AJRCCM Articles in Press. Published on 16-November-2017 as 10.1164/rccm.201709-1857LE

Systemic and airway inflammation after exposure to fumes from military small arms

Running Head: Gun smoke causes airway and systemic inflammation

Liv IB Sikkeland[1,2], Anne K Borander[3,4], Øyvind AVoie[5], Hans C D Aass[6], Reidun Øvstebø[6], Pål Aukrust[1,7,8] , Kjetil Longva[5], Neil E Alexis[10], Johny Kongerud[1,2] and Thor Ueland[1,7,9]

[1]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Olso, Norway
[2]Department of Respiratory Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway
[3]Department of Environmental and Occupational Medicine, Oslo University Hospital, Oslo, Norway
[4]The Occupational Health Service in the Norwegian Armed Forces, Sessvollmoen, Norway
[5]Norwegian Defence Research Establishment, Division Protection, Kjeller, Norway
[6]The Blood Cell Research Group, Department of Medical Biochemistry, Oslo University Hospital Ullevål, Oslo, Norway
[7]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway.
[8]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, Norway
[9]K. G. Jebsen Thrombosis Research and Expertise Center, University of Tromsø, Tromsø, Norway;
[10]Center for Environmental Medicine, Asthma and Lung Biology, UNC Chapel Hill, Chapel Hill, North Carolina, USA

Correspondence to: Liv Ingunn Bjoner Sikkeland, Department of Respiratory Medicine, Institute of Clinical Medicine, University of Oslo. N-0027 Oslo, Norway
l.i.b.sikkeland@medisin.uio.no

Author contributions: All authors contributed to the design of the study, to interpretation of the results, and in revising the manuscript. AKB, LIBS and ØAV participated in data collection. LIBS, AKB, HCD, TU and RØ performed the laboratory work. LIBS drafted the manuscript in collaboration with TU, and JK. The final version has been approved by all authors.
Support: This work was financed by the Norwegian Armed Forces.
Conflict of interest: Dr. Sikkeland reports grants from Boehringer Ingelheim, outside the submitted work.
Descriptor number: Environmental and Occupational Health. 6.16. Pulmonary Toxicology
Word count: 996

Key words: Induced sputum, CRP, leaded ammunition, unleaded ammunition, metal fume

Introduction

Worldwide millions of people train at firing ranges, but there are limited data on acute health effects of both leaded and unleaded ammunition. Norwegian Armed Forces report of soldiers complaining of general and respiratory symptoms after live-firing training with small arms, Heckler&Koch (HK) 416 assault rifle using lead-free ammunition. These soldiers are exposed to different gases, aerosols and associated metals such as copper, zinc and lead (1, 2). Many of these agents are pro-inflammatory and toxic when inhaled (3). We recently reported lung function decline, respiratory symptoms and general symptoms similar to metal fume fever, following exposure to fumes from shooting with small arms (4, 5). These symptoms were more frequent with use of unleaded ammunition.

We hypothesized that short-term exposure to fumes from firing small arms may induce both pulmonary and systemic inflammation at exposure levels commonly encountered at shooting ranges. In the same material as previously reported, we analyzed and examined the airway and systemic inflammatory responses in healthy volunteers following firing of small arms and compared the use of leaded vs. unleaded ammunition. Some of the results presented here have been previously reported in the form of abstracts (6, 7).

Material and Methods

As described previously (4, 5) fifty-four healthy non-smoking men (aged 19-62) were recruited and randomized to shooting sessions with small arms each using only one of the three types of ammunition: leaded, unleaded and modified unleaded. The two groups using unleaded ammunition were pooled in the analysis. Controlled shooting sessions were conducted outdoors in semi-enclosed tents. Questionnaire confirmed that none were exposed to fumes the last 14 days before baseline (BL) and between BL and shooting. Blood was collected 2-13 days before shooting (BL) and 24 hours after shooting (day one (d1)). Induced

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sputum was collected at BL and 48 hours after shooting (d2). The study was registered at ClinicalTrials.gov (NCT01477645) and approved by the Norwegian Regional Ethical Committee (REC2011/1335b). Exposure conditions have been previously described (4) In brief, concentrations of particulate matter (15.1mg/m^3±5.0), copper (Cu) (5.3mg/m^3±2.1) and zinc (Zn) (1.1mg/m^3±0.6). Particulate matter and Cu exceeded their respective threshold limit values. The mean temperature was -6°C (range -10.0°C to +8.8°C), but the subjects were properly attired and not subjectively cold (5). Spirometry and induced sputum were performed as described (5, 8). Nine subjects were excluded from sputum analysis due to poor sample quality (<50% cell viability or >50% squamous epithelial cells) or low cell number (<300 000 cells).

