ELSEVIER

European Journal of Pharmacology 320 (1997) 43–49

ejp

Hyperbaric oxygen worsens myocardial low flow ischemia-reperfusion injury in isolated rat heart

Sonia Radice [a], Giuseppe Rossoni [a], Giorgio Oriani [b], Michael Michael [b], Enzo Chiesara [a], Ferruccio Berti [a,*]

[a] *Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan, Via Vanvitelli 32, 20129 Milan, Italy*
[b] *Galeazzi Orthopedics Institute, Hyperbaric Service, Milan, Italy*

Received 24 June 1996; revised 4 November 1996; accepted 8 November 1996

Abstract

In these experiments rats were exposed to hyperbaric oxygen (100% oxygen; 2.5 atmospheres absolute pressure) for 1, 3 or 6 h. At the end of these periods the hearts were removed and subjected to low flow ischemia (perfusion rate from 12 ml/min to 2 ml/min for 40 min) and reperfusion. Hearts excised from control rats were subjected to the same procedure of ischemia and reperfusion. The data obtained from these experiments clearly indicate that the ischemic picture observed in control hearts is worsened in hearts obtained from hyperbaric oxygen-exposed animals. In fact, after ventricular standstill of the ischemic phase, the left ventricular end-diastolic pressure increased significantly and proportionally according to the time of hyperbaric oxygen exposure. The vasopressor activity of angiotensin II on coronary perfusion pressure was significantly changed, as compared to that in the control preparation: these alterations, well correlated to the time of hyperbaric oxygen exposure, seem to suggest impairment of the vascular endothelium-dependent relaxant function. Futhermore *N*-acetylcysteine and defibrotide, given orally to the rats before hyperbaric oxygen exposure, prevented the aggravation of the ischemic damage induced in ex vivo hearts.

Keywords: Hyperbaric oxygen; Heart, rat; Ischemia, low flow; *N*-Acetylcysteine; Defibrotide

1. Introduction

Experience has provided evidence for the strong benefit of hyperbaric oxygen therapy in the treatment of many severe human illnesses which have hypoxia as common feature (Heyman et al., 1966; Neubauer and End, 1980; Takahashi et al., 1992). This is particularly true during carbon monoxide poisoning, when a cascade of microvascular events leads to injury of endothelial cells. Under these circumstances the beneficial effects of hyperbaric oxygen application appear to involve the impairment of leukocyte binding, activation and subsequent lipid peroxidation (Thom, 1993). However, in spite of these advantageous findings, it has been reported that when the tissue oxygen tension is augmented, there may ensue marked hemodynamic alterations, such as a rise in peripheral resistance and a decrease of cardiac output and blood flow to various organs (Bergofsky and Bertum, 1966; Torbati et al., 1979). It has also been shown that hyperbaric conditions cause a variety of changes in the electrical activity of the mammalian heart; pacemaker automaticity, conduction velocity and repolarization are all affected in some way by hyperbaric oxygen exposure (Eckenhoff and Knight, 1984). Furthermore, there are indications from the literature that hyperbaric oxygen treatment may produce a tendency to coagulation (Flook, 1987; Harabin et al., 1990). This phenomenon has been explained by the capacity of hyperbaric oxygen to enhance the generation of oxygen free radicals which in turn may damage directly the vascular endothelial cells, thus damaging the homeostatic mechanism for regulation of platelet aggregability (Halliwell and Gutteridge, 1989). All these observations prompted us to investigate the course of an ischemic reperfusion event induced in isolated hearts obtained from rats subjected to various times of hyperbaric oxygen exposure. Even if oxygen therapy may be administered under hyperbaric conditions and is used in the management of several types of ischemic tissue injury, we provide evidence that, at least in

* Corresponding author. Tel.: (39-2) 749-0243; Fax: (39-2) 7014-6371.

PII S0014-2999(96)00885-0

the rat heart, this treatment may be detrimental for myocardial function particularly in the presence of moderate ischemic insult.

2. Materials and methods

2.1. Hyperbaric oxygen treatment

Sprague-Dawley male rats (Charles-River, Calco, Italy), weighing 170–180 g were fed a standard diet and water ad libitum. Groups of 5 rats, placed in a Plexiglas box, were introduced into a small (25 cm diameter, 50 cm long) hyperbaric chamber with one compartment (Sistemi Iperbarici Integrati, Rome, Italy) and exposed for a period of 1, 3 or 6 h to hyperbaric oxygen (100% O_2; 2.5 atm absolute pressure). In order to eliminate CO_2 accumulation, the chamber was flushed with 100% O_2 for 1 min every 20 min during exposure. A group of 8 animals was considered as controls not exposed to hyperbaric oxygen.

