Original Paper

Oxidative Stress in Fulminant Hepatic Failure: Comparison of Two Pig Models With and Without Liver Necrosis

Claudia Kusmic ScD, Ugo Boggi MD[1], Rosario Bellini MD[1], Fabio Vistoli MD[1]
Michele Castellari ScD, Gianni Taddei ScD[2], Andrea Minervini MD[1], Franco Filipponi MD[1]
Franco Mosca MD[1], Renata Barsacchi ScD[2]
Institute of Clinical Physiology, CNR; [1]Department of Oncology, Division of General Surgery and [2]Department of Physiology and Biochemistry, University of Pisa, Pisa, Italy
Corresponding Author: Claudia Kusmic, PhD, Istituto di Fisiologia Clinica, CNR, Via Savi, 8-56127 Pisa, Italy
Tel: +39 050 315 2670, Fax: +39 050 315 2166, E-mail:kusmic@ifc.cnr.pi.it

KEY WORDS:
Reactive oxygen species; Vitamin E; MDA; Total hepatectomy; Complete hepatic devascularization; Toxic liver syndrome

ABBREVIATIONS:
Bifurcated Vascular Prothesis (BVP); Common Bile Duct (CBD); Creatine phosphokinase (CPK); Electroencephalogram (EEG); Fulminant Hepatic Failure (FHF); Fluorescent Protein-Aldehyde Adducts (FPA); Hepatic Artery (HA); High-Performance Liquid Chromatography (HPLC); Heart Rate (HR); Hepatic Veins (HV); Inferior Vena Cava (IVC); Lactate-Dehydrogenase (LDH); Low-Density Lipoproteins (LDL); Mean Arterial Pressure (MAP); Malondialdehyde (MDA); Prothrombin Time (PT); Portal Vein (PV)

ABSTRACT

Background/Aims: No experimental study has clearly demonstrated how liver necrosis worsens the evolution of fulminant hepatic failure. Considering that several types of liver injury are associated with oxidative stress, we decided to measure plasma oxidative markers in two pig models of fulminant hepatic failure without and with liver necrosis.

Methodology: Fulminant hepatic failure was produced in two groups of six pigs each by either total hepatectomy or complete hepatic devascularization. The following parameters were recorded before and during the course of hepatic failure: electrocerebral activity, plasma vitamin E, malondialdehyde and fluorescent protein-aldehyde adducts, total cholesterol, lactate-dehydrogenase, creatine phosphokinase, and ammonium.

Results: Despite comparable survival periods, hepatic necrosis was associated with earlier electrocerebral deterioration. Plasma concentration of malondialdehyde and fluorescent protein-aldehyde adducts rose and vitamin E content decreased in both groups. However, while in the group without liver necrosis the rates of cholesterol and vitamin E decay were identical, in the group with liver necrosis cholesterol concentration decreased less than vitamin E concentration, strongly indicating a true intravascular oxidation of vitamin E. Interestingly, in both models the rise of oxidative parameters preceded the development of cell injury.

Conclusions: Oxidative stress, although present in both models, was significantly higher in the group with liver necrosis.

INTRODUCTION

Human fulminant hepatic failure (FHF) is a complex clinical syndrome in which the severe pathophysiologic consequences of failing liver function are combined with and amplified by the harmful effects of hepatic necrosis (i.e., the so called "toxic liver syndrome") (1,2).

In an attempt to reduce the harmful consequences of "toxic liver syndrome" several investigators have recently suggested the possibility of performing a total hepatectomy when the diagnosis of irreversible FHF has been firmly established and a liver graft cannot be obtained immediately (2,3). However, no experimental study has clearly demonstrated the reasons of the supposed detrimental effects of hepatic necrosis on the evolution of FHF.

Oxygen-derived free radicals are considered responsible for the oxidation of thiol groups, the induction of lipid peroxidation and the depletion of cellular antioxidants leading to cell death (4-7). Experimentally, oxidative stress has been associated with the acute hepatic injury produced by either intraperitoneal injection of CCl_4 (8) or oral administration of ethanol and diets high in polyunsaturated fatty acids (9). In humans, increased levels of oxygen-derived free radicals have been measured in patients with hepatic ischemia (10) and hepatic ischemia-reperfusion injuries (11). Moreover, preliminary reports on patients with severe forms of acute viral hepatitis B (12) and FHF (13) suggest that free-radical oxidation negatively affects the course of FHF. Should the presence of a failing necrotic liver be associated to an increased level of oxygen-derived free radicals this might explain some of the advantages empirically observed after total hepatectomy in humans (2) and contribute to clarify the pathogenesis of "toxic liver syndrome".

The main aim of this study was the analysis and the comparison of the patterns of oxidative stress occurring in two experimental models of FHF without and with hepatic necrosis. Oxidative stress is defined as a disturbance in the prooxidant-antioxidant balance in favor of the former. Amongst of many biological markers we chose to document the occurrence of oxidative stress by assessing plasma levels of malondialdehyde (MDA) and fluorescent protein-aldehyde adducts (FPA) - markers of oxidative damage of membrane phospholipids -, and plasma levels of vitamin E - the most important endogenous liposoluble antioxidant.