Leucocytes were analyzed in EDTA blood within 48h (Sysmex-haematology-system). Serum levels (gel-tubes centrifuged 1400xg, 15min within 60min after blood draw, stored at -80°C) of Surfactant protein-D (SpD), Club Cell protein-16 (CC16), CXC-Chemokine-Ligand-16 (CXCL16), soluble-CD14 (sCD14), sCD25, Myeloperoxidase (MPO), Pentraxin3 (PTX3) and YKL-40 (DuoSetELISA, R&Dsystems,USA), Von Willebrand Factor(vWF) (EIA,DakoCytomation), and C-reactive protein (CRP) (high-sensitive-immunoturbidimetric-assay Hitachi917,RocheDiagnostics) were measured. Sputum biomarkers were MPO, Matrix-metalloproteinase-8 (MMP-8), IL-1β and IL-8(DuoSetELISA). Intra- and inter-assay coefficients of variance were <10%.

Statistical comparison was performed using Mann-Whitney rank-sum test for non-parametric analysis (comparing leaded vs. non-leaded effects). Paired analysis was performed using Wilcoxen matched pairs test (pre- to post-exposure effects within individuals). Linear regression was used to assess predictors of change in sputum and blood neutrophils. Correlation between airborne exposures, as previously reported (4) and selected inflammatory markers was assessed by Spearman's rank correlation.

Results

Twenty-four hours after shooting all subjects regardless of ammunition type, showed a significant increase in total blood neutrophils (Table 1) and systemic inflammatory markers (Table 2), in particular, a 17-fold increase in CRP. The increase in blood neutrophils was correlated with airborne mass concentrations of dust (r=0.37,p=0.01) and associated metals Cu (r=0.43,p=0.002) and Zn (r=0.32,p=0.03) during shooting. The increase in number of blood neutrophils and CRP, but no other inflammatory markers, was greater in subjects using unleaded ammunition (Table 1 and Table 2). Analyzed separately and adjusting for age and outside temperature, Cu (beta=0.30,p=0.038) and Zn (beta=0.34,p=0.014) remained associated with change in neutrophils, but not when included together (p>0.15 for both, variance inflation factor p=1.86). No association between outside air temperature and inflammatory markers was observed except for sCD25 (r=0.378, p=0.005).

Similarly, percentage and cell count of sputum neutrophils increased following shooting (Table 1), Total neutrophil cell count in sputum correlated with exposure levels to Cu (r=0.33, p=0.038) and Zn (r=0.36, p=0.025). No significant changes were observed with sputum cytokines MPO, MMP-8, IL-1β and IL-8.

Discussion

We have previously reported that inhalation exposure of military personnel to fumes following firing of small arms resulted in lung function decline and increased general and respiratory symptoms (4, 5). Herein we show that exposure to fumes at levels commonly encountered at live firing training sessions induces acute airway and systemic inflammation that was more pronounced with unleaded versus leaded ammunition. The pronounced neutrophilia in blood and sputum and enhanced inflammatory soluble markers in blood were

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associated with exposure to dust particles and metals, in particular CuO and ZnO. These findings cohere with previous reports i*n vivo* and *in vitro* in rats and humans after exposure to nanoparticles such as CuO and ZnO (9, 10).

The increased inflammatory response we observed with unleaded ammunition may be due to higher emission of Cu and Zn in unleaded vs. leaded ammunition. The correlation between exposure levels of Cu and Zn and increase in neutrophils following shooting suggest that these metals may be a contributing factor, and in combination with other metals, might induce symptoms similar to metal fume fever (3, 10). Indeed, increased levels of neutrophils and inflammatory cytokines have been found to be a part of the pathogenesis of metal fume fever (11, 12), and as we were unable to pinpoint specific inflammatory pathway activation, our present findings further support such a notion. Finally, caution is needed when interpreting the results as unquantifiable physical influences could affect the results, and the experimental set-up with a semi-airtight tent may not always mimic real-life situations. We suggest that longitudinal studies are a necessary next step to clarify the potential long-term health consequences of exposure to fumes from the use of small arms fire.