2.2. Ischemia-reperfusion in isolated rat hearts

All the animals, not exposed and exposed to hyperbaric oxygen, were killed under anesthesia by cervical dislocation. The hearts were rapidly removed and perfused retrogradely through the aorta with gassed (95% O_2/5% CO_2) Krebs-Henseleit solution (37°C) of the following composi-

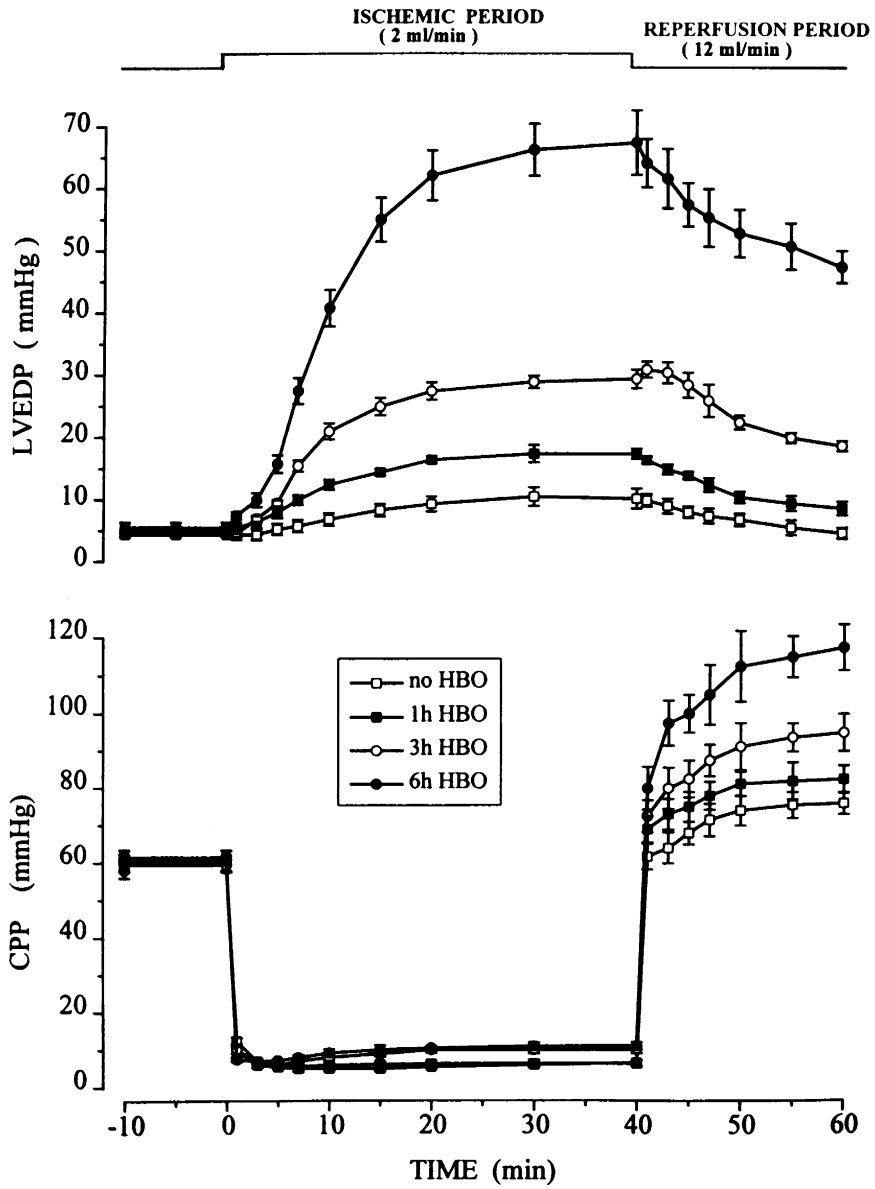

Fig. 1. Isovolumic left heart preparations submitted to 40-min low flow ischemia and reperfusion. Hearts were obtained from rats exposed to a hyperbaric oxygen (HBO) regimen. Each point represents the mean value ± S.E.M. for at least 5 hearts. Differences in left ventricular end-diatolic pressure (LVEDP) increase are given as area under the curve as given in Table 1. CPP, coronary perfusion pressure.

tion (mM): NaCl 118, KCl 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 5.5. After 30 min of equilibration, the pH of the heart perfusate was 7.4. According to the method described for rabbit heart preparations (Berti et al., 1988), left ventricular pressure was measured with a polyethylene catheter (with a small latex balloon on the top) inserted in the left ventricle cavity. The balloon was filled slowly with saline using a micrometer syringe until left ventricular end-diastolic pressure stabilized in the range of 5 mmHg. Coronary perfusion pressure and left ventricular pressure were monitored with a Statham transducer (HP-1280C) connected with a Hewlett-Packard (Waltham, MA, USA) dynograph (HP-7754A). The hearts were electrically paced at a frequency of 300 beats/min with rectangular pulses (1-ms duration, voltage 10% above threshold) by a Grass stimulator (mod S-88; Grass, Quincy, MA, USA). The perfusion rate of each heart was adjusted to yield a coronary perfusion pressure of 58–62 mmHg with a flow rate of 12 ml/min.