METHODOLOGY

Experimental Design

Two different surgical models of irreversible FHF were implemented into two experimental groups of six pigs each. Complete suppression of all hepatic functions, expected to occur in both groups, was achieved in two different ways. In the first group (FHF without necrosis group) the liver was removed (anatomic hepatectomy). In the second group (FHF with necrosis group) the liver was permanently deprived of both arterial and portal supply (functional hepatectomy) thus leaving *in situ* a dead organ capable of shedding its toxic products into the circulating stream through the hepatic veins. Pigs of both groups, weighing between 25 and 45kg (mean = 34.1±1.8kg), were kept under standard laboratory conditions and fasted overnight. The following parameters, thought to reflect the degree of oxidative damage, were recorded before surgery and timely reassessed thereafter: plasma vitamin E, MDA and FPA. The same plasma samples were also analyzed for: total cholesterol, lactate-dehydrogenase (LDH), creatine phosphokinase (CPK), and ammonium. Immediately after the end of the operation, all the pigs were woken and left under mild sedation on the operating table until death. Electrocardiogram, electroencephalogram, direct blood pressure and core body temperature were continuously monitored.

All the experiments were performed according to the International Principles governing research on animals under the supervision of a veterinarian.

Anesthesia

Pigs were pretreated with intramuscular atropine sulfate (0.01mg/kg), ketamine hydrochloride (10mg/kg), diazepam (0.3mg/kg) and azoperone (2.6mg/kg). Anesthesia was obtained by intravenous injection of fentanyl (1.0μg/kg), ketamine (5mg/kg) and propofol (2mg/kg). Pigs were arranged on an operating table equipped with thermic blanket (38°C), intubated orotracheally and ventilated with a mixture of oxygen and air (1:1) using a volume cycled mechanical ventilator preset to deliver a tidal volume of 280-300mL at a respiratory rate of 10-11 breaths/min. Muscle relaxation was induced with an intravenous bolus of panuronium bromide (0.1mg/kg) and maintained by continuous infusion (0.06mg/kg/h). Anesthesia was maintained by continuous infusion of fentanyl and propofol as described previously (14).

Surgical Models

1. **FHF without necrosis group:** Total hepatectomy with *en bloc* caval resection was performed in 6 pigs, according to the technique previously described by our group (14). In this model a caval-portal-jugular bypass was used to return portal and caval blood to the right heart during vascular reconstruction. A bifurcated dacron prosthesis was used to replace the retrohepatic segment of the vena cava and to construct a porta-caval shunt (**Figure 1A**).

2. **FHF with necrosis group:** Complete hepatic devascularization was achieved in 6 pigs, according to the technique originally described by Huguet *et al.* (15). After interruption of all sources of liver arterial supply, including the small branches running along the common bile duct and within the ligamentous attachments, an end-to-side porta-caval shunt was constructed (**Figure 1B**).

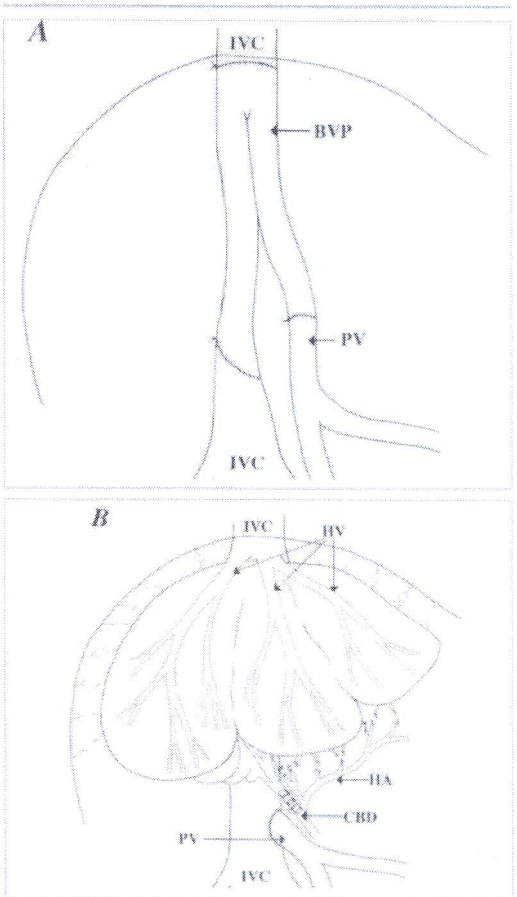

FIGURE 1 Models of FHF without (A) and with (B) liver necrosis. BVP: bifurcated vascular prosthesis; CBD: common bile duct; HA: hepatic artery; HV: hepatic veins; IVC: inferior vena cava; PV: portal vein.

During surgery, fluid losses, electrolyte abnormalities, serum glucose concentration changes and acid-base disturbances were serially monitored and immediately corrected by intravenous infusion of normal saline solution, lactated Ringer's solution, specific electrolyte solutions, concentrated glucose solutions, and sodium bicarbonate.

Supportive measures: Supportive measures were identical for both groups. Pigs received a continuous infusion of 10% dextrose solution at an infusion rate of 5mL/kg/h (0.5g/kg/h). Blood glucose levels were checked every 30min on a portable glucometer and infusion rates were regulated to maintain glucose levels between 75 and 100mg/dL (14). Fluid losses, serum electrolytes and acid-base balance were checked frequently and corrected as required. Pigs were continuously heated with warming blanket and received only

warm infusions.

Blood sample processing: Blood samples were timely collected from the jugular vein: before surgery (basal) and 60, 105, 150, 210, 330, 450, 570min after total hepatectomy or liver devascularization. Using ethylenediaminetetraacetic acid as anticlotting agent, the samples were immediately cooled in ice, centrifuged at 1000rpm for 10min and stored at -80°C as frozen plasma.