Acknowledgements. Rita Tansø and Ingjerd Thrane Occupational Health Service, Norwegian Armed Forces and Mayes Kasem, Department of Respiratory Medicine, Oslo University Hospital are acknowledged for excellent laboratory technical assistance. We thank the management and employees at the Norwegian Defence Research Establishment and the Norwegian Armed Forces for their considerable co-operation and for taking part in this study.

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At a glance commentary

Scientific knowledge

Worldwide, millions of military and non-military people conduct live-fire training regularly, and some experience general and respiratory symptoms afterwards. They are exposed to different gases, vapors, combustion products, particulate matter and different metals in the size fraction of nanoparticles. To date, health concerns have focused on lead exposure and subsequent lead poisoning, but toxic agents other than lead are also emitted. We have previously shown that exposure to fumes from military small arms gives general and respiratory symptoms, and acute decline in lung function that appeared within 90 minutes and lasting more than 24 hours. The symptoms are similar to those reported in metal fume fever, while the decline in lung function might be an irritant effect.

What this study adds

We have shown that exposure to fumes from small arms triggers airway and systemic inflammation, reflected by changes in acute stress response proteins, immune cell activation, altered lung epithelial integrity and vascular inflammation.

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The inflammation was more pronounced in unleaded ammunition which emits higher levels of copper and zinc, compared to leaded ammunition. More research is needed to better understand the potential long-term health consequences of exposure to fumes from using small arms fire. However, a pre-cautionary initiative by reducing exposure during shooting practice would be of importance.

Table 1. Levels and delta values of neutrophils in lung and blood for the different ammunition types.

		All		**Leaded ammunition**		**Unleaded ammunition**		
		Levels	delta	Levels	delta	Levels	delta	P-value†
Blood		n=54		n=17		n=37		
Blood neutrophils (10^6 cells/mL)	BL	2.8 (2.2,3.5)	4.6 (2.4, 5.7)	2.4 (2.2,2.9)	2.2 (1.4, 3.2)	2.9 (2.0,4.0)	5.1 (3.7,7.1)	<0.001
	d1	7.3 (5.0,8.8)*		5.0 (3.7,5.4)*		8.3 (7.0,9.6)*		
Blood neutrophils %	BL	51 (46,57)	17 (12,24)	47 (45,54)	13 (6,22)	52 (76,57)	20 (14,24)	0.157
	d1	70 (64,75)*		62 (60,69)*		73 (68,78)*		
Sputum		n=45		n=15		n=30		
Sputum neutrophils (cells/mg sputum)	BL	949 (429,1711)	383 (-98,1699)	1042 (433,2524)	18 (-521,666)	942 (427,1711)	442 (95,1828)	0.166
	d2	1320 (619,3392)*		744 (421,2233)		1456 (725,3730)*		
Sputum neutrophils %	BL	49 (28,64)	27 (10,43)	47 (28,64)	18 (2,38)	49 (27,64)	30 (15,43)	0.268
	d2	80 (70,88)*		80 (48,83)*		79 (70,89)*		

Delta is the difference between day one (d1) or day two (d2) and baseline (BL). Data is presented as median (25th to 75th percentile). †P-value represent Mann-Whitney rank sum test using delta values of blood or sputum cell comparing leaded vs. non-leaded ammunition types. *$p<0.05$ represent the comparison between BL and d1 or d2.

