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High-mobility group box 1 protein levels in serum of subjects after exposure to fire smoke — short communication

Stężenie białka HMGB1 w surowicy osób narażonych na dymy pożarowe

The authors declare no financial disclosure

Abstract

Introduction: Fire smoke inhalation a recognized etiologic factor of airway injuries. The objective of this study was evaluation of serum high-mobility group box 1 (HMGB1) protein concentration in subjects exposed to fire smoke (SEFS).

Material and methods: The study group consisted of 40 consecutive patients admitted to the Toxicology Unit, Lodz, Poland after exposure to fire smoke. Serum HMGB1 concentrations were measured upon admission to hospital and rechecked on the 2nd and on the day of discharge. Patients also underwent routine toxicological diagnostic procedures applied in case of those exposures, such as carboxyhaemoglobin (COHb) levels and urinary thiocyanate concentrations. The same diagnostic tests were performed in 10 healthy volunteers not exposed to smoke of the control group.

Results: The average serum SEFS concentration of HMGB1 protein was not significantly higher on admission in comparison with the respective values recorded on the 2nd day and on the day of discharge. The mean serum level of HMGB1 protein of exposed group was higher than that one in the control group, however the difference was not statistically significant. The highest concentration of HMGB1 protein was noted in serum of 28 subjects exposed to fire smoke reporting at least one symptom and the difference was statistically significant in a comparison with the control group.

Conclusion: As indicated, an acute exposure to smoke may lead to transient increase of HMGB1 in serum in exposed subjects. Further studies are necessary in order to confirm the importance of this protein in pathogenesis of acute airway injury due to exposure to fire smoke.

Key words: HMGB1 protein, lung toxicity, fire

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Streszczenie

Wstęp: Narażenie na dymy pożarowe jest udokumentowaną przyczyną toksycznego uszkodzenia układu oddechowego. Celem badania była ocena stężeń białka HMGB1 w surowicy osób narażonych na dymy pożarowe.

Materiał i metody: grupę badaną stanowiło 40 osób narażonych na dymy pożarowe, które zostały przyjęte na oddział toksykologii w Łodzi. Stężenia białka HMGB1 oznaczono w dniu przyjęcia do szpitala oraz ponownie w drugim i ostatnim dniu hospitalizacji. U pacjentów wykonano także badania toksykologiczne zlecane przy tego typu narażeniu: stężenie karboksyhemoglobiny i stężenie rodanów w moczu. Podobny panel badań zlecono u 10 zdrowych osób (grupa kontrolna) nienarażonych na dymy pożarowe.

Wyniki: Stężenia białka HMGB1 w grupie pacjentów narażonych na dymy pożarowe nie różniły się istotnie statystycznie w analizowanych dniach hospitalizacji, jak i z wartością wymienionego parametru ocenianego w surowicy osób z grupy kontrolnej. Stężenie białka HMGB1 w surowicy krwi 28 osób narażonych na dymy pożarowe zgłaszających obecność co najmniej jednego objawu chorobowego w pierwszej dobie hospitalizacji było istotnie wyższe niż w materiale biologicznym osób z grupy kontrolnej.

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Wnioski: Prezentowane wyniki badań mogą wskazywać na specyficzność działania HMGB1 jako mediatora reakcji zapalnej w drogach oddechowych osób narażonych na czynniki drażniące uwalniane podczas pożaru.

Słowa kluczowe: białko HMGB1, uszkodzenie płuc, pożar

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Introduction

Exposure to the variety of chemical agents emitted during fire accidents may result in acute airway injuries [1]. Nitrogen and sulphur dioxides, aldehydes, carbon monoxide (CO) and halogenated hydrocarbons constitute the major volatile toxins released during the combustion and pyrolysis processes [1]. Such exposures may lead to the development of inflammation of upper and lower airways. According to Abraham et al. HMGB1 protein is a mediator of acute inflammatory lung injury [2]. Intratracheal instillation of HMGB1 in mice produces acute injury that is manifested by neutrophil accumulation, lung edema and increase in pulmonary cytokine levels, such as: TNF, IL-1 β and MIP-2 [2]. This pro-inflammatory cytokine is actively released from such cells as: macrophages, monocytes, NK cells, dendritic cells, endothelial cells and platelets [3]. HMGB1 is also released into the extracellular compartment by necrotic and damaged cells. Then it consequently modulates the innate immune response as a nuclear protein [4].

The question addressed by the present study was whether the extent of acute respiratory tract injury caused by acute exposure to fire smoke corresponds with alterations in serum HMGB1 levels.

Material and methods

Subjects and exposure

Forty consecutive patients from a large town were admitted to the Toxicology Unit between January and December 2009 directly after accidental exposure to fire smoke (subjects exposed to fire smoke, SEFS). The main source of exposure was uncontrolled fire in dwelling house or flat, resulting from careless handling stoves, cigarettes or charcoal grills. Patients spent at least 15–20 minutes in the atmosphere containing a mixture of toxic gases and fumes produced by the combustion and pyrolysis of some plastics, wood, fabric and other substrates. No assessment of smoke particles diameter was performed at the place of accident.