Biochemical Parameters of Oxidative Stress

1. Vitamin E assay: After addition of 50µL of 50-mMol/L ethanol solution of butylated hydroxytoluene to 150µL of plasma, vitamin E was extracted according to the procedure described by Lang *et al.* (16). Plasma aliquots were mixed with 1mL of 100-mMol/L sodium dodecylsulfate solution in water, 2mL of ethanol/isopropanol (95:5 v/v), 2mL of *n*-hexane and mixed with a vortex mixer for 2min. The hexane phase was separated by centrifugation, 1.5mL of aliquots were evaporated under nitrogen flux and resuspended in 110µL of methanol. Determinations by high-performance liquid chromatography (HPLC) were performed utilizing a 5-µm ODS C-18 reverse phase column (Beckman) using 1mL/min of methanol as eluent and a fluorimeter operating at 286nm excitation and 330nm emission wavelengths.

2. Malondialdehyde assay: Thiobarbituric acid reactive species were quantified as an index of MDA production using the spectrophotometric technique of Jentzsch *et al.* (17). Plasma was heated at 90°C for 45min and mixed with 25µL of 50-mMol/L ethanol solution of butylated hydroxytoluene, 400µL ortophosphoric acid (0.2 moles/L), and 50µL of 0.67% thiobarbituric acid. Samples were subsequently extracted with *n*-butanol and read at 535nm wavelength.

3. Fluorescent protein-aldehyde adducts assay: Samples were tested according to the method of Tuschida *et al.* (18). One hundred and fifty µL of plasma aliquots were washed 3 times with 6mL of ethanol/diethyl ether solution (3:1 v/v). The pellet was evaporated under nitrogen flux, 50µL of 350-mMol/L sodium dodecylsulfate were added and the mixture was resuspended in 3mL of bidistilled water. The samples were read with a fluorimeter operating at 360nm excitation and 430nm emission wavelengths to measure the total FPA.

Hematochemical Analysis

All plasma samples were also routinely analyzed for: total cholesterol, LDH, CPK, and ammonium. Assays were performed using a Beckman CX5CE instrument, with the corresponding commercial kits, from Beckman Analytical s.p.a.. Coagulative parameters (prothrombin time and fibrinogen concentration) were determined using a Behring Coagulation Timer, with corresponding commercial kits from Behring.

Electrophysiological Recordings

Electrical activity of the brain was monitored and timely recorded by means of seven needle electrodes secured to different scalp areas (frontal, central, and temporal areas) on an electroencephalogram (EEG) apparatus (OTE BIOMEDICA, 1230 Neurograph 18®). EEG recordings were analyzed by a neurologist and graded according to the 6 level rank proposed by Huguet *et al.* (15).

Autopsy

Postmortem examinations were routinely carried out in order to verify the presence of brain edema and of any other sign consistent with the occurrence of intracranial hypertension, and the absence of surgical complications. Brain and liver (FHF with necrosis group) histologies were obtained in every case. At least 6 large biopsy specimens were taken from different parts of the liver and processed for routine light microscopy. Hepatocytes with and without signs of necrosis were counted in at least 3 fields for each biopsy. The degree of liver injury was therefore expressed as the percentage of necrotic hepatocytes.

Statistical Analysis

Data are presented as means ±standard errors (s.e.). The statistical significance of the differences between experimental groups as well as between different times within each group were assessed by factorial analysis of variance, *post hoc* Fisher's test and *t* test (2-tailed) using STATWIEV 4.0 program (Abacus Concepts, Inc.).

RESULTS

FHF Without Necrosis Group

Total hepatectomy was carried out expeditiously and with negligible blood loss in every case. No pig required blood transfusion and hematology parameters measured after the procedure showed no significant variations when compared to their basal values. The operation was completed in 135±16min and the veno-venous bypass ran for 47±5min. All the pigs were readily extubated within 30min of the end of the operation.

The pigs died of progressive liver failure 1013±122min after hepatectomy. Supportive measures allowed the animals to remain hemodynamically stable until the last phases of the coma. Respiratory arrest, preceded by a period of tachypnoea and diffuse electrophysiologic suffering, was the terminal event in all the animals.

At autopsy brains were clearly swollen with flattened cortical gyri. The abdomen was clean and no surgical complication was identified. Histology confirmed the presence of cerebral edema.

Plasma concentration of α-tocopherol decreased significantly during the anhepatic phase, reaching 83% ±4 ($P<0.02$) and 53% ±4 ($P<0.001$) of its basal value 105 and 570min after hepatectomy, respectively. Interestingly enough, vitamin E reduction overlapped cholesterol decay (**Figure 2A**).

Plasma concentrations of the oxidative markers MDA and FPA rose significantly during anhepaty (**Figure 3A**). One hundred and five minutes after hepatectomy MDA plasma concentration significantly exceeded its basal value of 33% ±9 ($P<0.05$) and

