Int J Legal Med (1998) 111 : 256–260

ORIGINAL ARTICLE

E. Osuna · M. D. Pérez-Cárceles · A. García-Lorente
M. Sánchez-Hanke · D. N. Vieira · L. Carvalho
K. Püschel · A. Luna

Lipid peroxidation in lung tissue after chest trauma and correlation with the duration of the post-trauma survival period

Received: 5 December 1997 / Received in revised form: 18 February 1998

Abstract Blunt chest trauma is a frequently encountered cause of death in forensic pathology and one of the organs most likely to be affected are the lungs. Assuming that the victim survives the initial trauma, reperfusion processes take place, free radicals are formed and lipid peroxidation occurs. The aim of this study was to ascertain whether the length of the survival time is correlated with the extent of lipid peroxidation in the lung tissue following such ischaemia-reperfusion processes. A study of 470 samples taken from all five pulmonary lobes from 94 cadavers was carried out. Cases were allocated to different groups according to whether there was chest trauma and/or a known survival period. Lipid peroxidation was investigated by determining malondialdehyde (MDA) levels. The lowest mean level of peroxidation was found in control cases showing no evidence of chest trauma at autopsy and no apparent survival period. Our results suggest that the level of lipid peroxidation in lung tissue can be a reliable indicator of survival processes.

Key words Chest trauma · Lipid peroxidation · Reperfusion · Lung · Postmortem

Introduction

The study of the free radicals of oxygen is becoming increasingly important in clinical situations (Bulkley 1983; McCord 1985; Southorn and Powis 1988; Halliwell et al. 1992). It has been suggested that the sudden presence of oxygen during reperfusion after a period of ischemia may be toxic for cells. The oxygen molecule is capable of producing reactions in a cell, forming highly reactive free radicals and inducing lipid peroxidation of membranes, whose integrity is altered and permeability increased (McCord 1985).

Blunt chest trauma, resulting mostly from car accidents, is a frequent cause of death in forensic pathology (Calhoon et al. 1992). The organ most likely to be damaged in such accidents is the lungs. The readily observable macroscopical changes often associated with histopathological changes such as edema, congestion, hemorrhage, contusion, etc. (Janssen 1984) can be regarded as processes which are later expressed by changes in lung perfusion and intrapulmonary circulatory disturbances (Eddahibi et al. 1996). Such anatopathological alterations are non-specific and are subject to different variables such as the intensity and localization of the trauma, anatophysiological characteristics of the blood vessels and the previous state of the lung tissue. These factors, together with the fact that a minimum period is necessary for them to be expressed, lead to significant inter- and intraindividual variations.

Following the initial phase of micro-circulatory shock, a number of lung reperfusion processes take place, assuming that the subject survives the initial trauma. These processes may cause a number of local changes due to the formation of free radicals (Halliwell and Gutteridge 1990; Halliwell et al. 1992), the consequent lipid peroxidation leading to membrane damage. The reaction of lipid peroxides with thiobarbituric acid (TBA) has been widely adopted as a sensitive assay method for lipid peroxidation in animal tissues (Ohkawa et al. 1979; Esterbauer and Cheeseman 1990; Hageman et al. 1992; Schweich et al. 1994). Malondialdehyde (MDA) is a secondary product of lipid peroxidation and can be complexed with two molecules of TBA to yield and adduct showing maximum absorption at 532 nm.

The objective of this study was to ascertain whether the length of the survival time is related to the degree of

E. Osuna (✉) · M. D. Pérez-Cárceles · A. García-Lorente · A. Luna
Department of Forensic Medicine, University of Murcia,
E-30100 Espinardo, Murcia, Spain
Tel +34-68-363956/363957; Fax +34-68-364150

M. Sánchez-Hanke · K. Püschel
Institute of Legal Medicine, University of Hamburg,
D-22529 Hamburg, Germany

D. N. Vieira · L. Carvalho
Institute of Legal Medicine, University of Coimbra,
P-3000 Coimbra, Portugal

lipid peroxidation in the lung tissue following such ischaemia-reperfusion processes.

