Int J Legal Med (2004) 118 : 19–23
DOI 10.1007/s00414-003-0410-7

ORIGINAL ARTICLE

P. Doménech · L. Carbonell · M. D. Pérez Cárceles
M. Falcón · A. Luna · E. Osuna

Application of postmortem lipid peroxidation in heart tissue to the diagnosis of myocardial damage

Received: 18 March 2003 / Accepted: 14 October 2003 / Published online: 18 November 2003
© Springer-Verlag 2003

Abstract The response by the myocardial tissue to injury may be manifested as macroscopical or microscopical lesions or, following an ischemia-reperfusion process, by the formation of reactive oxidant substances (ROS) which, in turn, may increase lipid peroxidation levels in myocardial tissue. The aim of this study was to determine whether the levels of lipid peroxidation in myocardial tissue from subjects who had died from different causes are related to cardiac lesions of ischemic or traumatic aetiology. We studied 825 hypostasis-free samples taken from the myocardial tissue of 75 cadavers. In all cases, a survival period was known to have existed. Lipid peroxidation in myocardial tissue was estimated by the colorimetric determination of malondialdehyde. The highest levels of tissue peroxidation were observed in the group of subjects who died from myocardial infarction. We observed a statistically significant correlation between the extent of peroxidation and the presence of myocardial damage of ischemic or traumatic aetiology. In our study, lipid peroxidation was associated with myocardial injury. The data suggest that the extent of lipid peroxidation in myocardial tissue may be used as a reliable indicator of myocardial damage in support of data obtained by conventional microscopy.

Keywords Lipid peroxidation · Myocardial infarction · Chest trauma · Postmortem diagnosis

Introduction

Myocardial infarction and chest trauma are common in forensic pathology. Following myocardial injury and during the survival period, reperfusion mechanisms and inflammatory processes produce reactive oxidant substances (ROS) resulting in, among other processes, the peroxidation of cell membranes (Zweier 1988; McCord 1985; Southorn and Powis 1988; Lunec 1990; Ambrosio et al. 1991; Griffiths and Halestrap 1995; Lucas and Szweda 1998; Paradies et al. 1999; Fineschi et al. 2001; Dröge 2002; Suematsu et al. 2002). In a previous study (Osuna et al. 1998) we emphasised the usefulness of the postmortem determination of lipid peroxidation levels in lung tissue to demonstrate survival after a chest trauma-related lung injury.

The aim of this preliminary study was to determine whether the levels of lipid peroxidation in myocardial tissue from subjects who had died from different causes are related to cardiac lesions of ischemic or traumatic aetiology.

P. Doménech · M. D. Pérez Cárceles · M. Falcón · A. Luna
E. Osuna (✉)
Department of Forensic Medicine, University of Murcia,
30100 Espinardo, Murcia, Spain
Tel.: +34-968-363956, Fax: +34-968-364338,
e-mail: eosuna@um.es

L. Carbonell
Department of Physiology, University of Murcia,
30100 Espinardo, Murcia, Spain

Materials and methods

We studied 825 hypostasis-free samples taken from the myocardial tissue of 75 cadavers (64 males and 11 females) with a mean age of 51 years (SD 19; range 12–87 years). The study was approved by the Ethics Committee of the Institute of Forensic Medicine and the Ethics Committee of the University of Murcia. Cases were chosen according to the postmortem interval, cause and circumstances of death. We included only cadavers with a postmortem interval of less than 24 h. To minimise postmortem artifacts the bodies were refrigerated, the average interval between death and refrigeration was 3 h and the mean postmortem interval was 7.4 h (SD 4.1, range 2–18 h). In all cases, a survival period was known to have existed.