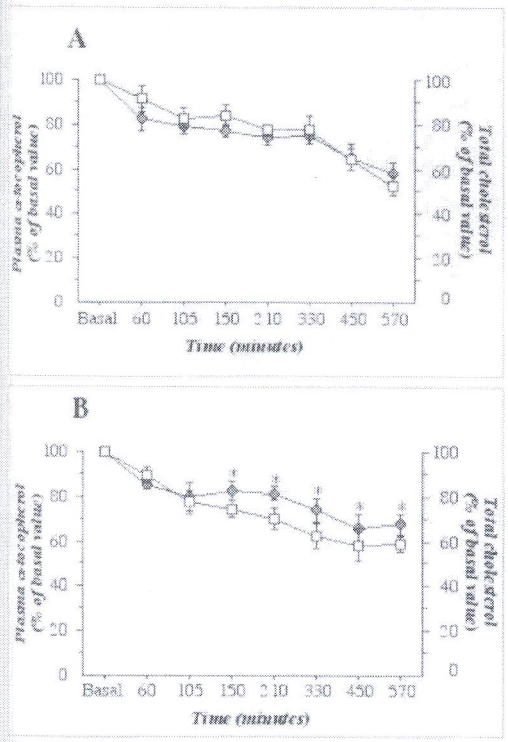

FIGURE 2 Time course of plasma total cholesterol and vitamin E decays in the two pig models of FHF without (A) and with (B) liver necrosis. Total cholesterol (filled circles) and α-tocopherol (empty squares) are expressed as percentage of the basal values. Basal samples were collected before surgery. Results are presented as mean ±s.e. of six animals for each group. *$P<0.05$ cholesterol *vs.* α-tocopherol concentration.

reached the maximum increase of 55% ±7 ($P<0.005$) at 570min.

The rise of plasma concentration of FPA was more rapid and 60min after hepatectomy it exceeded the basal value of 120% ±21 ($P<0.005$). Statistical significance was maintained until the end of the experiment **(Figure 3A)**.

CPK release, considered a marker of cellular injury, occurred well after the rise of oxidative markers and reached the statistical significance 330min after hepatectomy. Plasma LDH release was constant throughout the experiment **(Figure 3A)**.

FHF With Necrosis Group

Hepatic devascularization was completed in 105±14min without significant blood losses. No pig was transfused and hematology parameters measured before and after the procedure were unchanged. All the pigs were readily extubated within 3min of the end of the operation.

The pigs died of progressive liver failure 872±133min after hepatic devascularization. Death was preceded by a short period of hemodynamic instability and occurred after severe EEG deterioration.

At autopsy brains were clearly swollen with flattened cortical gyri. The failing livers had a decreased consistency and showed a spotty bluish discoloration. The porta-caval shunts were always patent and no surgical complication was noticed. Brain histology confirmed the presence of severe edema. The percentage of necrotic hepatocytes was 50% ±7.

A significant decrease in vitamin E content was first measured 105min after hepatectomy (78% ±5 of the basal level; $P<0.005$) and reached its maximum at 570min (59% ±3; $P<0.005$). Noteworthy, cholesterol decline did not overlap vitamin E decay. As depicted in the graph of **Figure 2B**, cholesterol decay was less evident than that of vitamin E. Consequently, factorial analysis of variance showed a significant difference between plasma levels of vitamin E and cholesterol ($F=6.07$; $df=1$; $P<0.02$) and Fisher's *post hoc* test indicated that vitamin E consumption was higher than cholesterol reduction ($P<0.05$) 150min after complete hepatic devascularization.

The rise of both MDA and FPA was markedly increased in this model **(Figure 3B)**. MDA increased constantly after devascularization reaching a significant value at 150min (40% ±10 over the basal value; $P<0.05$). FPA showed a significant rise early after devascularization (42% ±8 at 60min, $P<0.05$) and remained at a high level until the end of the experiment **(Figure 3B)**.

CPK release reached high significant levels ($P<0.005$) 330min after the beginning of warm hepatic ischemia. As shown in **Figure 3B**, also in this model, CPK release occurred well after oxidative damages arose.

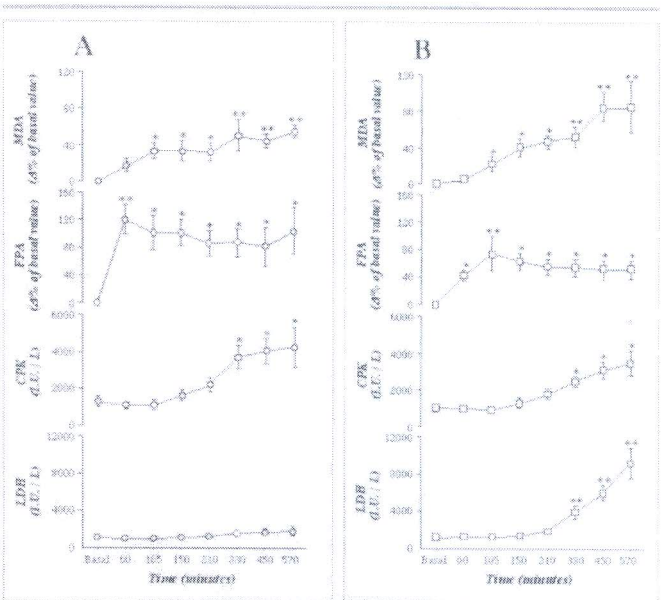

FIGURE 3 Time course of plasma oxidative parameters and cellular damage indices in the two pig models of FHF without (A) and with (B) liver necrosis. The oxidative markers malondialdheyde (MDA) and fluorescent protein adducts (FPA) are expressed as difference per cent (Δ%) of the basal values. Cellular injury is expressed as release of creatine phosphokinase (CPK) and lactate-dehydrogenase (LDH) in IU/L. Results are presented as mean ±s.e. of six animals for each group. *$P<0.05$, **$P<0.005$ *vs.* basal value.