Ischemia was induced by reducing the coronary flow to 2 ml/min (coronary perfusion pressure, 4–6 mmHg) for a period of 40 min. At the end of this period, reperfusion at the initial flow rate (12 ml/min) was resumed for 20 min.

The vasopressor activity of angiotensin II (1 μg as a bolus in the perfusion system) was routinely recorded at the beginning of each experiment.

Hearts obtained from rats that had been exposed to hyperbaric oxygen for 6 h were subjected to ischemia-reperfusion experiments after 1, 3 or 6 days.

2.3. Animal treatment

Groups of rats were treated with *N*-acetylcysteine given orally (1 g/kg once a day for 2 days and immediately before hyperbaric oxygene exposure) and defibrotide (100 mg/kg twice a day for 2 days) and exposed to hyperbaric oxygen for 6 h. Control rats received a corresponding amount of saline.

Defibrotide is known for its ability to protect from ischemic myocardial insults (Berti et al., 1986), whereas *N*-acetylcysteine has been shown to protect the heart by scavenging reactive oxygen species (Brunet et al., 1995).

2.4. Statistical analysis

Differences between groups in individual experiments were analyzed for statistical significance by one-way analysis of variance (ANOVA) and Student's *t*-test (two-tailed) for unpaired samples. A value of $P < 0.05$ was considered significant. Results are expressed as means ± S.E.M.

2.5. Drugs

The following drugs were used: *N*-acetylcysteine (Zambon, Milan, Italy), defibrotide (Crinos, Villa Guardia, Italy), angiotensin II (Sigma, St. Louis, MO, USA).

3. Results

3.1. Ischemia-reperfusion in isolated rat hearts

When the rate of perfusion (12 ml/min) of isovolumic left heart preparations paced at 300 beats/min was reduced to 2 ml/min, the left ventricular developed pressure (peak left ventricular systolic pressure minus left ventricular end-diastolic pressure) and left ventricular dP/dt_{max} declined rapidly. At the same time, the phasic contractility of the hearts slowed until complete ventricular arrest (standstill) was achieved. Afterwards, only a minimal elevation of left ventricular end-diastolic pressure was recorded at the end of 40-min ischemia. Reperfusion (12 ml/min) caused a substantial recovery of cardiac contractility (75%; $P < 0.01$) with prompt return of the electrical pacing. Coronary perfusion pressure was only minimally affected, indicating a modest increase in coronary resistance as well as a lower degree of ischemic damage (Fig. 1). In these control preparations, the pressor effect of angiotensin II (1 μg as a bolus) was consistent with an increase in coronary resistance which was in the range of 20 mmHg (Fig. 2). When the ischemia-reperfusion experiments were repeated with hearts excised from rats subjected to hyperbaric oxygen for 1, 3 or 6 h, worsening of the ischemic picture, depending on the time of exposure in the hyperbaric chamber, was observed (Fig. 1). Aggravation of the ischemic damage was particularly significant in the hearts from rats subjected to hyperbaric oxygen for 6 h. In these cases, the ventricular contracture at the end of the ischemic period was 6 times higher ($P < 0.001$) than that

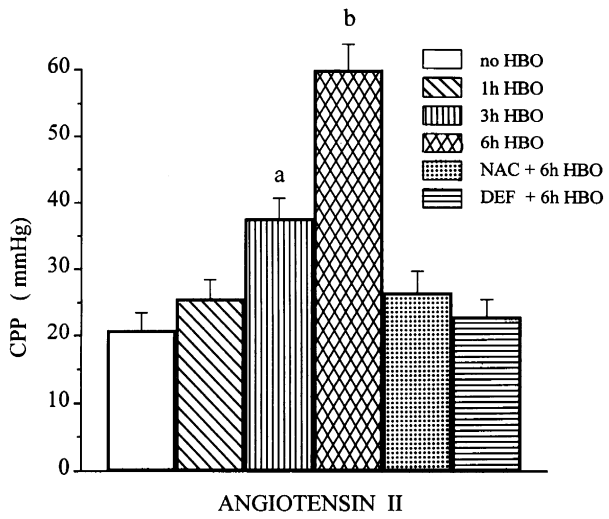

Fig. 2. Vasopressor activity of angiotensin II (1 μg as a bolus in the perfusion system) in isovolumic left heart preparations before reduction of the flow rate. *N*-Acetylcysteine (NAC, 1 g/kg p.o.; once a day for 2 days and immediately before hyperbaric oxygen exposure) and defibrotide (DEF, 100 mg/kg p.o.; twice a day for 2 days) were given orally before exposure of the rats to hyperbaric oxygen (HBO) for 6 h. Columns represent the mean values and vertical bars the S.E.M., of at least 5 experiments. [a] $P < 0.05$; [b] $P < 0.01$ versus control (no HBO).