Materials and methods

The cases used in this study were 94 cadavers (66 males and 28 females) submitted for medicolegal autopsy at the Institutes of Forensic Medicine of Coimbra (Portugal) and Hamburg (Germany). The mean age of the subjects was 50.06 years (SD 21.82 years, range 6–89 years). During the autopsy 470 samples free from hypostasis phenomena were taken from each of the five pulmonary lobes and stored at –70 °C prior to analysis and transportation to Murcia (Spain) in dry ice (transit time less than 9 h). The 94 cases were allocated to one of four groups depending on whether or not there was an apparent survival period and whether or not the victim suffered chest trauma. The groups were as follows: (group 1) cases with no chest trauma and with no known survival period (n = 7) (2 cases of cerebrovascular disease and 5 of acute haemorrhage), (group 2) cases with no chest trauma but with a survival period of varying length and probable lung distress (n = 16) (4 hanging, 2 fat embolism, 6 adult respiratory distress syndrome and 4 pneumonia), (group 3) cases with chest trauma and no apparent survival period (n = 24) (21 motor vehicle collisions and 3 jumping from a height) and (group 4) cases with chest trauma and with a survival period (n = 47) (all motor vehicle collisions). The age of the victims, survival periods and postmortem intervals (mean hours ± SD) for the four groups are shown in Table 1. Histological studies with hematoxylin-eosin (H&E) staining were performed on all samples of lung tissue taken from the pulmonary lobes. The levels of lipid peroxidation in each pulmonary lobe and mean peroxidation level from the five pulmonary lobes of the four differents groups were also analysed.

Lipid peroxidation in postmortem lung tissues was estimated by the colorimetric determination of MDA. After washing with 9 volumes of bidistilled water, a sample of lung tissue (1 g) was homogenized for 10 min with chloroform-methanol solution (2:1, v/v) in an ice bath so that the lipids could be extracted; 0.15 mg of butylatedhydroxytoluene (BHT) was added to ensure that no lipid oxidation occurred during the assay (Radin 1969; Ohkawa et al. 1979). Homogenisation was followed by centrifugation at 1500 g for 15 min. The organic layer was pipetted off, washed with water and evaporated in a stream of cold nitrogen. The extract was weighed and redissolved in methanol (1 ml/mg) before being mixed with 2.5 volumes of cold 20% (w/v) trichloroacetic acid (TCA) to precipitate protein and one volume of 0.67% (w/v) TBA. The reaction mixture was heated for 10 min in boiling water and the pink chromogen was measured by spectrophotometry at 532 nm. MDA was estimated using a standard curve of 1,1,3,3-tetraethoxypropane and the MDA levels were expressed as nmol/g tissue.

For statistical analysis of the data, a multivariate analysis and a non-parametric test (Kruskal-Wallis test) were used to compare groups. In addition, specific contrast tests for each variable grouped according to diagnostic category were carried out using the Mann-Whitney test.

Table 1 Age, survival period and postmortem interval (in hours). Mean and standard deviation (SD) values for the four groups

Groups	N	Age (years old)		Survival time (h)		Postmortem interval (h)	
		Mean	SD	Mean	SD	Mean	SD
1	7	42.71	17.06	0.0	0.0	40.1	23.1
2	16	38.62	16.98	80	155.2	28.1	19.8
3	24	49.33	21.53	0.0	0.0	36.8	19.2
4	47	55.42	21.82	95.7	199.1	30.1	17.3

Results

Table 2 shows the lipid peroxidation levels (mean, SD and SE) obtained for the four groups. A non-parametric test (Kruskal-Wallis Test) was used to compare the mean values within the groups (Table 3). Except in the case of the lower left lobes, lipid peroxidation in the other lobes differed significantly between groups and the mean level of peroxidation showed statistically significant differences ($P < 0.025$) between groups. The lowest mean values of peroxidation were found in cases involving no chest trauma and no apparent survival period (group 1) while the highest values were found in the group of subjects who died due to chest trauma after a survival period (group 4), although this observation did not apply to the upper lobes.

We contrasted the mean peroxidation levels of different diagnostic groups and observed statistically significant differences between the group of subjects with no chest trauma and no apparent survival period (group 1) and the group of subjects with no chest trauma but with a survival period (group 2) ($P = 0.0450$) and the group of subjects with chest trauma and with a survival period (group 4) ($P = 0.0048$) (Table 4). There were no significant differences between group 1 and group 3 (chest trauma without survival period). It is important to emphasize the importance of the variable “survival period” in our study since the values obtained seem to mainly depend on the survival time and not on the existence of chest trauma.

