Journal of Forensic and Legal Medicine 38 (2016) 93–96

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Short report

Pulmonary macrophages activity in CO intoxication

Maria Pieri [a, *], Pasquale Giugliano [b], Giuseppe Vacchiano [c]

[a] Department of Advanced Biomedical Science-Legal Medicine Section, University of Naples "Federico II", Naples, Italy
[b] AORN "Sant'Anna e San Sebastiano" Caserta- Legal Medicine Section, Caserta, Italy
[c] Department of Law, Economics and Mathematical Methods, University of Sannio, Benevento, Italy

ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form
19 November 2015
Accepted 3 December 2015
Available online 17 December 2015

Keywords:
CO intoxications
Macrophages
Prolonged asphyxia

ABSTRACT

The presence of macrophages and their activation on the pulmonary tissues of 21 subjects deceased after CO intoxication has been studied. A notable number of activated macrophages, especially in the interstitial level, have been evidenced, and such phenomenon supports the hypothesis of a possible association between CO intoxication and pulmonary macrophages activity. The highlighted association could be mediated by changes of the surfactant, by impairing of mitochondrial respiration and by release of pro-inflammatory cytokines.

1. Introduction

Pulmonary histology in subjects deceased after asphyxiation has been extensively investigated since '60. Many authors investigated the presence of pulmonary macrophages as a possible indicator of prolonged asphyxia.[1–4]

Some Authors reported a relevant number of macrophages in pulmonary tissue in subjects deceased after prolonged asphyxiation[5–7]; while others have emphasised the possible pre-existence of pathological phenomena, which may have brought to such observation.[8,9] Results obtained by the different Authors seem to be controversial and not definitive.

Recently, Strunk et al.[10] reported as prolonged asphyxiation is associated with:

- a significant increase of intra-alveolar mature macrophages;
- an increase of intra-alveolar giant cells;
- the relevant strong presence of "*young macrophages*".

In a study on deaths due to protracted asphyxia, Vacchiano et al.[11] considered particularly useful to focus on the number and degree of activation of interstitial macrophages, unlike the alveolar ones, are responsible for the phagocytic activity.

Given this, it would seem useful to investigate the presence and the activation of macrophages in the pulmonary tissues of subjects deceased after CO asphyxiation, in order to verify the relationship between this particular type of asphyxia, characterized by slow course, and the pulmonary macrophages activity. In fact, macrophages are the long-lived effector cells within the lung. They are reactive, responding to endogenous and exogenous stimuli, as well as proactive mediators, able to modulate the behaviour of surrounding cells. They play a critical role in the pathogenesis of many pathological conditions of lungs and their number is elevated in lung walls in areas of known pathology.[12] They are the sentinels of healthy state of pulmonary tissue[13] and they have a particular plasticity adapting themselves at the ever-changing needs of pulmonary tissue.[14]

2. Materials and methods

2.1. Study design

Pulmonary tissues of 21 CO intoxicated subjects have been investigated. Age, sex and circumstances of death were known. Smokers, subjects aged >35 years, and subjects exposed to environmental and working pollution were excluded from the study. Subjects with pre-existing cardiopulmonary diseases were also excluded. Details of enrolled subjects are schematically reported in Table 1.

* Corresponding author. Department of Advanced Biomedical Science-Legal Medicine Section, University of Naples "Federico II", Via S. Pansini, 5, II Policlinico Ed. 20, 80131 Naples, Italy. Tel.: +39 0817463474; fax: +39 0817464726.
E-mail addresses: maria.pieri@unina.it (M. Pieri), dottgiugliano@tiscali.it (P. Giugliano), givacchi@tin.it (G. Vacchiano).

http://dx.doi.org/10.1016/j.jflm.2015.12.002
1752-928X/

Table 1
Details of enrolled CO-intoxication cases.