Data concerning the initial causes of death and autopsy records were unknown to the persons who performed the biochemical and histological analyses. Cases were assigned to one of four diagnostic groups based on the cause of death according to the patient's medical records, scene of death, autopsy, and toxicological and complementary histological findings. The groups were as follows:

1. Myocardial infarction (n=19): 12 deaths witnessed, in 7 cases death was not witnessed, but previous history and subsequent data obtained from an autopsy pointed to myocardial infarction.
2. Multiple trauma (n=19): all motor vehicle collisions witnessed and with chest trauma.
3. Asphyxia (n=18): 12 cases of hanging and 6 of drowning, in the latter all 6 deaths were witnessed.

4. Natural deaths excluding myocardial infarction (n=19): all deaths witnessed, 12 cases of cerebrovascular disease, 2 of pneumonia and pulmonary embolism, 3 of acute renal failure, and 2 of acute gastric hemorrhage.

Cardiopulmonary resuscitation was applied in 22 subjects (7 cases of myocardial infarction, 7 of asphyxia, 5 of multiple trauma, 2 of cerebrovascular disease and 1 of pulmonary embolism.

During the autopsy 11 samples of myocardial tissue (weight 0.5 g) were taken from each cadaver from the following zones: 2 samples from the right ventricle (the middle of the fat-free part of the anterior and posterior walls), 2 samples from the septum (border of upper and lower third of the septum), 6 samples from the left ventricle (upper anterior and upper posterior, lower anterior and lower posterior, upper lateral and lower lateral) and 1 sample from the apex. Samples were stored at –80°C prior to analysis. Histological studies with hematoxylin-eosin (HE) staining were carried out on cardiac tissue. Also acridine orange fluorescent staining in formalin-fixed paraffin sections was performed. In 19 cases evidence of significant stenosis in one or more of the epicardial coronary arteries was found and HE staining showed specific signs of necrosis. Acridine orange classified 10 of these cases as strongly positive (++) and 9 as positive (+), all corresponding to cases classified as myocardial infarction. In 32 cases, HE staining showed interstitial oedema, 22 cases congestion and 9 cases focal fibrosis. Contraction band necrosis was found in 31 cases, in 10 of which cardiopulmonary resuscitation had been applied.

Lipid peroxidation in myocardial tissue was estimated by the colorimetric determination of malondialdehyde (MDA) (Ohkawa et al. 1979; Esterbauer and Cheeseman 1990) after extracting lipids from the tissue (Radin 1969) and MDA levels were expressed as nmol/g tissue.

For statistical analysis of the data, multivariate and discriminant analyses were used. Continuous variables not normally distributed were analysed non-parametrically using the Kruskal-Wallis test to compare groups. In addition, specific contrast tests for each variable (grouped according to the diagnostic category) were carried out using the Mann-Whitney test. For each of the variables studied a ROC (receiver operating characteristic) curve was drawn and the area under the curve was measured using a non-parametric method (Griner et al. 1981; Hauley and McNeil 1983; Zweig and Campbell 1993). The cut-off point for each variable was taken as the point nearest the ideal point of the ROC curve at which sensitivity would be 1 and 1–specificity would be 0.

Results

Table 1 shows the lipid peroxidation levels (mean±SD and range) obtained in the four diagnostic groups. As can be seen, statistically significant differences between groups were obtained. The highest overall levels were found in the group of subjects who had died from myocardial infarction except for the samples from the lower posterior zone of the left ventricle, where the highest levels corresponded to the group of subjects dying of chest trauma, the second highest group with regards to overall lipid peroxidation levels. No statistically significant correlation was observed between the mean levels of lipid peroxidation and the postmortem interval. In subjects where cardiopulmonary resuscitation had been applied, the mean level of lipid peroxidation was 192.48±11.78 nmol/g tissue (DS 55.28; range 92.15–303.66). No statistically significant differences were observed between these and the group of subjects where no cardiopulmonary resuscitation was applied.