observed in the corresponding control preparations (Fig. 1). During reperfusion, there was poor recovery of mechanical activity, associated with persistent rhythm disturbances due to a marked elevation of left ventricular end-diastolic pressure. Moreover, at the end of 20-min reperfusion, coronary perfusion pressure was still significantly increased (153% of that of controls; $P < 0.01$), possibly due to a certain degree of heart stiffness (Fig. 1). Challenge of these preparations with a bolus injection of angiotensin II in the pre-ischemic period brought about a remarkable increase in coronary perfusion pressure values, which was particularly significant in the heart from animals exposed to hyperbaric oxygen for 1, 3 or 6 h (Fig. 2). In this last group of rats, the activity of angiotensin II on coronary perfusion pressure was increased 2.9 times ($P < 0.01$) in comparison to that of control (not exposed to hyperbaric oxygen) preparations. These results seem to reflect impaired function of the endothelial cells lining the coronary vasculature.

The negative influence exerted over the ischemic damage by hyperbaric oxygen treatment in the rat involves a reversible process, since the aggravation reported above was reduced with time. In fact, when the hearts were used for ischemia reperfusion experiments 3 or 6 days after the

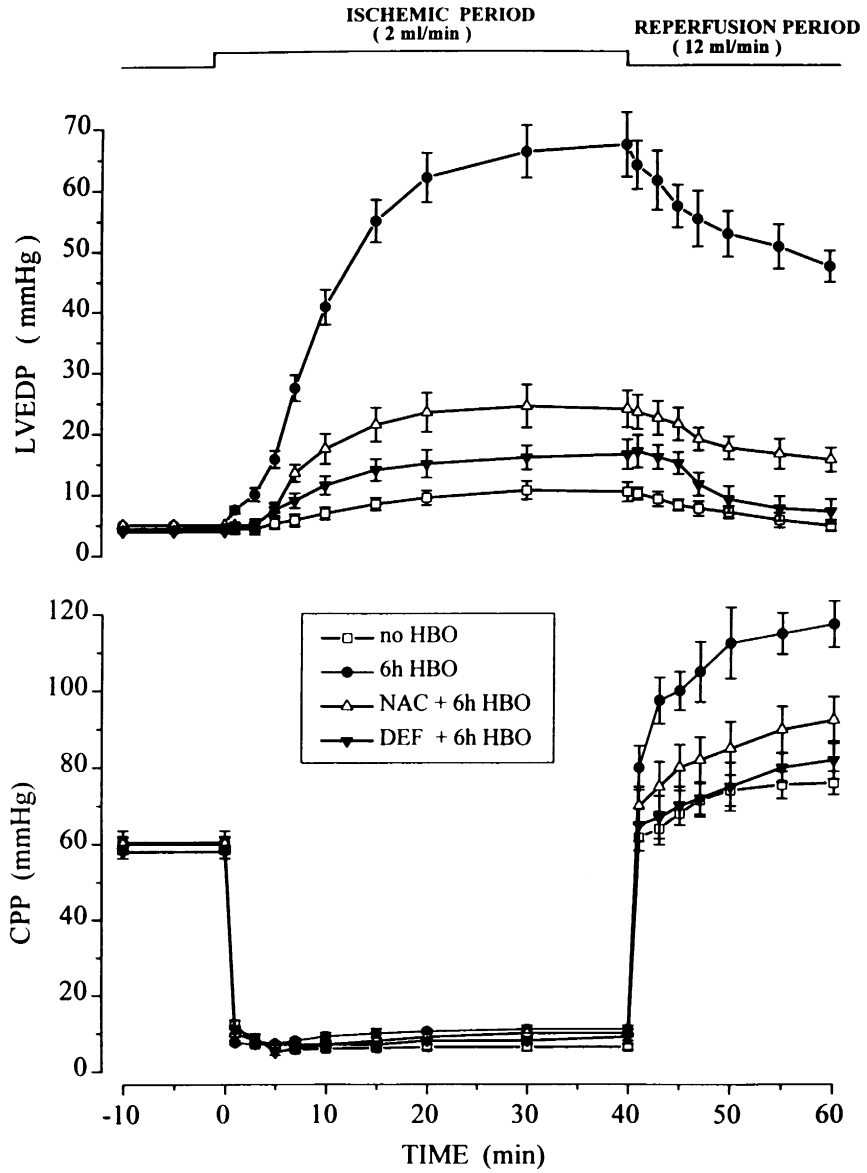

Fig. 3. Isovolumic left heart preparations submitted to 40-min low flow ischemia and reperfusion. Hearts were obtained from rats exposed to hyperbaric oxygen (HBO) for 6 h (6 h HBO). *N*-Acetylcysteine (NAC, 1 g/kg p.o.; once a day for 2 days and immediately before hyperbaric oxygen exposure) and defibrotide (DEF, 100 mg/kg p.o.; twice a day for 2 days) were given orally before exposure to 6 h HBO. The trend to a left ventricular end-diastolic pressure (LVEDP) increase is shown as area under the curve evaluated according to the trapezoid method (see Table 1). Control (no HBO): 147 ± 16 ($n = 8$); 6 h HBO: 1092 ± 88 [c] ($n = 6$); NAC + 6 h HBO: 381 ± 48 [b] ($n = 6$); DEF + 6 h HBO: 213 ± 32 [b] ($n = 6$). Differences versus control (no HBO): [c] $P < 0.001$; [b] $P < 0.01$.

animals had been exposed for 6 h to hyperbaric oxygen, there was a progressive improvement of the hearts resistance to the effect of a global reduction of perfusion rate (Table 1). At the same time, the response of the coronary vasculature to angiotensin II regressed gradually to the range of the control hearts response (data not shown).

3.2. N-Acetylcysteine and defibrotide activity

Treatment of the rats with *N*-acetylcysteine (1 g/kg p.o.; once a day for 2 days and immediately before hyperbaric oxygen exposure) before 6-h hyperbaric oxygen exposure resulted in a beneficial effect on the isolated hearts in ischemia-reperfusion experiments. In fact, in this sequence of experiments, after the perfusion rate through the hearts was reduced (2 ml/min), the increase of left ven-

Table 1
Trend of left ventricular end-diastolic pressure (LVEDP) increase in isovolumic left heart preparations (obtained from rats exposed to hyperbaric oxygen (HBO)) submitted to 40-min low flow ischemia and reperfusion

Experimental groups	No. of experiments	AUC of LVEDP	% Increase
No HBO	8	147 ± 16	–
1 h HBO	5	249 ± 27	69.4 [a]
3 h HBO	5	490 ± 60	233.2 [b]
6 h HBO	6	1092 ± 88	642.5 [c]
6 h HBO after 3 days	5	537 ± 57	265.0 [b]
6 h HBO after 6 days	6	181 ± 20	23.5

Data are mean values ± S.E.M. AUC: area under the curve of LVEDP (see Fig. 1) evaluated according to the trapezoid method (in ordinate, LVEDP in mmHg; in abscissa, time from 0 to 60 min). [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ vs. control (no HBO).