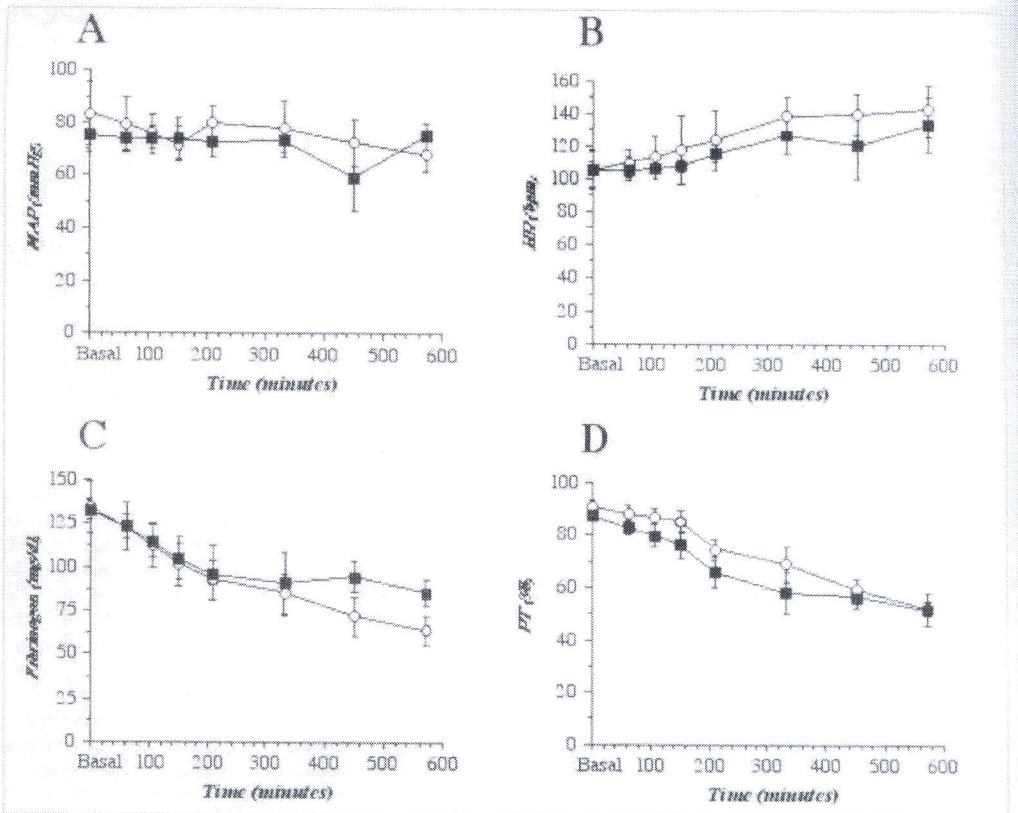

FIGURE 4 Time course of mean arterial pressure (MAP) (A), heart rate (HR) (B), fibrinogen (C) and percentage of prothrombin time (PT%) (D) in the two pig models of FHF without (empty circles) and with (filled squares) liver necrosis. Results are presented as mean ±s.e. of six animals for each group.

A significant release of LDH ($P<0.005$), indicating the incoming liver necrosis, was first recorded 330min after hepatic devascularization. This sharp release of LDH corresponded to a further sudden rise of MDA level **(Figure 3B)**.

FHF Without Necrosis Group *vs.* FHF With Necrosis Group

No significant difference was recorded between the two groups with respect to: operative times, intraoperative hemodynamics **(Figure 4A and B)**, coagulative parameters **(Figure 4C and D)**, time required to resume to spontaneous ventilation and postoperative survival.

The patterns of hepatic coma produced in the two experimental groups were similar **(Figure 5)**. However, pigs included in the FHF with necrosis group showed a more rapid deterioration of their EEG activity **(Figure 5A)** and a sharper increase of serum ammonia **(Figure 5B)** after the first phases of hepatic coma. Although postoperative survival was similar in the two groups (1013±122min *vs.* 872±133min), the absence of liver necrosis allowed the animals to maintain a satisfying EEG activity for a significantly longer period of time.

Vitamin E consumption occurred in both groups. However, while in the FHF without necrosis group the reduction of the levels of vitamin E corresponded to an identical decrease of total cholesterol, in the FHF with necrosis group cholesterol decay did not overlap the lowering of vitamin E. On the whole, 150min after the beginning of FHF the animals of the later group had consumed significantly more vitamin E than the anhepatic pigs **(Figure 2)**.

MDA rise was increased in both groups albeit hepatic devascularization was characterized by an additional sharp rise, documented 330min after the beginning of FHF, corresponding to a well evident peak of LDH concentration **(Figure 6A)**.

The time course of FPA increase was not significantly different in the two groups with the only exception of first sampling time (60min), when the FHF without necrosis group peaked to a level about 3 times higher than the FHF with necrosis group **(Figure 6B)**.

The pattern of CPK release was similar in the two groups **(Figure 6C)**. On the contrary, LDH release, a well-known marker of liver necrosis, was much more evident in the FHF with necrosis group **(Figure 6D)**.