Table 2 shows the lipid peroxidation levels (mean±SD and range) obtained when the sample was divided into two groups, one of subjects who died from myocardial infarc-

Table 1 Mean values (nmol/g) ± standard deviation (SD) of lipid peroxidation and statistical significance in the Kruskall-Wallis test for the four diagnostic groups

Zones	Myocardial infarction N=19 Mean ± SD (range)	Multiple Trauma N=19 Mean ± SD (range)	Asphyxia N=18 Mean ± SD (range)	Natural deaths N=19 Mean ± SD (range)	*P*
RPV	428.4±387.9 (142.8–1531.5)	357.1±220.2 (134.7–923.8)	397.3±564.9 (44.2–2515.8)	177.7±64.7 (46.9–302.7)	0.002
RAV	443.9±425.8 (124.5–1813.3)	414.7±310.0 (151.2–1076.8)	362.4±419.6 (86.4–1824.7)	180.9±48.3 (118.2–306.1)	0.000
LULV	361.8±120.5 (130.6–668.5)	281.2±113.2 (98.4–438.2)	257.5±89.3 (78.1–435.6)	181.6±68.7 (62.7–366.2)	0.000
LUAV	319.3±119.3 (113.6–617.4)	319.2±139.7 (132.4–574.2)	278.6±124.5 (86.4–536.9)	182.4±75.2 (94.9–357.7)	0.001
LUPV	333.1±171.9 (162.9–948.5)	322.2±131.2 (144.3–599.2)	254.8±112.1 (86.4–513.0)	178.6±70.0 (44.1–362.8)	0.000
LLAV	317.3±113.3 (137.3–626.1)	305.3±111.8 (141.7–569.0)	256.7±135.7 (18.2–558.4)	189.5±71.6 (88.7–390.3)	0.001
LLLV	336.6±112.2 (175.6–719.7)	298.7±134.4 (142.7–686.1)	274.8±131.3 (114.5–535.1)	191.2±73.9 (70.9–416.1)	0.001
LLPV	305.8±110.9 (150.0–641.7)	316.5±111.0 (140.0–548.8)	268.8±158.6 (68.1–701.3)	188.6±72.7 (43.0–380)	0.000
US	305.3±83.0 (104.4–497.7)	296.0±119.1 (111.0–550.1)	251.1±99.7 (99.6–397.5)	176.0±70.5 (70.9–345.6)	0.000
LS	335.1±121.5 (130.5–667.7)	305.9±117.4 (111.0–517.4)	247.1±111.2 (51.5–479.9)	195.0±73.2 (51.1–354.2)	0.000
A	405.4±332.7 (231.4–1512.6)	317.3±119.8 (134.8–531.4)	289.0±195.8 (98.9–831.1)	174.3±76.6 (40.5–367.5)	0.000
Mean perox	353.8±157.0 (148.8–774.4)	321.3±130.2 (142.2–592.2)	285.3±168.6 (92.1–797.2)	183.2±59.2 (83.1–346.7)	0.000

RPV Right posterior ventricle.
RAV Right anterior ventricle.
LULV Left upper lateral ventricle.
LUAV Left upper anterior ventricle.
LUPV Left upper posterior ventricle.
LLAV Left lower anterior ventricle.
LLLV Left lower lateral ventricle.
LPV Left lower posterior ventricle.
US Upper septum.
LS Lower septum.
A Apex.
Mean perox Mean peroxidation.

Table 2 Mean values (nmol/g) ± standard deviation (SD) for lipid peroxidation in the groups of myocardial infarction+trauma (*N*=38) and no myocardial infarction+no trauma (*N*=37) and the probability obtained in the Mann-Whitney Wilcoxon test to contrast the values of peroxidation for the two diagnostic groups