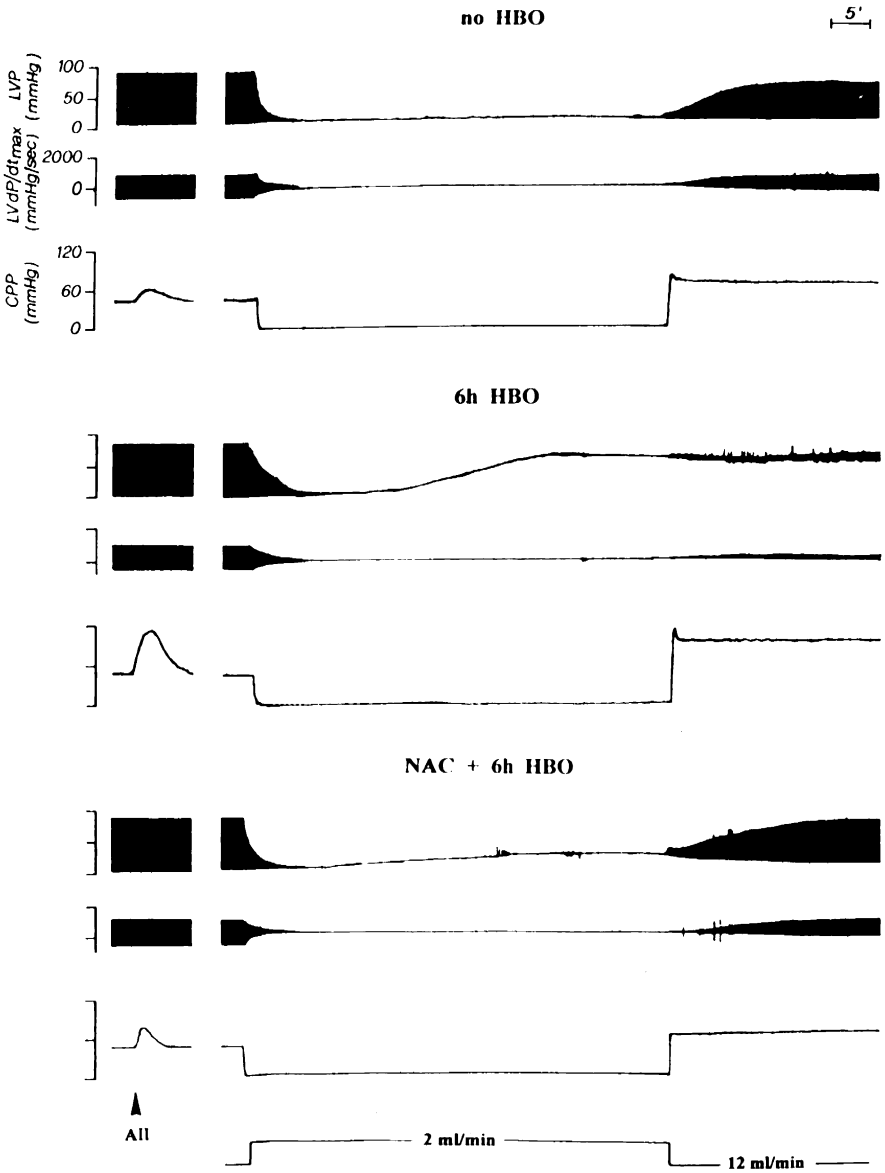

Fig. 4. Mechanograms related to isovolumic left heart preparations from rats not exposed to hyperbaric oxygen (no HBO) and exposed to hyperbaric oxygen for 6 h (6 h HBO). *N*-Acetylcysteine (NAC, 1 g/kg p.o.; once a day for 2 days and immediately before hyperbaric oxygen exposure) was given orally before exposure of the rat to 6-h hyperbaric oxygen. At the arrow, angiotensin II (AII, 1 μg as a bolus in the perfusion system).

tricular end-diastolic pressure, reflecting ventricular contracture, was diminished by 64% ($P < 0.01$) of that recorded in hearts from saline-treated animals. Moreover, on reperfusion, the appearance of electrical pacing favored an almost complete recovery of heart contractility. At the same time, coronary perfusion pressure was very little affected, being 21 ± 3 mmHg ($P < 0.01$) over the values for the pre-ischemic phase (Figs. 3 and 4).

A similar trend of protecting activity was obtained with defibrotide (100 mg/kg p.o.; twice a day for 2 days). In this case, the ventricular contracture during ischemia was modest and, on reperfusion, the cardiac mechanical activity regained its strength following the regular pacing and coronary perfusion pressure was preserved to near basal values (Fig. 3).

As shown in Fig. 2, pretreatment of the rats with *N*-acetylcysteine or defibrotide maintained the vasopressor activity of angiotensin II at the same potency as that recorded in hearts from saline-treated animals not exposed to hyperbaric oxygen.

4. Discussion

The present findings clearly demonstrated that the acute exposure of rats to a hyperbaric oxygen regimen is responsible for the aggravation of the ischemic picture monitored in ex vivo hearts submitted to 40-min low flow ischemia and reperfusion.

This phenomenon, paralleled by impairment of the endothelium-dependent relaxing function of the coronary vasculature, appears to involve a reversible process, since the worsening of the ischemic damage had almost vanished within a few days. The marked elevation of resting tension (left ventricular end-diastolic pressure), reflecting a certain degree of stiffness, and the related depression of cardiac mechanics on reperfusion, recorded in hearts from rat exposed to hyperbaric oxygen for 6 h, may be the results of a chain of biochemical events, triggered by the rise in tissue oxygen tension particularly in the intracardiac tissues. Such an increase of oxygen tension, secondary to the high concentration of oxygen inspired by the animals at ambient pressure, has been shown to be the cause of relevant hemodynamic alterations, including an increase of peripheral vascular resistance and a decrease of blood flow through the heart (Bergofsky and Bertum, 1966; Torbati et al., 1979). Moreover, experimental studies have shown that hyperoxia causes deleterious effects such as an oxygen free radical production increase (Halliwell and Gutteridge, 1989), compromised organ perfusion and an increase in myocardial ischemia (Neill, 1969). Furthermore, studies in open-chest dogs demonstrated that the decrease of isometric systolic tension and coronary flow alterations observed with a high oxygen tension (100%) are manifestations of early oxygen toxicity (Daniell and Bagwell, 1968). On this basis, it is reasonable to speculate that, during exposure to hyperbaric oxygen, the rat may undergo hemodynamic changes, such as pulmonary hypertension, and other pathological alterations (proton accumulation, acidosis, increased production of superoxide anion (O_2^-) and the more toxic hydroxyl radicals (–OH) and perhaps neutrophil activation) predisposing the heart to a greater sensitivity to superimposed driving by low flow ischemia. Therefore, the relevant increase in left ventricular end-diastolic pressure with depression of contractility noted in these ex vivo studies could ensue from the interactions, already initiated in vivo, between free radicals with the unsatured fatty acids of phospholipid membranes, leading to the generation of new radicals which, in turn, could have affected the integrity of the sarcolemma, sarcoplasmic reticulum and mitochondria. Similar damage has been documented during the course of myocardial ischemia (Hess et al., 1982; Henry et al., 1977). The beneficial results of the present experiment with rats treated with *N*-acetylcysteine or defibrotide favor the above hypothesis. Both these compounds, given to the animals before their exposure to hyperbaric oxygen, were able to keep the myocardial ischemic damage to the same degree of severity as that observed in hearts of animals not exposed to hyperbaric oxygen. In this regard, it has been reported (Brunet et al., 1995) that *N*-acetylcysteine could directly scavenge –OH produced by the isolated rat hearts reperfused after 90 min of low flow ischemia, whereas others (Pelaia et al., 1995) showed that this compound inhibits hyperbaric oxygen lipid peroxidation in man. Regarding defibrotide, its protecting activity may lie in its well known capacity to enhance the generation of prostacyclin and its likely constituent, nitric oxide, from vascular endothelial cells (Berti et al., 1987; Lefer et al., 1990; Hohlfeld et al., 1993). This natural polydeoxyribonucleotide of mammalian origin has been shown to be very effective in vitro and in vivo to preserve myocardial integrity following myocardial ischemia and reperfusion (Berti et al., 1986; Palmer and Goa, 1993).

