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IL-6, a central acute-phase mediator, as an early biomarker for exposure to zinc-based metal fumes

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ABSTRACT

Aims: Systemic C-reactive protein (CRP) increases 1 day after short-term inhalation of welding fumes containing zinc and/or copper. The aim of the current study was to find further, possibly earlier systemic biomarkers after inhalation of different welding fumes containing zinc and traces of aluminum, with or without copper, as these metal combinations become more common in modern joining technology.
Methods: The study group consisted of 15 non-smoking male volunteers with healthy lung function data and without any occupational metal fume exposure. On 4 different exposure days, the members of the study group were exposed under controlled conditions to ambient air or 3 different welding fumes for 6 h. Spirometric and impulse oscillometric measurements and differential blood counts were performed and serum samples were collected before exposure and 6, 10 and 29 h after start of exposure. The biomarker concentrations in serum were measured by electrochemiluminescent assays.
Results: Systemic increases of IL-6 peaked significantly at 10 h compared to baseline ("ZincZinc": P = 0.0005 (median increase (m. incr.) = 1.36 pg/mL); "ZincAlu": P = 0.0012 (m. incr. = 1.48 pg/mL); "AluBronze": P = 0.0005 (m. incr.=2.66 pg/mL)). At 29 h, CRP and serum amyloid A (SAA) increased distinctively ("ZincZinc": P = 0.032 (m. incr. = 0.65 μg/mL) [CRP], 0.077 (m. incr. = 0.61 μg/mL) [SAA]; "ZincAlu": P = 0.001 (m. incr. = 1.15 μg/mL) [CRP], 0.0024 (m. incr. = 0.94 μg/mL) [SAA]; "AluBronze": P = 0.002 (m. incr. = 2.5 μg/mL) [CRP], 0.002 (m. incr. = 0.97 μg/mL) [SAA]). The median increases of CRP and IL-6 were most pronounced for the welding fume which contained besides zinc also copper (AluBronze). For differentiating AluBronze from control exposure, receiver operating characteristic (ROC) curve analysis was performed and the area under the ROC curve (AUC) for the IL-6 increases (10 h versus 0 h) was 0.931. The additional inflammatory mediators [vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), interferon-γ (IFN-γ), cell counts] and the lung function parameters did not show any significant changes after exposure.
Conclusions: Consistent with its role of the mediation of the acute-phase response, systemic increases of IL-6 after welding fume exposure peak at 10 h before the increases of the acute-phase reactants CRP and SAA at 29 h. IL-6 may represent a highly sensitive and early biomarker for the exposure to metal fumes containing zinc and copper. As IL-6, CRP and SAA are independent, strong risk markers for future cardiovascular diseases, these data may particularly be important for long-term welders with respect to their cardiovascular health.

1. Introduction

Welding fumes contain a wide range of metals and non-metals with varying toxic effects (Antonini, 2003; Antonini et al., 2003; Erdely et al., 2011). Epidemiological studies have demonstrated an increase of the prevalence in pulmonary diseases, inflammation and cardiovascular diseases after exposure to welding fumes (Antonini, 2003; Antonini et al., 2003; Ibfelt et al., 2010). An improved understanding of possible adverse health effects as well as underlying mechanisms after exposure to welding fumes is important for the risk assessment and the development of prevention strategies and will impact a large population of workers (Antonini, 2003).

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Zinc, copper, aluminum or combinations thereof become more common in modern joining technology, especially in the automotive industry (Reisgen et al., 2013). Whereas zinc oxide contained in the particulate fraction of welding fumes is generally regarded as a constituent causing acute respiratory illness (Antonini, 2003), copper fume-induced inflammation was judged to be a rare event, on the basis of insufficient scientific literature about possible adverse health effects in the year 2000 (Borak et al., 2000). Toxic effects of copper and zinc have been described by in vitro cell culture experiments (Karlsson et al., 2013; Lanone et al., 2009). Recently, a distinct increase of systemic C-reactive protein (CRP) has been shown one day after short-term inhalation of zinc- and copper-containing welding fume, from a metal inert gas brazing (MIG) process with a copper containing welding wire on zinc coated steel, at an average fume concentration of 2.5 mg m^{-3} (Hartmann et al., 2014). A subsequent study showed that similar CRP-effects were caused by welding fumes containing only zinc or, alternatively, only copper (Markert et al., 2016). In contrast to the heavy metals copper and zinc, exposure to MIG welding fumes of the light metal aluminum, a common welding additive, caused no changes of CRP (Hartmann et al., 2014). However, studies on workers exposed to aluminum welding fumes or aluminum dust have revealed disturbances of cognitive processes and changes in mood and EEG (Polizzi et al., 2002; Riihimaki and Aitio, 2012).