DISCUSSION

In an attempt to reduce the harmful consequences

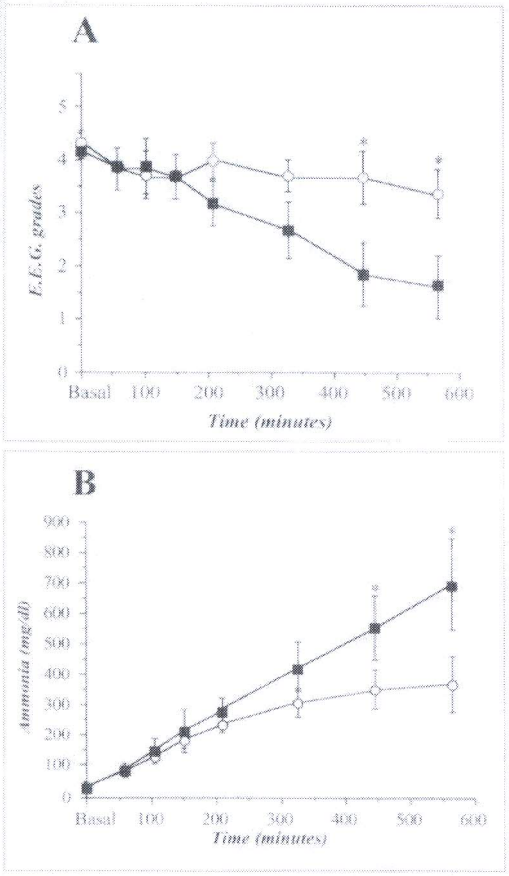

FIGURE 5 Time course of EEG grades (A), plasma ammonia nitrogen (B) in the two pig models of FHF without (empty circles) and with (filled squares) liver necrosis. Results are presented as mean ±s.e of six animals for each group. *$P<0.05$ between groups.

of "toxic liver syndrome" several investigators have recently described the possibility of performing a total hepatectomy when the diagnosis of irreversible FHF has been firmly established and a liver graft cannot be obtained immediately. This approach, so far reserved only to highly selected series of patients with FHF (2,3) and primary graft nonfunction (19-21), has the theoretical advantage of removing an endogenous source of toxic products. The results of these clinical experiences, although interesting, are difficult to evaluate due to the rarity of the disease and to the impredictability of its course. Moreover, the rationale of "early hepatectomy" in FHF has not been supported by any specific study designed to demonstrate the mechanisms through which hepatic necrosis *per se* would worsen the course of FHF. A better understanding of the pathophysiologic role of hepatic necrosis in FHF would be required not only to provide some scientific justification to early total hepatectomy but also, and perhaps more importantly, to allow the identification of specific medical treatments capable of delaying or reversing the detrimental effects of "toxic liver syndrome".

Earlier animal studies showed that the pattern of hepatic coma produced in models without and with necrosis was different in respect to several hematochemical, hemodynamic and metabolic parameters, but failed to identify the underlying factor(s) potentially responsible for such differences (22-24). The observation that increased levels of oxygen-derived free radicals were measured in animal models of liver injury (8,9) as well as in patients with hepatic ischemia (10) and hepatic ischemia-reperfusion injuries (11), severe acute hepatitis B (12), chronic active hepatitis (7) and FHF (13), prompted us to verify if hepatic necrosis was associated with an increased level of oxidative stress. To verify this hypothesis we analyzed the pattern of oxidative stress measured in two surgical models of FHF in which the suppression of all hepatic functions was obtained by either total hepatectomy (FHF without necrosis group) or complete hepatic devascularization (FHF with necrosis group).

Despite the technical difficulties entailed by total hepatectomy in the pig, especially in terms of reduced blood loss and maintenance of hemodynamic stability throughout the procedure, the surgical results obtained with our two models were equivalent. Standardization of the operative results is the first, indispensable, condition to be achieved to use a surgical model for subsequent meaningful experimentation. This issue should not be underestimated since many of the parameters that need to be evaluated in these studies may be influenced by either the hepatic coma or the surgical trauma. Indeed, animal models play a fundamental role in the development of many new medical technologies and, from this point of view, nowadays animal models themselves can be considered a medical technology (14).

Although survival was similar in the two experimental groups, the absence of liver necrosis was associated to a significantly longer period of good EEG activity. These results are similar to those reported by Mazziotti *et al.* (24) in the pig and agree with the study of Holmin *et al.* (25) demonstrating no changes on cerebral energy state during the initial phases of anhepatic coma in the rat.

Our results demonstrate that surgical procedures per se give rise to an oxidative stress. In fact, a consistent and constant increase of all oxidative parameters was measured in both experimental groups as a consequence of surgical tissue handling.

The comparison of our two models clearly showed that oxidative damage was enhanced by liver necrosis. Indeed, plasma concentration of MDA was higher in the group with liver necrosis and increased proportionally to the degree of hepatocyte injury, as documented by LDH release.

Under our experimental conditions FPA were an early index of oxidative damage. In fact, their increase was already evident 1 hour after the induction of FHF and could be documented in both groups throughout the experiments. Surprisingly, the peak of FPA rise was observed in the anhepatic animals 60min after hepatectomy. This early remarkable increase of FPA can be possibly related to an oxidative stress (data not