Therefore, under our experimental conditions, defibrotide, without directly scavenging free signals, may have inhibited the possible adhesion and activation of polymorphonuclear leukocytes which are thought to be involved in the pathogenesis of ischemia and reperfusion (Di Perri and Pasini, 1988).

Another point of interest emerging from the present studies concerns the remarkable vascular hyperreactivity to angiotensin II in perfused hearts from hyperbaric oxygen-exposed rats, indicating that the regulatory function of coronary endothelium (prostacyclin and nitric oxide release) against vasopressor stimuli, was impaired. This would imply that the vascular endothelium damage, which took place during exposure of the animals to high pressure oxygen, could again have involved the direct generation of free radicals by the endothelial cells, caused by the increase in oxygen tension in these cells via xanthine oxidase (Downey et al., 1988), and consequent activation of neutrophils. These proinflammatory cells are known to be

involved in the pathogenesis of the ischemia reperfusion injury (Mullane, 1988) by adhering to vascular endothelium and releasing cytotoxic materials (oxygen-derived free radicals, tumor necrosis factor and leukotrienes) that damage the endothelial cells (Rossoni et al., 1996).

Therefore, it is tempting to speculate that protecting the rats with *N*-acetylcysteine or defibrotide before hyperbaric oxygen exposure, which attenuates the formation of a toxic mediator, creates conditions that lead to preservation of endothelial cell integrity. This could be the reason why the potency of angiotensin II to increase coronary perfusion pressure in the isolated hearts of rats pretreated with *N*-acetylcysteine and defibrotide and exposed to hyperbaric oxygen, was similar to that recorded in the hearts from control animals not exposed to hyperbaric oxygen. In conclusion, even if oxygen therapy may be regularly administered under hyperbaric conditions and is used in several types of ischemic organ injury, evidence has been provided that, at least in the normal rat without any preexisting hypoxic condition, it may be harmful for the myocardium in the presence of moderate ischemic injury. In this respect, Neill (1969) showed that in patients with coronary heart disease, and chronic hypoxia of the myocardium at rest, arterial hyperoxia did not change the blood lactate-pyruvate concentration ratio.

References

Bergofsky, E.H. and P. Bertum, 1966, Response of regional circulations to hyperoxia, J. Appl. Physiol. 21, 567.

Berti, F., G. Rossoni, R. Niada, G.C. Folco, C. Omini and C. Tondo, 1986, Defibrotide, an antithrombotic substance that preserves postsynaptic α and β-adrenergic function in post acute infarcted rabbit heart, J. Cardiovasc. Pharmacol. 8, 235.

Berti, F., G. Rossoni, C. Omini, G.C. Folco, L. Daffonchio, T. Viganò and C. Tondo, 1987, Defibrotide, an antithrombotic substance which prevents myocardial contracture in ischemic rabbit heart, Eur. J. Pharmacol. 135, 375.

Berti, F., G. Rossoni and F. Magni, D. Caruso, C. Omini, L. Puglisi and G. Galli, 1988, Nonsteroidal antiinflammatory drugs aggravate acute myocardial ischemia in the perfused rabbit heart: a role for prostacyclin, J. Cardiovasc. Pharmacol. 12, 438.