No statistically significant correlation was obtained between the mean level of lipid peroxidation in lung tissue and the age of the victims, postmortem interval or the presence of chest trauma. Statistically significant correla-

Table 2 Mean, standard deviation (SD) and standard error of the mean (SE) values (nmol/g) for lipid peroxidation in the four groups of lung tissue

Groups	Upper right			Middle right			Lower right			Upper left			Lower left			Mean peroxidation		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
1	4.9	9.0	3.4	9.8	15.6	65.9	5.9	9.2	3.5	5.9	6.9	2.6	9.3	10.4	3.9	7.2	10.0	3.8
2	18.1	11.8	2.9	15.0	11.1	2.7	13.1	7.1	1.7	17.9	9.8	2.4	15.2	9.6	2.4	16.0	8.4	2.1
3	12.8	11.7	2.3	11.0	10.1	2.0	12.4	10.2	2.0	10.5	10.1	2.0	12.5	10.9	2.2	11.7	9.8	2.0
4	15.9	12.0	1.7	18.6	13.9	2.0	16.3	12.1	1.7	15.9	13.5	1.9	16.8	12.0	1.7	16.6	11.4	1.6

Table 3 Kruskal-Wallis Test for the four groups

Variable	df	Statistic	*P*
Upper right	3	12.45	0.006
Middle right	3	10.08	0.017
Lower right	3	8.13	0.043
Upper left	3	13.18	0.004
Lower left	3	5.37	0.146
Mean peroxidation	3	9.64	0.021

Table 4 The Mann-Whitney test to contrast the values of mean peroxidation in diagnostic groups

Variable	Groups	U Statistic	Z Statistic	*P*
Mean peroxidation	1–2	26	–2.0045	0.045
	1–3	60	–1.1339	0.256
	1–4	55	–2.8198	0.004
	2–3	137	–1.5184	0.128
	2–4	342	–0.5369	0.591
	3–4	413	–1.8355	0.066

Table 5 Kruskal-Wallis Test to compare lipid peroxidation levels in different lung lobes according to whether or not there was a survival period

Variable	df	Statistic	*P*
Upper right	1	7.28	0.007
Middle right	1	8.55	0.003
Lower right	1	4.52	0.033
Upper left	1	9.91	0.001
Lower left	1	4.52	0.033
Mean peroxidation	1	7.72	0.005

tions were obtained between the survival period and the values of lipid peroxidation in the lower left ($P = 0.002$), middle right ($P = 0.007$) and lower right ($P = 0.013$) lobes and with the mean level of peroxidation ($P = 0.011$).

We then divided our 94 cases into two categories depending on whether or not there was an apparent survival period and a Kruskall-Wallis test was used to compare lipid peroxidation levels in different lung lobes. Significant statistical differences were found between these two groups in the five different pulmonary lobes and in the mean peroxidation levels (Table 5). The highest values were found in cases involving a survival period (Figs. 1 and 2).

According to the length of the survival period, we established two groups (no known survival period or with a survival period equal to or less than 1 h, and a survival period of more than 1 h). A Kruskal-Wallis test was used to compare the mean peroxidation levels in the groups and significant differences were found ($P = 0.005$). The lowest mean values of peroxidation (12.26 nmol/g) were found in cases with no apparent survival time or with a survival period below 1 h, while higher values (18.45 nmol/g) were found for victims showing a survival period of more than 1 h.

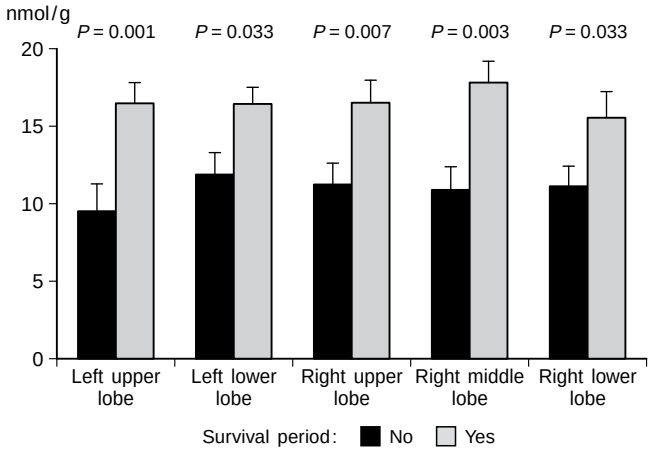

Fig. 1 Levels (nmol/g) of lipid peroxidation (mean and standard error of the mean) in lung lobes compared to the presence or absence of a survival period