Case	Sex	Age	Body found in	Hb-CO%
1	M	34	Car	74.0
2	M	31	Bedroom	79.9
3	F	21	Bathroom	91.6
4	M	31	Car	84.6
5	F	35	Car	89.0
6	F	31	Bedroom	88.6
7	M	16	Bedroom	79.0
8	M	27	Bathroom	86.5
9	M	21	Car	80.0
10	M	30	Living room	82.2
11	M	28	Car	87.0
12	F	27	Bathroom	79.0
13	F	18	Car	78.0
14	M	29	Bedroom	74.0
15	F	20	Car	84.0
16	M	22	Car	88.0
17	F	25	Bedroom	76.0
18	M	27	Bathroom	80.0
19	F	32	Kitchen	68.0
20	F	30	Car	76.0
21	M	29	Kitchen	54.0

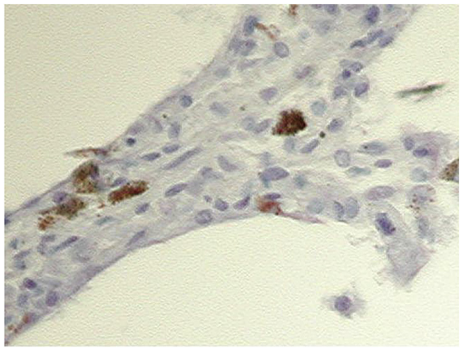

Fig. 1. Activated macrophages on the septa and the interstitial tissue. APAAP Method. MMP-2, 200X.

2.2. HbCO quantification

The CO ematic levels were defined by detection of HbCO doses by spectrophotometry, according to the method proposed by Luchini et al.[15]

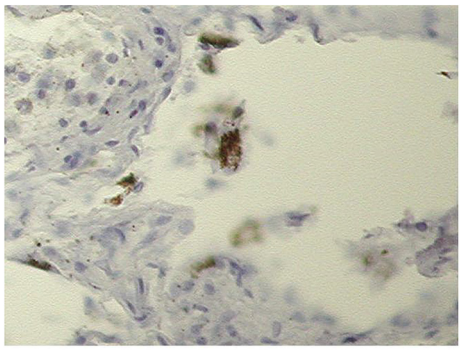

Fig. 2. An activated macrophage in an alveolar cavity of exfoliated cells. APAAP Method. MMP-2 200X.

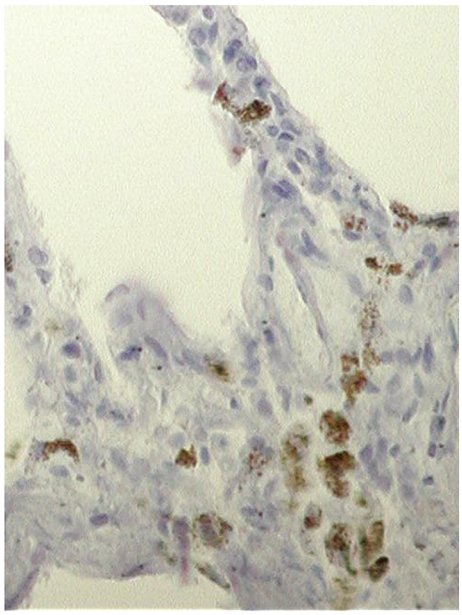

Fig. 3. Numerous activated macrophages on the interstices and the septa. APAAP Method. MMP-2, 200X.

2.3. Histological analysis

Specimens of lungs were obtained by 21 autopsies performed between 24 and 36 h post-mortem. The pulmonary tissues were selected random from the lungs (apex, base or middle lobe). Samples were fixed in buffered formalin and embedded in paraffin. Control cases (n = 12) were selected among traumatic sudden deaths due to traffic accidents or gunshot wounds. The same exclusion criteria (as regards age and chronic or acute pathology) defined for enrolled CO-intoxicated subjects were adopted also for the control group. Sections 2 and 3 μm thick were prepared and stained with H/E.

In line with previous findings,[16] the macrophages were identified using monoclonal antibody to human metallo proteinases (MMP-2), which highlights precocious macrophages activity (APAAP Method). It is generally accepted that MMP-2 is expressed

Table 2
Activated macrophages count (mean cell number for field).

Case	Total macrophages (alveolar + interstitial)	Interstitial macrophages
1	13	8
2	18	11
3	29	16
4[a]	30	19
5	19	11
6	15	9
7[a]	34	20
8[a]	35	22
9[a]	38	25
10	20	12
11	21	11
12	25	13
13	28	15
14	25	14
15	28	16
16[a]	40	21
17	20	13
18	28	13
19	20	11
20	24	13
21	31	18

[a] Autholytic phenomena evidenced.