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Table 2. Levels and delta values of the different inflammation markers in lung and blood for the different ammunition types

		All		Leaded ammunition		Unleaded ammunition		
		Levels	delta	Levels	delta	Levels	delta	P-value[†]
Blood		n=54		n=17		n=37		
Leukocyte markers								
sCD14 (µg/mL)	BL	1.26 (1.03, 1.41)	0.28 (0.03, 0.54)	1.18 (1.07, 1.51)	0.23 (-0.02, 0.46)	1.28 (1.03, 1.39)	0.37 (0.08, 0.55)	0.376
	d1	1.47 (1.33, 1.75)*		1.42 (1.30, 1.66)*		1.49 (1.35, 1.75)*		
MPO (ng/mL)	BL	327 (192, 429)	340 (121 ,474)	267 (192, 397)	317 (121, 478)	360 (201, 461)	342 (154, 469)	0.918
	d1	705 (407, 903)*		660 (366, 874)*		712 (408, 903)*		
sCD25 (ng/mL)	BL	326 (231, 424)	85 (23, 149)	332 (264, 424)	90 (33, 127)	324 (222,424)	82 (23, 149)	0.985
	d1	392 (339, 476)*		397 (354, 490)*		388 (333,435)		
Acute phase proteins								
CRP (mg/L)	BL	0.9 (0.5,1.8)	14.8 (9.0, 21.4)	0.8 (0.5, 1.1)	11 (7.3, 12.5)	1.1 (0.5, 2.2)	17.9 (12.4, 24.8)	0.014
	d1	15.8 (10.4,23.2)*		12.1 (7.8, 14.1)*		20.3 (14.2,25.3)*		
PTX3 (ng/mL)	BL	0.8 (0.6, 1.1)	0.5 (0.3, 0.7)	0.8 (0.6, 1.0)	0.4 (0.2, 0.6)	0.8 (0.6, 1.2)	0.5 (0.3, 0.8)	0.099
	d1	1.4 (1.1, 1.7)*		1.3 (1.1, 1.5)*		1.4 (1.1, 1.8)*		
Vascular inflammation								
YKL40 (ng/mL)	BL	34 (24, 47)	38 (17, 76)	32 (23, 52)	23 (13, 57)	34 (26, 42)	39 (25, 76)	0.165
	d1	69 (51, 135)*		58 (43, 135)*		70 (56, 119)*		
CXCL16 (ng/mL)	BL	5.0 (3.9, 5.6)	0.8 (0.9, 1.5)	4.5 (3.9, 5.2)	0.5 (-0.4, 1.4)	5.2 (4.1, 5.9)	0.9 (0.2, 1.6)	0.545
	d1	5.6 (4.9, 6.5)*		5.1 (4.6, 5.7)*		5.7 (5.2, 6.6)*		
vWF (AU)	BL	383 (204, 647)	287 (-83, 776)	249 (188, 393)	208 (-35, 412)	454 (269,695)	372 (-133, 884)	0.407
	d1	749 (247, 1239)*		457 (242, 747)*		997 (615,1365)*		
Lung biomarkers								
CC16 (ng/mL)	BL	17.3 (15.0, 21.0)	4.2 (-3.4, 8.5)	17.8 (15.0, 20.9)	4.4 (1.2, 12.4)	17.2 (15.1, 21.0)	4.1 (-0.8, 7.8)	0.397
	d1	22.3 (15.6, 28.8)*		25.5 (20.0, 29.6)*		22.1 (14.9, 27.8)*		
SPD (ng/mL)	BL	6.3 (2.2, 13.8)	2.7 (-0.02, 5.8)	5.2 (1.1, 12.3)	2.3 (-1.5, 4.9)	7.6 (2.4, 15.0)	2.8 (0.0, 6.7)	0.386
	d1	8.6 (5.4, 17.9)*		8.2 (3.3, 12.3)*		10.2 (5.5, 19.9)*		

sCD14 (soluble CD14), Myeoloperoxidase (MPO), sCD25/Interleukin 2 receptor alpha (IL-2 Rα), C-reactive protein (CRP), Pentraxin 3 (PTX3), YKL-40/ Chitinase-3-like protein 1 (CHI3L1), CXC Chemokine Ligand 16 (CXCL16), Von Willebrand Factor (vWF), Club Cell protein16 (CC16), Surfactant protein D (SpD). Delta is the difference between day one (d1) and baseline (BL). Data is presented as median (25th to 75th percentile). †P-value represent Mann-Whitney rank sum test using delta values of inflammatory markers comparing leaded vs. non-leaded ammunition types. *$p<0.05$ represent the comparison between BL and d1 or d2.