The aim of this study was to find further and if possible earlier biomarkers than the acute-phase protein CRP after exposure to zinc- and/or copper-containing welding fumes at a controlled average fume concentration of 2.0 or 2.5 mg m^{-3}. Zinc and copper are often used in combination, and hence we investigated the following 3 different MIG-welding/brazing fumes, which contained also traces of aluminum: i) brazing of galvanized steel using an aluminum bronze wire (Alubronze), used often for vehicle construction, especially for vehicle interiors; ii) joining of galvanized steel and aluminum using a zinc wire (ZincAlu), used more and more for multi material joints of steel and aluminum; and iii) brazing of galvanized steel using a zinc wire (ZincZinc), used more and more for brazing of components with the purpose of maintaining the zinc coating and hence corrosion protection, e.g. for cable channels.

Earlier biomarkers might be helpful for the occupational health surveillance for metal workers, particularly as potential preventive personalized biomarkers for the early recognition of inflammatory effects of concerned individuals. To this aim, we included, in contrast to earlier studies (Brand et al., 2013a; Hartmann et al., 2014; Markert et al., 2016), beside 6 h also an additional time point at 10 h after start of the short-term welding fume exposure.

Beside CRP, we included the biomarkers serum amyloid A (SAA), IL-6, interferon-γ (IFN-γ), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). CRP and SAA are positive acute phase reactants and sensitive markers of an acute inflammation. Their levels usually increase within hours after inflammatory stimuli. IL-6 is a pleiotropic cytokine that plays a central role in inflammation, host defense and tissue injury. It is a central stimulator of the acute phase response (Ganapathi et al., 1988; Kishimoto et al., 1992; Koj et al., 1988; Moshage et al., 1988). Reports about systemic IL-6 levels after exposure to zinc-oxide fume are rare and inconsistent (Blanc et al., 1993; Fine et al., 1997). CRP, SAA and IL-6 are independent, robust biomarkers which indicate an increased risk for cardiovascular risk (Johnson et al., 2004; Kaptoge et al., 2012; Ridker et al., 2000a, 2000b), and all 3 markers may functionally contribute to this type of diseases (Dong et al., 2011; Pepys et al., 2006; Schieffer et al., 2000; Thompson et al., 2015; Yamagami et al., 2004). IFN-γ is a mainly T-cell derived cytokine that is critical for innate and adaptive immunity. VCAM-1 and ICAM-1 are cell adhesion molecules (CAMs) expressed on endothelial cells under proatherosclerotic conditions (Ley and Huo, 2001), which mediate the adhesion of leukocytes to vascular endothelium. Soluble forms of these molecules (sVCAM-1, sICAM-1) are shed from endothelial cell surfaces and are detectable in serum (Byrne et al., 2000). The investigation of the performance of these biomarkers after short-term exposure with mainly copper- and/or zinc-containing welding fumes is needed to further elaborate on potential harmful effects of these metal fumes, also on long-term cardiovascular health.

2. Material and methods

2.1. Subjects

The study group consisted of 15 non-smoking male volunteers with healthy lung function data and without any occupational metal fume exposure. These volunteers did not suffer from asthma or any other lung or cardiac disease based on a physical examination including blood pressure measurements, electrocardiogram (ECG), lung function (bodyplethysmography) measurements and an assessment of the medical history via a questionnaire. All volunteers did not take any medication prior to or during the study. None of the volunteers was obese. The current study investigated exclusively male volunteers, as the essential stratification after inclusion of both genders would have diminished the statistical power. The study protocol was approved by the Ethics Committee of the Medical Faculty of the RWTH Aachen University and all study subjects provided written informed consent prior to inclusion. Two subjects had to be excluded due to flu infection and/or initially distinctively elevated serum CRP concentrations. The anthropometric and lung function data of the study population are shown in Table 1.

2.2. Study design

The controlled exposures to welding fumes were performed in the “Aachen Workplace Simulation Laboratory” (AWSL), which consists of 2 units: i) the emission room in which the welding fumes are generated, and ii) the exposure room in which the test subjects are exposed. Both units are connected by a ventilation system which allows a controlled exposure by regulating the fume concentration in the exposure room (Brand et al., 2013b; Hartmann et al., 2014). Each subject was exposed to either ambient air or one

Table 1
Anthropometric and lung function data of the study population.

Parameter	Average	Std. Dev.	Min	Max
age [years]	26	4	19	36
height [cm]	183	9	170	199
body mass [kg]	85	8	75	100
VC [L]	6.00	0.80	4.72	7.01
VC% pred	104	12	83	130
FEV1 [L]	4.78	0.49	3.95	5.57
FEV1 pred	106	10	91	123

VC, vital capacity; FEV1, forced expiratory volume; % pred, Percent of the index value; Std. Dev., Standard deviation.

Table 2
Average particle mass concentration and content of zinc, copper and aluminum in the 3 welding fumes used for exposure.

Welding fume	Mass Conc. [mg/m^3]	Al [%]	Zn [%]	Cu [%]	Al [mg/m^3]	Zn [mg/m^3]	Cu [mg/m^3]
AluBronze	2.5	1.3	57	15	0.032	1.43	0.375
ZincAlu	2	2.9	70	0	0.058	1.4	0
ZincZinc	2	1.2	70	0	0.024	1.4	0

Al, Aluminum; Zn, Zinc; Cu, Copper.