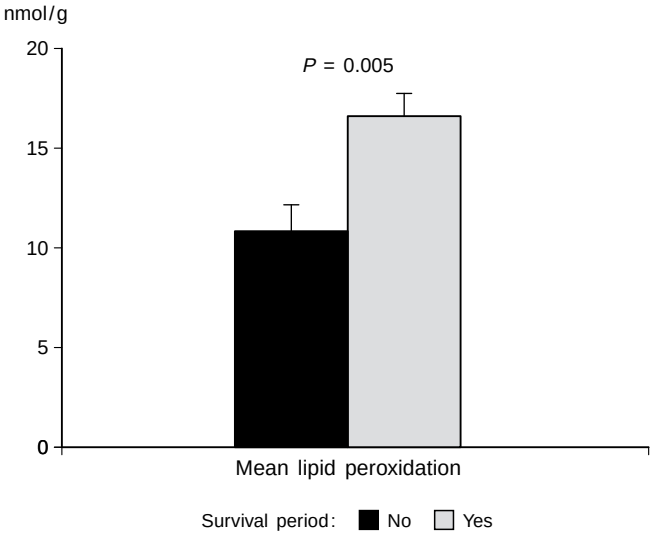

Fig. 2 Mean levels and standard error of the mean lipid peroxidation in all samples compared to the presence or absence of a survival period

Finally, we established two groups (one normal and one showing morphological lung abnormalities such as edema, congestion, hemorrhage, etc.) according to the histological findings. No significant differences in the peroxidation values were found between the groups.

Discussion

Among the medicolegal questions for which an autopsy should provide an answer is whether or not there was a survival period following the traumatic episode. The response by the body to lung tissue injury and, as in our case, following chest wall trauma, can be manifested as macroscopical or microscopical lesions or, following ischemia-reperfusion phenomena, by the formation of oxygen free radicals which, in turn, may increase lipid

peroxidation levels in lung tissue (Brigham 1986; McCord 1987; Klausner et al. 1989; Schweich et al. 1994). However, both processes require a minimum period of development before they can be observed. Lipid peroxidation is probably an intra-vital process taking place during the reperfusion period and which may be correlated with the duration and intensity of the above processes, longer survival periods resulting in a higher degree of lipid peroxidation. This was clearly demonstrated when a correlation matrix was applied. However, no significant correlation was found between the existence of chest trauma or subsequent lesions and the levels of lipid peroxidation. In our study, the values obtained were closely related to the existence of a survival time and not to the existence of chest trauma.

Establishing precise thresholds with a margin of variability which permits forensic conclusions to be reached is very difficult. In our study the lowest mean peroxidation levels were observed in the victims with no survival period. However, when the sample was divided according to the approximate length of the survival time and the mean peroxidation levels were compared, there were significant differences between the victims with no known survival period or with a survival time of less than 1 h and the group of victims who had survived for more than 1 h. This classification was taken as the closest possible measure of the real survival time. It must be emphasised that we depended on the information in the autopsy reports and we established a survival time with a margin of 1 h in an attempt to establish an approximate threshold for forensic use. However, it is really necessary to treat each case individually since individual circumstances (smoking, previous lesions of lung tissue etc.) may obscure the interpretation of our results. As we have found no published studies on lipid peroxidation in lung tissue from cadavers with which our observations could be compared, it is difficult to discuss and interpret some of our findings.

We found no correlation between the survival period and the values of lipid peroxidation in the upper lobes. Analysis of our results points to varying levels of lipid peroxidation in the different lobes. This variation was also observed in the histological study and suggests that the mean level of peroxidation in the lung tissue from the same individual should be taken into account since any variation would then be minimized. This interlobe variation may be the consequence of differences in the blood flow, which is greater in the lower lobes, or be due to the differing alveolocapillary permeability of different pulmonary areas. Ramos et al. (1997) found regional variations in glutathione peroxidase, superoxide dismutase and malondialdehyde between different myocardial zones. These variations suggest the existence of a different antioxidant metabolism in each zone according to metabolic and functional requirements (Andersen et al. 1989; Ramos et al. 1997). We avoided any interference due to hypostasis and postmortem vascular congestion since we only collected lung tissue which was free of the hypostasis phenomena and which was also washed. However as hypostasis is a postmortem process it would not induce undue modifications since peroxidation is an active and vital process.

Another variable which might intervene in peroxidation is the agonal process itself which must be considered as a vital process.