Brunet, J., M.J. Boily, S. Cordeau and C. Des Rosiers, 1995, Effects of *N*-acetylcysteine in the rat heart reperfused after low-flow ischemia: evidence for a direct scavenging of hydroxyl radicals and a nitric oxide-dependent increase in coronary flow, Free Radic. Biol. Med. 19, 627.

Daniell, H.B. and E.E. Bagwell, 1968, Effects of high oxygen on coronary flow and heart force, Am. J. Physiol. 214, 1454.

Di Perri, T. and F.L. Pasini, 1988, Effect of defibrotide on polymorphonuclear leucocytes: modulation of calcium entry and availability, Semin. Thrombosis Hemostasis 14, 23.

Downey, J.M., D.J. Hearse and D.M. Yellon, 1988, The role of xantine oxidase during myocardial ischemia in several species including man, J. Mol. Cell. Cardiol. 20, 55.

Eckenhoff, R.G. and D.R. Knight, 1984, Cardiac arrhythmias and heart rate changes in prolonged hyperbaric air exposures, Undersea Biomed. Res. 11, 355.

Flook, V., 1987, Physics and physiology in the hyperbaric environment, Clin. Phys. Physiol. Meas. 8, 197.

Halliwell, B. and J.M. Gutteridge, 1989, The superoxide theory of oxygen toxicity, in: Free Radicals in Biology and Medicine, eds. B. Halliwell and J.M. Gutteridge (Clarendon Press, Oxford) p. 136.

Harabin, A.L., J.C. Braisted and E.T. Flynn, 1990, Response of antioxidant enzymes to intermittent and continuos hyperbaric oxygen, J. Appl. Physiol. 69, 328.

Hess, M.L., N.H. Manson and E. Okabe, 1982, Involvement of free radicals in the pathophysiology of ischemic heart disease, Can. J. Physiol. Pharmacol. 60, 1382.

Henry, P.D., R. Shuchleib, J. Davis, E.S. Weiss and B.E. Sobel, 1977, Myocardial contracture and accumulation of mitochondrial calcium in ischemic rabbit heart, Am. J. Physiol. 233, H677.

Heyman, E., H.A. Salzman and R.E. Whalen, 1966, The use of hyperbaric oxygenation in the treatment of cerebral ischemia and infarction, Circulation 33, 20.

Hohlfeld, T., H. Strobach and K. Schrör, 1993, Stimulation of endogenous prostacyclin protects the reperfused pig myocardium from ischemic injury, J. Pharmacol. Exp. Ther. 264, 397.

Lefer, A.M., N. Aoki and D. Mulloy, 1990, Coronary endothelium-protective effects of defibrotide in ischaemia and reperfusion, Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 246.

Mullane, K.M., 1988, Myocardial ischemia-reperfusion injury. Role of neutrophils and neutrophil derived mediators, in: Human Inflammatory Disease: Current Concepts in Immunology. Clinical Immunology, Vol. 1, ed. G. Marone (B.C. Decker, Burlington) p. 143.

Neill, W.A., 1969, Effects of arterial hypoxemia and hyperoxia on oxygen availability for myocardial metabolism, Am. J. Cardiol. 24, 166.

Neubauer, R.A. and E. End, 1980, Hyperbaric oxygenation as an adjective therapy in strokes due to thrombosis: a view of 122 patients, Stroke 11, 297.

Palmer, K.J. and K.L. Goa, 1993, Defibrotide: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in vascular disorders, Drugs 45, 259.

Pelaia, P., M. Rocco, R.A. De Blasi, G. Spadetta, D. Alampi, F.S.M. Araimo and S. Nicolucci, 1995, Valutazione della perossidazione lipidica in corso di ossigenoterapia iperbarica: ruolo protettivo della *N*-acetilcisteina, Minerva Anestesiol. 61, 133.

Rossoni G., A. Sala, F. Berti, T. Testa, C. Buccellati, C. Molta, R. Müller-Peddinghaus, J. Maclouf and G.C. Folco, 1996, Myocardial protection by the leukotriene synthesis inhibitor BAY X1005; importance of transcellular biosynthesis of cysteinyl-leukotrienes, J. Pharmacol. Exp. Ther. 276, 335.

Takahashi, M., N. Iwatsuki and K. Ono, 1992, Hyperbaric oxygen therapy accelerates neurologic recovery after 15 minute complete global cerebral ischemia in dogs, Crit. Care Med. 20, 1588.

Thom, S.R., 1993, Functional inhibition of leukocyte B_2 integrins by hyperbaric oxygen in carbon monoxide-mediated brain injury in rats, Toxicol. Appl. Pharmacol. 123, 248.

Torbati, D., D. Parolla and S. Lavy, 1979, Organ blood flow, cardiac output, arterial blood pressures and vascular resistance in rats exposed to various oxygen pressures, Aviat. Space Environ. Med. 50, 256.