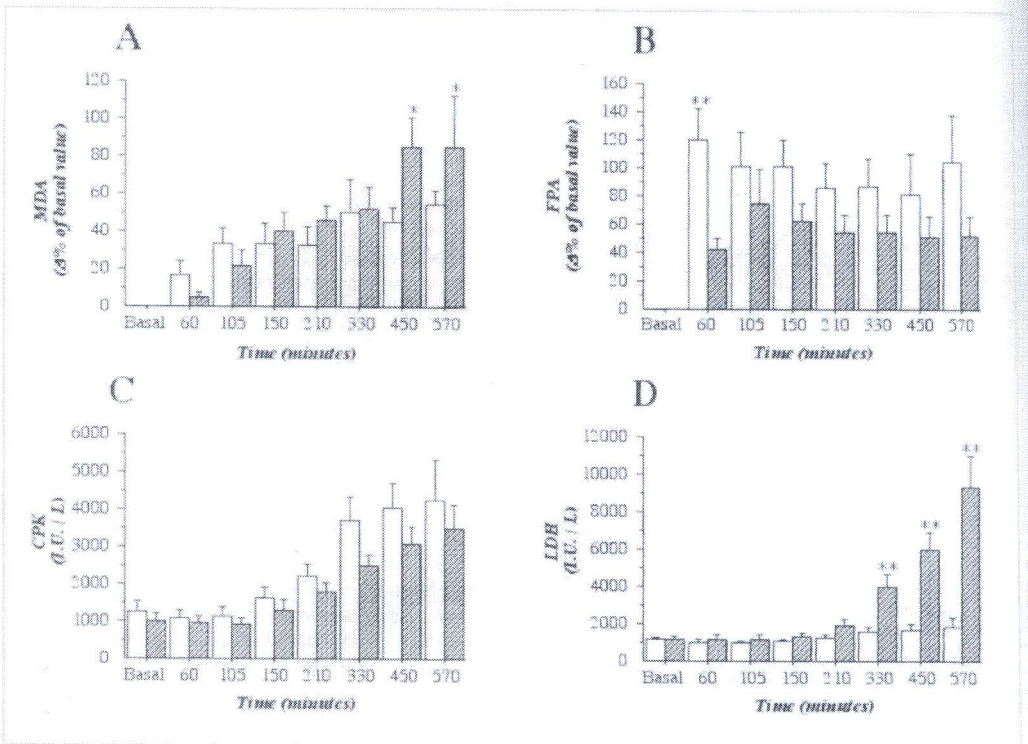

FIGURE 6 Comparison of oxidative markers and cellular injury indexes between the two pig models of FHF without (open columns) and with (dashed columns) liver necrosis. Plasma MDA (A) and plasma FPA (B) concentrations are expressed as difference per cent (Δ%) of the basal value; CPK (C) and LDH (D) release are measured in IU/L. Results are presented as mean ±s.e. of six animals for each group. $^{*}P<0.05$, $^{**}P<0.005$ between groups.

shown) caused by the extracorporeal circulation required to allow hemodynamic stability during total hepatectomy.

The most striking proof that oxidative damage is more severe when FHF is accompanied by liver necrosis comes from the assays of vitamin E. In fact, although a decay of plasma concentration of α-tocopherol, indicating a remarkable decrease of the antioxidant power, was documented in both experimental groups, the relationships between the decay of vitamin E and total cholesterol differed markedly in the two groups (**Figure 2**). In FHF without liver necrosis vitamin E and cholesterol decreased at a similar pace indicating the presence of an increased cellular uptake of LDL. On the contrary, in FHF with necrosis vitamin E concentration decreased more expeditiously than that of cholesterol strongly suggesting the existence of a true intravascular oxidation of vitamin E, possibly related to the release of free radicals from the ischemic liver into the circulating stream. This hypothesis is also supported by the sharp increase of MDA concentration measured after the release of the biochemical markers of liver necrosis.

Interestingly, in both experimental models the rise of oxidative indexes preceded the development of cell injury documented by CPK and LDH release. This observation provides a possible rationale for the use of antioxidant agents to prevent, block or reverse the arising of cellular injury in FHF. Indirect evidence supporting this hypothesis comes from the well-established use of *N*-acetylcysteine, a drug with known antioxidant properties, in the treatment of FHF due to paracetamol poisoning (26-28). The demonstration of the existence of significant oxidative stress also in viral FHF adds further interest to this suggestion. Promising experimental results have already been obtained in a toxic model of acute hepatic injury (intraperitoneal injection of CCl_4 in CSF-1 mice) with the use of vitamin E (8).

In conclusion, although the patterns of hepatic coma produced in the two experimental models of FHF implemented in this study were similar, the absence of hepatic necrosis seemed to delay the occurrence of irreversible brain damage. Accordingly, anhepatic pigs could have been rescued by a liver transplantation for a longer period of time when compared with devascularized livers. The reasons of this different behavior are difficult to be evaluated and their detailed analysis is well beyond the purposes of this study. Nonetheless, our data give further credit to the hypothesis that the presence of a necrotic liver *in situ* can influence negatively the course of FHF.

Significant oxidative stress was documented in both experimental models, but it was clearly more

severe in the FHF with necrosis group. Accordingly, a treatment with antioxidant agents, beneficial in both conditions, would be particularly suitable in case of a FHF with liver necrosis.

REFERENCES

1 **Maddrey WC, Friedman LS, Munoz SJ, Kahn EG:** Selection of the patient for liver transplantation and timing of surgery. In: Maddrey WC (Ed.). Transplantation of the liver. New York: Elsevier Science Publishing Co Inc, 1988; pp. 23-58.

2 **Ringe B, Lübbe N, Kuse E, Frei U, Pichlmayr R:** Total hepatectomy and liver transplantation as two-stage procedure. Ann Surg 1993; 218:3-9.

3 **Rozga J, Podesta L, LePage E, Hoffman A, Morsiani E, Sher L, et al:** Control of cerebral edema by total hepatectomy and extracorporeal liver support in fulminant hepatic failure. Lancet 1993; 342:898-899.

4 **Barsacchi R, Coassin M, Maiorino M, Pelosi G, Simonelli C, Ursini F:** Increased ultra weak chemiluminescence emission from rat heart at post ischemic reoxygenation. Free Radic Biol Med 1989; 6:573-579.