The average age in the examined group was 49.75 ± 16.48 years. Sixteen of them (40%) were smokers at the time of study or in the past.

The control group consisted of 10 healthy individuals and 5 of them (50%) smoked cigarettes at the moment of study or before.

The Regional Bioethical Committee approved the study protocol and all the participants submitted their written consent prior enrolling them to the study.

Clinical symptoms

Each patient was examined for the presence and intensity of following symptoms: cough, wheeze, sputum production, exertional dyspnoea, and ocular, nasal and pharyngeal symptoms as well. The intensity of these symptoms was evaluated using scoring: 0 points for lack of symptom, 1 point — for mild expression, 2 points — for strong manifestation.

Laboratory tests

Carboxyhemoglobin (COHb) level and urinary thiocyanate levels were evaluated on admission to the hospital.

HMGB1 determination

HMGB1 in serum was measured using commercially available enzyme-linked immunosorbent assay (ELISA) (IBL International GMBH, Germany). Sensitivity of the method was 1.0 ng/ml.

Statistical analysis

Results are expressed as mean values \pm SD.

Results of toxicological tests at the 1st day of hospitalization in the group of SEFS and control group were compared using the Mann-Whitney test.

The results of tests for HMGB1 performed at the 1st, 2nd day and the day of discharge for the studied group were matched against those one obtained in the control group using the Tamhane's test.

The p values < 0.05 were considered significant.

Results

In the studied group of 40 subjects, twenty eight patients (70%) complained of at least one symptom associated with the exposure. The most frequently observed initial clinical pathology

in this group was a manifestation of lower airways pathology that was reported by 21 patients (52.5% of studied cohort). Nasal or pharyngeal symptoms were recorded in 14 (35%) cases and symptoms associated with conjunctivitis in 9 (22.5%) cases (all data presented in the article published in 2013 [5]). The intensity of symptoms on admission was characterised as mild by 18 subjects and as abundant was marked by 10 patients, respectively. Only 5 subjects reported the intensity of analyzed symptoms as mild on the day of discharge.

Discussed symptoms occurred in the group of 16 smokers with different frequency. Particularly, ocular ones were reported by 6 (37.5%) subjects, those of upper airways (nasal/pharyngeal) by 10 (62.5%) cases and dyspnea/cough by 8 (50%) subjects, respectively. Six smokers did not complain of any symptom due to exposure to fire on the day of admission (the first day of hospitalization).

The presence of thermal injury to the face and/or nose was reported in 8 (20%) subjects among the analyzed group of forty patients admitted to hospital immediately after accidental exposure to fire smoke.

The average level of COHb in the studied group constituted $6.77 \pm 6.37\%$ of total haemoglobin, whereas in the control group it was $1.63 \pm 0.57\%$, respectively. The mean concentrations of thiocyanates in the group of SEFS were 5.84 ± 7.12 mg/l.

COHb levels and thiocyanates concentrations were significantly higher in the group of SEFS compared to control ($p < 0.05$) (all data presented in the article published in 2013 [5]).

The highest concentration of HMGB1 in the examined group of 40 subjects exposed to fire smoke was noted at the admission day (the 1st day of hospitalization) — 3.41 ± 4.48 ng/ml. It tended to decrease gradually to 2.86 ± 1.7 ng/ml on the second day of hospitalization and subsequently to 2.17 ± 1.93 ng/ml on the day of discharge. Mean serum HMGB1 levels in the hospitalized patients did not differ significantly from those found in serum of the controls (Fig. 1).

The concentration of HMGB1 in the serum of 28 symptomatic subjects from the exposed group was higher comparing to that of 12 asymptomatic subjects from the same group, however the observed difference did not reach statistical significance (Fig. 2). The symptomatic group included all — 8 (28.57%) patients with thermal injury of the face and/or nose.

There was observed statistically significant difference between the concentration of HMGB1 in the serum of 28 symptomatic subjects from the

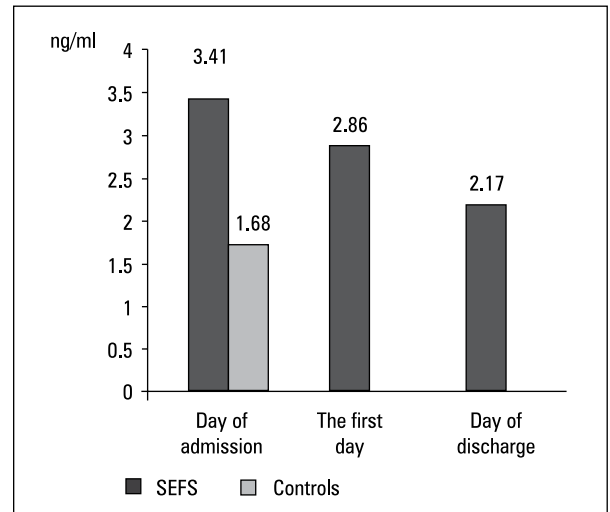


Figure 1. The concentration of HMGB1 in serum of subjects exposed to fire smoke (SEFS) and in the controls