Zones	MI+trauma N=38 Mean ± SD (range)	No MI + no trauma N=37 Mean ± SD (range)	*P*
RPV	392.7±313.2 (134.7–1531.4)	284.5±406.4 (44.2–2515.8)	0.002
RAV	429.3±367.6 (1224.5–1813.3)	269.3±304.6 (86.4–1824.7)	0.000
LULV	321.5±122.3 (98.4–668.4)	218.5±87.2 (62.7–435.6)	0.000
LUAV	319.3±127.9 (113.5–617.4)	229.2±111.9 (86.4-536.9)	0.002
LUPV	327.6±150.9 (144.3–948.5)	215.6±99.4 (44.1–513.0)	0.000
LLAV	311.3±111.2 (137.3–626.1)	222.2±111.5 (18.2–558.4)	0.000
LLLV	317.7±123.6 (142.7–719.7)	231.9±112.6 (70.9–535.1)	0.001
LLPV	311.2±109.6 (140.0–641.7)	227.6±127.1 (43.0–701.3)	0.000
US	300.7±101.4 (104.4–550.1)	212.5±92.9 (70.9–397.5)	0.000
LS	320.5±118.2 (111.0–667.7)	220.4±96.0 (51.1–479.9)	0.000
A	361.4±250.6 (134.8–1512.6)	230.1±156.2 (40.5–831.1)	0.000
Mean perox.	337.6±143.2 (142.2–774.4)	232.9±133.6 (83.1–797.2)	0.000

RPV Right posterior ventricle.
RAV Right anterior ventricle.
LULV Left upper lateral ventricle.
LUAV Left upper anterior ventricle.
LUPV Left upper posterior ventricle.
LLAV Left lower anterior ventricle.
LLLV Left lower lateral ventricle.
LLPV Left lower posterior ventricle.
US Upper septum.
LS Lower septum.
A Apex.
Mean perox Mean peroxidation.

tion or multiple trauma (*n*=38) and the other comprising the remaining cases (*n*=37). Statistically significant differences were observed between these groups. The highest values were found in the group of subjects who had died of myocardial infarction or chest trauma. We obtained a statistically significant correlation between the levels of peroxidation and the presence of myocardial lesions in all sites except the posterior part of the right ventricle.

We considered the ROC curves reflecting the levels of peroxidation and determined the cut-off point on each curve (Table 3) and compared the areas below the different curves. All the areas under the curves where significantly different from 0.5. If we take the diagnostic categories, myocardial infarction+trauma and no myocardial infarction+no trauma, in the discriminant analysis, correct classification was found in 78.7% of the cases (81.1% for the group of subjects who did not die from ischemic or traumatic cardiac lesions) (Fig. 1).

Table 3 Cut-off points established according to the use of a receiver operator characteristic (ROC) curve

Variable	Cut-off point	Sensitivity of the cut-off point (%)	Specificity of the cut-off point (%)
RPV	260.0	63.2	75.7
RAV	213.6	76.3	78.4
LULV	291.4	71.1	81.1
LUAV	231.0	81.6	64.9
LUPV	268.5	65.8	81.1
LLAV	256.7	71.1	78.4
LLLV	261.9	73.7	78.4
LLPV	239.6	76.3	73.0
US	207.8	86.8	59.5
LS	218.4	84.2	62.2
A	232.7	81.6	73.0
Mean perox.	264.4	73.7	78.4

RPV Right posterior ventricle.
RAV Right anterior ventricle.
LULV Left upper lateral ventricle.
LUAV Left upper anterior ventricle.
LUPV Left upper posterior ventricle.
LLAV Left lower anterior ventricle.
LLLV Left lower lateral ventricle.
LLPV Left lower posterior ventricle.
US Upper septum.
LS Lower septum.
A Apex.
Mean perox Mean peroxidation.

Discussion

The response of myocardial tissue to injury may manifest itself as macroscopical or microscopical lesions or, following an ischemia-reperfusion process, by the formation of ROS which in turn, may increase lipid peroxidation levels in the myocardial tissue (Kloner et al. 1998; Jordan et al. 1999; Powell et al. 2001). Fineschi et al. (2001) assessed the effects of cocaine on the cardiovascular system in a rat model and found that its administration compromised the antioxidant defence system of the heart.