5 **Salaris SC, Babbs CF:** Effect of oxygen concentration on the formation of malonaldheyde-like materials in a model of tissue ischemia reoxygenation. Free Radic Biol Med 1989; 7:603-609.

6 **Stein HJ, Oosthuizen MMJ, Hinder RA, Lamprechts H:** Oxygen free radicals and glutathione in hepatic ischemia-reperfusion injury. J Surg Res 1991; 50:398-402.

7 **Yamamoto Y, Yamashita S, Fujisawa A, Kokura S, Yoshihawa T:** Oxidative stress in patients with hepatitis, cirrhosis and hepatoma evaluated by plasma antioxidants. Biochem Biophys Res Com 1998; 247:166-170.

8 **Liu SL, Degli Espositi S, Yao T, Diehl AM, Zern MA:** Vitamin E therapy of acute CCl_4 induced acute hepatic injury in mice is associated with inhibition of nuclear factor K binding. Hepatology 1995; 22:1474-1481.

9 **Polavarapu R, Spitz DR, Sim JE, Follansbee MH, Oberley LW, Rahaemtulla A, et al:** Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil. Hepatology 1988; 27:1317-1323.

10 **Biasi F, Chiarpotto E, Lanfranco G, Capra A Zummo U, Chiappino I, et al:** Oxidative stress in the development of human ischemic hepatitis during circulatory shock. Free Radic Biol Med 1994; 17:225-233.

11 **Biasi F, Bosco M, Chiappino I, Chiarpotto E, Lanfranco G, Ottobrelli A, et al:** Oxidative damage in human liver transplantation. Free Radic Biol Med 1995; 19:311-317.

12 **Pak SG, Nikitin EV:** Status of the process of free radical oxidation and the antioxidation system in patients with severe course of hepatitis B. Klin Med (Mosk) 1991; 69:54-57.

13 **Evans PJ, Evans R, Bomford A, Williams R, Halliwell B:** Metal ions catalytic for free radical reactions in the plasma of patients with fulminant hepatic failure. Free Radic Res 1994; 20:139-144.

14 **Filipponi F, Boggi U, Meacci L, Burchielli S, Vistoli F, Bellini R, et al:** A new technique for total hepatectomy in the pig for testing liver support devices. Surgery 1999; 125:448-455.

15 **Huguet C, Opolon P, Bloch P, Gallot D, Morisot P, Hadchouel P, et al:** Coma hépatique expérimental par nécrose ischémique aiguë du foie chez le porc. J Chir 1972; 103:63-76.

16 **Lang JK, Gohill R, Packer L:** Simultaneous determination of tocopherols, ubiquinols and ubiquinones in blood, plasma, tissues and subcellular fractions. Anal Biochem 1986; 157:106-116.

17 **Jentzsch AM, Bachmann H, Fürst P, Biesalski HB:** Improved analysis of malondialdehyde in human body fluids. Free Radic Biol Med 1996; 20:251-256.

18 **Tsuchida M, Miura T, Mizutani K, Aibara K:** Fluorescent subsatances in mouse and human sere as a parameter of *in vivo* lipid peroxidation. Biochim Biophys Acta 1985; 834:196-204.

19 **Ringe B, Pichlmayr R, Lübbe N, Bornscheuer A, Kuse E:** Total hepatectomy as temporary approach to acute hepatic or primary graft failure. Transpl Proc 1988; 20:552-557.

20 **So SKS, Barteau GA, Perdrizet GA, Marsh JW:** Successful retransplantation after 48-hour anhepatic state. Transpl Proc 1993; 25:1962-1963.

21 **Ringe B, Lübbe N, Kuse E, Frei U, Pichlmayr R:** Management of emergencies before and after liver transplantation by early total hepatectomy. Transpl Proc 1993; 25:1090.

22 **Tonnesen K:** Experimental liver failure. A comparison between hepatectomy and hepatic devascularization in the pig. Acta Chir Scand 1977; 143:271-277.

23 **Tonnesen K, Balling H:** Cerebral flow and metabolism in experimental liver failure. A comparison between hepatectomy and total hepatic devascularization in pigs. Acta Chir Scand 1986; 152:439-445.

24 **Mazziotti A, Bernardi M, Antonini L, Dioguardi FS, Bellusci R, Papa V, et al:** Plasma amino acid patterns in experimental acute hepatic failure: comparison between hepatectomy and liver devascularization in pigs. Surgery 1981; 90:527-534.

25 **Holmin T, Agardh CD, Alinder G, Herlin G, Hultberg B:** The influence of total hepatectomy on cerebral energy state, ammonia-related amino acids of the brain and plasma amino acids in the rat. Eur J Clin Invest 1983; 13:215-220.

26 **Prescott LF, Illingorth RN, Critchley JA, Stewart MJ, Adam RD, Proudfoot AT:** Intravenous *N*-acetylcysteine: the treatment of choice for paracetamol poisoning. Br Med J 1979; 2:1097-1100.

27 **Harrison PM, Keays R, Bray GP, Alexander GJ, Williams R:** Improved outcome of paracetamol-induced fulminant hepatic failure by late administration of acetylcysteine. Lancet 1990; 335:1572-1573.

28 **Harrison PM, Wendon JA, Gimson AE, Alexander GJ, Williams R:** Improvement by acetylcysteine of hemodynamics and oxygen transport in fulminant hepatic failure. N Engl J Med 1991; 324:1852-1857.