According to Ramos et al. (1997), no changes were detected in MDA levels in relation to the postmortem interval, which means this assay may be useful in postmortem studies. Lipids are highly resistant to autolysis (Enticknap 1960) and lipid peroxidation is an oxygen-dependent process (Halliwell and Gutteridge 1984, 1990; Gutteridge 1988). In our opinion, such peroxidation does not occur in a corpse because of the progressive decrease in the partial oxygen pressure and the extinction of the redox potential. However, we have been unable to find studies comparing the peroxidation levels antemortem and postmortem. It might be possible to analyse lung samples from thoracotomy after removal during surgical treatment of trauma. However, it is practically impossible to design a model to approach this problem, bearing in mind ethical, clinical and legal implications.

For the postmortem intervals involved in our study no statistically significant differences between the mean levels of lipid peroxidation in the different lobes were observed and we concluded that, at least in our study, this factor did not affect the results.

Other factors (smoking, infections, bronchial asthma, etc.) may generate free radicals leading to lipid peroxidation of the respiratory epithelial membranes and these could have interfered with the levels we found. However, since the study was intended only as an initial approach to understanding lung lipid peroxidation in its application to forensic pathology, we did not take such factors into account. Our study was based on autopsy data and the histological findings and no other variables were considered. Nevertheless, we have correlated the peroxidation levels with the histological studies performed on all samples of lung tissue taken from the pulmonary lobes and which must in one way or another , reflect previous alterations of the pulmonary tissue. As already stated, no significant differences in peroxidation were observed between the two groups which were established (normal and those showing morphological alterations). In our opinion, peroxidation is the expression of a biochemical process which occurs earlier and in a more generalised form than structural histological changes. In this respect, the non-specificity of structural data makes them difficult to apply, justifying the validity of using peroxidation as opposed to anatopathological findings to demonstrate our objectives.

In our opinion the peroxidation phenomena seems to be associated with lung reperfusion in victims who have suffered chest trauma or lung injury. The data seem to confirm our initial hypothesis and enable us to propose lung peroxidation as an index of lung damage in support of data obtained by conventional microscopy and as a reliable indicator of survival processes.

References

Andersen HR, Nielsen D, Falke E (1989) Right ventricular infarction: larger enzyme release with posterior than with anterior involvement. Int J Cardiol 22:347-355

Brigham KL (1986) Clinical implications of basic research. Role of free radicals in lung injury. Chest 89:859-863

Bulkley GB (1983) The role of oxygen free radicals in human disease processes. Surgery 94:407-411

Calhoon JH, Grover FL, Trinkle JK (1992) Chest trauma. Approach and management. Clin Chest Med 13:55-67

Eddahibi S, Raffestin B, Tayarani Y, Carville C, Adnot S (1996) Hypoxia-reoxygenation impairs NO-mediated vasodilation in rat lungs. Am J Physiol 271:L441-L449

Enticknap JB (1960) Biochemical changes in cadaver sera. J Forensic Med 7:135-146

Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 186:407-431

Gutteridge JMC (1988) Lipid peroxidation: some problems and concepts. In: Halliwell B (ed) Oxygen radicals and tissue injury. Allen Press, Kansas, pp 9-19

Hageman JJ, Bast A, Vermeulen NPE (1992) Monitoring of oxidative free radical damage in vivo: analytical aspects. Chem Biol Interact 82:243-293

Halliwell B, Gutteridge JMC (1984) Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet 1:1396-1398

Halliwell B, Gutteridge JMC (1990) Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 186:1–85

Halliwell B, Gutteridge JMC, Cross CE (1992) Free radicals, antioxidants, and human disease: where are we now? J Lab Clin Med 119:598–620

Janssen W (1984) Forensic histopathology. Springer, Berlin Heidelberg New York

Klausner JM, Paterson IS, Kobzik L, Valeri CR, Shepro D, Hechtman HB (1989) Oxygen free radicals mediate ischemia-induced lung injury. Surgery 105:192–199

McCord JM (1985) Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 312:159–163

McCord JM (1987) Oxygen-derived radicals: a link between reperfusion injury and inflammation. Federation Proc 46:2402–2406

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Radin NS (1969) Preparation of lipids extracts. Methods Enzymol 14:245–254

Ramos V, Valenzuela A, Villanueva E, Miranda MT (1997) Antioxidant-related enzymes in myocardial zones and human pericardial fluid in relation to the cause of death. Int J Legal Med 110:1–4

Schweich MD, Lison D, Lauwerys R (1994) Assessment of lipid peroxidation associated with lung damage induced by oxidative stress. In vivo and in vitro studies. Biochem Pharmacol 47: 1395–1400

Southorn PA, Powis G (1988) Free radicals in medicine. II. Involvement in human disease. Mayo Clin Proc 63:390–408