Lipid peroxidation is a vital process taking place during the reperfusion period. Postischemic reperfusion may occur after resuscitation attempts or reperfusion treatment (angioplasty, fibrinolysis, coronary by-pass). The ischemia-reperfusion process may also appear after restoration of the blood flow in ischemic tissue as a result of collateral circulation or the end of vasospasm. In addition, the inflammatory processes around the necrotic area give rise to oxidative stress mechanisms that result in lipid peroxidation (McCord 1985; Southorn and Powis 1988; Lunec 1990; Dröge 2002; Suematsu et al. 2002).

In our study cardiopulmonary resuscitation had been applied in 22 subjects. In these cases, myocyte necrosis with contraction bands were usually found in the myo-

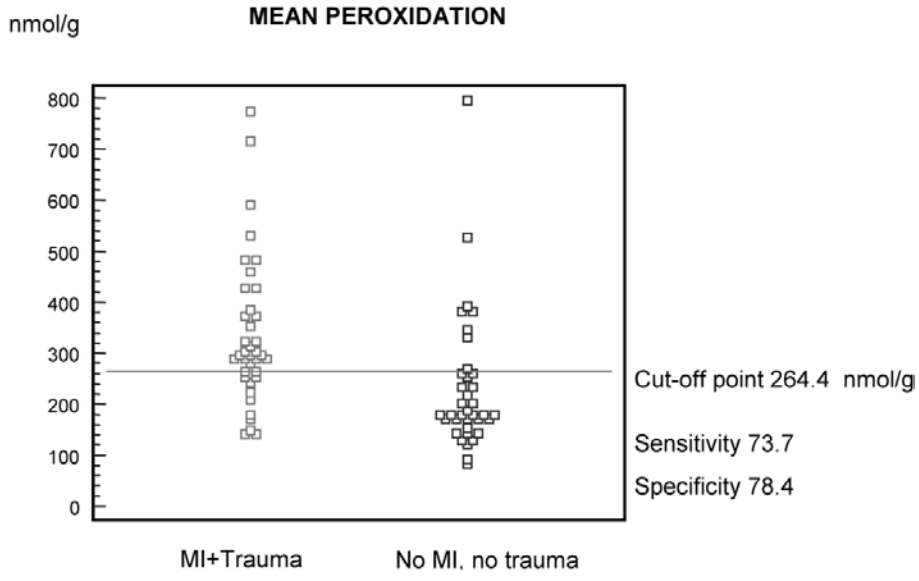

Fig. 1 Distribution of the cases into myocardial infarction+trauma and no myocardial infarction+no trauma according to the peroxidation values and taking 264.4 nmol/g as cut-off value

cardium (Baroldi et al. 1974, 2001; Silver et al. 1980). In our study contraction bands were observed in 10 of these subjects. Contraction band necrosis is a marker of cell death and can be identified as such a short time after irreversible myocyte injury (Virmani et al. 1996; Baroldi et al. 2001; Ortmann et al. 2001). Ortmann et al. (2001) demonstrated its presence in antemortem mechanical heart trauma. For some authors contraction band necrosis is a consequence of reperfusion per se (Braunwald 1990). Fineschi et al. (2001) postulated that the presence of contraction bands is not a result of ischemia but rather a result of direct cathecolamine release whose oxidation results in the formation of aminochromes and free radicals. For Baroldi et al. (2001) the significantly greater extent of contraction band necrosis in cases of sudden unexpected coronary death involving resucitation attempts seemed more likely to be due to the result of prolonged survival rather than due to any iatrogenic effects. Apoptosis also occurs in myocardial infarction (Misao et al. 1996; Saraste et al. 1997; James 1998). The study of myocardial tissue taken from forensic autopsy cases and examined by immunohistochemical and molecular biological methods using the terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end-labelling (TUNEL) is a useful method for the diagnosis of early ischaemic injury (Edston et al. 2002; Nakatome et al. 2002).

No statistically significant differences in lipid peroxidation were obtained between the group of subjects where cardiopulmonary resuscitation was applied, and those where it was not applied. In our study it was difficult to assess the effectiveness of resuscitation attempts and their repercussion on the ischemia-reperfusion process. The levels of peroxidation observed in these subjects varied greatly. For example, the lowest value corresponded to a drowned subject and the highest value to a person who died of acute myocardial infarction with a survival period of 2 h and 15 min.