Rycina 1. Stężenie HMGB1 w surowicy osób narażonych na dymy pożarowe oraz w grupie kontrolnej

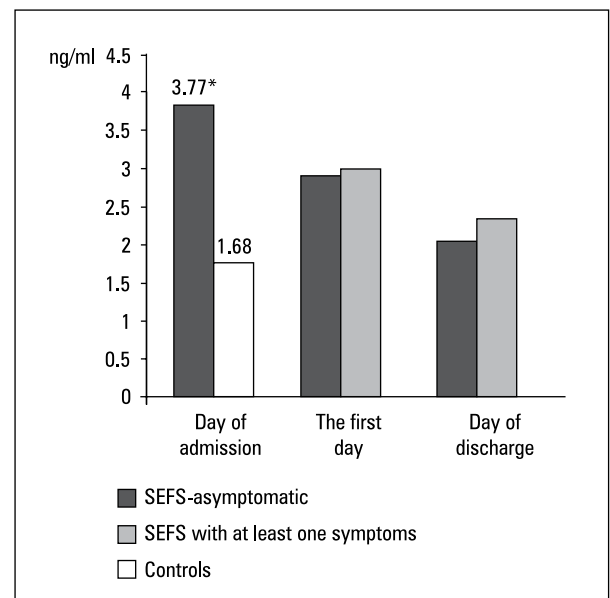


Figure 2. The concentration of HMGB1 in serum of subjects exposed to fire smoke (SEFS) (asymptomatic and with at least one symptom) and in the controls

Rycina 2. Stężenie HMGB1 w surowicy osób narażonych na dymy pożarowe (osoby bez objawów klinicznych i z co najmniej jednym objawem chorobowym) oraz w grupie kontrolnej

exposed group comparing to the concentration of this protein in the control group ($p < 0.05$) (Fig. 2).

Discussion

A number of studies have dealt with the search for diagnostic tools measuring non-invasi-

vely the extent of lung inflammation for over the last decades [6, 7]. There are data showing that HMGB1 can be inducer of inflammation [8, 9]. High HMGB1 concentrations were found in the pulmonary epithelial lining fluid of patients with sepsis [10]. This protein was found as a mediator of burn injury [11] and rheumatoid arthritis [12]. Some studies have focused on promoting role of HMGB1 in cancer development and progression [13].

HMGB1 is a nuclear non-histone DNA-binding protein that serves as a structural co-factor for transcriptional regulation in somatic cells [14, 15].

There are two pathway mechanisms for cells to liberate HMGB1 into the extracellular milieu. The first one is a passive release from damaged or necrotic cells, and in this scenario HMGB1 acts as intracellular marker responsible for recognizing tissue damage and initiating reparative responses [16]. Another scenario assumes that HMGB1 may be actively secreted from immune cells and works as a proinflammatory cytokine [17]. In our study, the highest concentration of HMGB1 in the group of 40 subjects exposed to fire smoke was noted at the admission day (the 1st day of hospitalization) and it tended to decrease gradually on the second day of hospitalization and on the day of discharge, however the average concentration of HMGB1 did not differ significantly between analyzed days of hospitalization in patients exposed to fire smoke. No significant difference was also found between HMGB1 concentrations in serum of exposed subjects to fire smoke and the control ones. The average concentration of HMGB1 in the control group was significantly lower than that one measured on the first day of hospitalization in 28 symptomatic subjects. It is important to notice that in the aforesaid group there were all subjects with thermal injury of the face and/or nose. As mentioned above some authors [11] reported that up-regulation of HMGB1 expression may be involved in the pathogenesis of multiple organ damage secondary to major burns as a result of releasing endogenous endotoxins.

The higher increase of HMGB1 concentration in our study directly after the exposure to fire smoke may be explained by damage of cells in the respiratory tract, which resulted from exposure to the toxic agents emitted during the fire.

Unfortunately no data about the nature of inhaled smoke directly in the atmosphere at the place of accident were obtainable.

Relatively low levels of COHb and lack of clinical signs of carbon monoxide poisoning observed in the presented study may suggest that

in our group this kind of exposure did not play an important role, so that fact resulted in the lack of increasing HMGB1 concentrations on the following days of hospitalization.

The most frequent complaints at admission in the group of subjects exposed to fire included lower airway symptoms and similar findings were described by other authors [18, 19].

The group of SEFS showed significantly higher COHb level and thiocyanate concentration on admission compared with the control group. These two gases are released during uncontrolled fire at varied concentrations depending on the kind of substrates undergoing combustion and they may contribute to overall toxicity of fire smoke [20–22].

Conclusion

As indicated, acute exposure to smoke may lead to transient increase of HMGB1 in serum of exposed subjects. Further studies are necessary to confirm the important role of this protein in pathogenesis of acute airway injury.

Conflict of interest

The authors declare no conflict of interest.

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