruloplasmin. Currently, there are no other studies related to thiol-disulfide exchanges in acute CO poisoning, and this study comprises the first report in this area.

2. Materials and methods

2.1. Study protocol

Forty-three patients (20 male, 23 female) with acute CO poisoning when admitted to the emergency department were enrolled in the study. Diagnosis of acute CO poisoning was based on the patients' history, clinical and laboratory findings, and patients' COHb levels [3]. Subjects were excluded from the study if they exhibited ischemic or hemorrhagic stroke, central nervous system disorders, head trauma, or other known probable unconsciousness causes, were suffering from any existing systemic, infectious, or inflammatory diseases, such as diabetes mellitus, cardiac disorders, anemia, hypertension, or showed evidence of any renal, hepatic, or gastrointestinal diseases. Additionally, all patients who were smokers, abused alcohol, or took any medications were excluded from the study. The control group comprised 35 healthy individuals (14 male, 21 female): a detailed medical history was taken and a normal physical examination performed for each subject. None of the subjects was taking vitamin supplements. Study groups were matched in terms of age and sex. The study protocol was approved by the local ethics committee, and written informed consents were obtained from all participants prior to involvement in the study.

Venous blood samples were taken via venipuncture from all of the subjects and drawn into tubes. Specimens were processed by centrifugation at 1800*g* for 10 min. After the separation, serum samples were instantly frozen and kept at −80 °C until testing.

Serum thiol-disulfide pair tests were measured through a novel spectrophotometric method [12] with an automated analyzer (Roche, cobas 501, Mannheim, Germany). In this method, disulfide bridges were reduced to free thiols via the reductant sodium borohydride. The remaining sodium borohydride was exhausted using formaldehyde. Then, the reduced thiol groups and existing native thiols were analyzed with 5, 5′-dithiobis-(2-nitrobenzoic) acid (DTNB). This resulting measurement gave the total thiol amounts. The disulfide amounts were calculated as half of the subtraction of native thiol from total thiol concentrations. Once the native thiol levels, total thiol levels, and disulfide amounts were determined, the disulfide/native thiol [(–S–S–)/(–SH)], disulfide/total thiol [(–S–S–)/(–SH + –S–S–)], and native thiol/total thiol [(–SH)/(–SH– + –S–S–)] ratios were calculated.

Total antioxidant response, total oxidant status, and serum ceruloplasmin levels were determined by the automated methods described by Erel [16–18]. PON and ARES serum activity were measured by using commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey).

Albumin and total protein levels were detected with commercially available assay kits (Roche, Mannheim, Germany) with an auto-analyzer (cobas 501, Roche, Mannheim, Germany). COHb level measurements were performed in a blood gas analyzer (Rapid point500, Siemens, Munich, Germany). Normobaric oxygen treatment using 100% oxygen was provided to all patients with acute CO poisoning, and none of the participants received hyperbaric oxygen therapy.

2.2. Statistical analysis

SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Data distribution was examined by both visual (histograms, probability plots) and statistical methods (the Kolmogorov-Smirnov test and the Shapiro-Wilk test). As the variables showed normal distribution, independent sample t tests were applied to compare the parameters between groups using mean and standard deviation. Correlation analyses were performed using Pearson's correlation co-efficient. In all analyses, the outcomes were considered statistically significant if p value was <0.05.

3. Results

The subjects' demographic and clinical characteristics appear in Table 1. There were no statistically significant differences between the groups in terms of age and sex. Serum albumin and total protein levels were similar between the two groups, and the differences between mean albumin and total protein levels were not statistically significant in the two groups ($p > 0.05$). In all patients, CO exposure occurred as a result of defective heating systems. There was no loss of consciousness when patients arrived at the hospital. In addition, no mortality occurred.

Table 2 and Fig. 1 indicate the thiol-disulfide profiles of all participants. In the acute CO poisoning study group, native and total thiol levels were significantly lower than in the control group ($p < 0.001$, for both). When the two groups were compared, based on disulfide levels, there was a significant difference between the groups ($p =$

Table 1
Clinical characteristics and some laboratory findings of the study population.

	CO poisoning ($n = 43$)	Control group ($n = 35$)	p Value
Age (year)	34.56 ± 19.05	33.83 ± 12.53	NS*
Gender (male/female)	20/23	14/21	NS
Albumin (g/dl)	4.41 ± 0.47	4.53 ± 0.24	NS
Total protein (g/dl)	7.38 ± 0.63	7.37 ± 0.36	NS
COHb (%)	19.68 ± 6.94	0.89 ± 0.86	$p < 0.001$

Values are mean ± SD.
$p < 0.05$ was accepted as statistically significant.
CO, carbon monoxide; COHb, carboxyhemoglobin.
* NS, non-significant.

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