The highest levels of tissue peroxidation were observed in the group of subjects who died from myocardial damage (ischemic or traumatic). The use of a ROC curve permits us to choose the best possible cut-off point (Altman 1991; Zweig and Campbell 1993), in our case 264.4 nmol/g for mean peroxidation levels with a sensitivity of 73.7% and specificity of 78.4%. The discriminant analysis pointed to the important negative predictive value. Only 7 of the 37 cases comprising the group of subjects who did not die from ischemic or traumatic cardiac lesions were wrongly classified. These could be further broken down into 6 cases of asphyxia and 1 of pulmonary embolism, in both of which cardiac damage may have been substantial due to intense agony phenomena. For the individuals presenting anatomopathological alterations compatible with acute myocardial infarction, 13 of the 19 subjects showed high lipoperoxidation values and 14 of the 19 subjects whose death was due to chest traumatism followed by a survival period also showed high values. The mean peroxidation value in the group of subjects who died of asphyxia was below the mean value observed in subjects who died of acute myocardial infarction or chest trauma, perhaps due to the decreased levels of oxygen in the asphyxia and the attenuation of peroxidation processes.

The absence of a significant degree of correlation between peroxidation levels and the presence of myocardial damage in the posterior zone of the right ventricle may in our opinion, be due to the low incidence of infarction in this zone. The presence of anastomotic channels between the left circumflex and right coronary arteries, presumably contributes to the better survival of these areas (James 1961; Camps et al. 1976; Knight 1996).

A study by Ramos et al. (1997) pointed to local variations in glutathione peroxidase and superoxide dismutase from different myocardial zones. Glutathione peroxidase activities were higher in the interventricular septum from the group of cardiac deaths. The superoxide dismutase activity was higher in the anterior wall of the left ventricle and in the interventricular septum, which are areas with a higher frequency of ischemic myocardial damage (Andersen et al. 1989; Knight 1996).

Another variable which, it was thought, might influence the results is the postmortem interval. In our study we used a short postmortem interval (mean 7.48, SD 4.16 h) and no statistically significant correlation was obtained between this and the mean levels of lipid peroxidation. Ramos et al. (1997) found no correlation between MDA levels and the postmortem interval and, in a previous study (Osuna et al. 1998), we found no statistically significant correlation between the levels of lipid peroxidation in lung tissue and the postmortem interval. In our opinion, the absence of oxygen means that conditions do not exist for postmortem peroxidation to proceed.

In our study, lipid peroxidation was associated with myocardial injury. The data suggest that the concentration of lipid peroxidation in myocardial tissue may be used as a reliable indicator of myocardial damage in support of data obtained by conventional microscopy, a procedure we consider indispensable at a routine level. However, we must remember that on some occasions, especially under those circumstances in which the evolution of the lesion is insufficient to produce conclusive findings, the interpretation of data obtained in an anatomopathological study must be supported by these complementary tests.

References

Altman DG (1991) Practical statistical for medical research. Chapman and Hall, London, pp 416–418

Ambrosio G, Zweier JL, Flaherty JT (1991) The relationship between oxygen radical generation and impairment of myocardial energy metabolism following post-ischemic reperfusion. J Mol Cell Cardiol 23:1359–1374

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

Baroldi G, Radice F, Schmid G, Leone A (1974) Morphology of acute myocardial infarction in relation to coronary thrombosis. Am Heart J 87:65–75

Baroldi G, Mittleman RE, Parolini M, Silver MD, Fineschi V (2001) Myocardial contraction bands. Definition, quantification and significance in forensic pathology.Int J Legal Med 115: 142–151

Braunwald E (1990) Textbook of cardiology, 3rd edn. WB Saunders, Philadelphia, pp 1225–1226

Camps FE, Robinson AE, Lucas BGB (1976) Gradwohl's legal medicine, 3rd edn. John Wright, Bristol, pp 220–254

Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95

Edston E, Gröntoft L, Johnsson J (2002) TUNEL: a useful screening method in sudden cardiac death. Int J Legal Med 116:22–26

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

Fineschi V, Baroldi G, Centini F et al. (2001) Markers of cardiac oxidative stress and altered morphology after intraperitoneal cocaine injection in a rat model. Int J Legal Med 114:323–330

Griffiths EJ, Halestrap AP (1995) Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open after reperfusion. Biochem J 307:93–98

Griner PF, Mayewski RJ, Mushlin AI, Greenland P (1981) Selection and interpretation of diagnostic test and procedures. Principles and applications. Ann Intern Med 94:557–592

Hauley JA, McNeil BJ (1983) The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 143:29–36

James TN (1961) Anatomy of the coronary arteries. Harper and Row, Hagerstown, pp 51–60

James TN (1998) The variable morphological coexistence of apoptosis and necrosis in human myocardial infarction: significance for understanding its pathogenesis, clinical course, diagnosis, and prognosis. Coron Artery Dis 9:291–307

Jordan JE, Zhao ZQ, Vinten-Johansen J (1999) The role of neutrophils in myocardial ischemia-reperfusion injury. Cardiovasc Res 43:860–878

Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E (1998) Medical and cellular implications of stunning, hibernation and preconditioning: an NHLBI workshop. Circulation 97:1848–1867

Knight B (1996) Forensic pathology. Arnold, London, pp 490–495

Lucas DT, Szweda LI (1998) Cardiac reperfusion injury: aging, lipid peroxidation, and mitochondrial dysfunction. Proc Natl Acad Sci U S A 95:510–514

Lunec J (1990) Free radicals: their involvement in disease processes. Ann Clin Biochem 27:173–182

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

Misao J, Hayakawa Y, Ohno M, Kato S, Fujiwara T, Fujiwara H (1996) Expression of bcl-2 protein, an inhibitor of apoptosis, in ventricular myocytes of human hearts with myocardial infarction. Circulation 94:1506–1512

Nakatome M, Matoba R, Ogura Y et al. (2002) Detection of cardiomyocyte apoptosis in forensic autopsy cases. Int J Legal Med 116:17–21

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–158

Ortmann C, Pfeiffer H, Brinkmann B (2001) Immunohistochemical alterations after intravital and post-mortem traumatic myocardial damage. Int J Legal Med 115:23–28

Osuna E. Pérez-Cárceles MD, García Lorente A, Sánchez Hanke M, Vieira DN, Carvalho L, Püschel K, Luna A (1998) Lipid peroxidation in lung tissue after chest trauma and correlation with the duration of the post-trauma survival period. Int J Legal Med 111:256–260

Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Serena D, Ruggiero FM (1999) Lipid peroxidation and alteration to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. Free Radic Biol Med 27: 42–50

Powell SR, Gurzenda EM, Wahezi SE (2001) Actin is oxidised during myocardial ischemia. Free Radic Biol Med 30:1171–1176

Radin N (1969) Preparation of lipid 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

Saraste A, Pulkki K, Kallajoki M, Henriksen K, Parvinen M, Volpo-Pulkki LM (1997) Apoptosis in human acute myocardial infarction. Circulation 95:320–323

Silver MD, Baroldi G, Mariani F (1980) The relationship between acute occlusive coronary thrombi and myocardial infarction studied in 100 consecutive patients. Circulation 61:219–227

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

Suematsu M, Suzuki H, Delano FA, Scmid-Schonbein GW (2002) The inflammatory aspects of the microcirculation in hypertension: oxidative stress, leukocytes/endothelial interaction, apoptosis. Microcirculation 9:259–276

Virmani R, Farb A, Burke A (1996) Contraction band necrosis: a new use for an old friend. Lancet 347:1710–1711

Zweier JL (1988) Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. J Biol Chem 263:1353–1357

Zweig MH, Campbell G (1993) Receiver operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 39:561–577