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# Role of hypotension in brain-death associated impairment of liver microcirculation and viability

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**Abstract** Hypotension in brain-dead organ donors is considered a determinant factor of graft viability. The aim of this study was to elucidate the role of hypotension in brain-death associated impairment of hepatic microcirculation and function. Male Sprague-Dawley rats with an intracranial balloon were used. Group I ($n = 7$) served as sham controls. In group II ($n = 7$) brain death was induced through inflation of an intracranial balloon. In group III ($n = 7$) hypotension without brain death was induced by means of pentobarbital. In group II, a steep rise of arterial pressure was followed by a fall to a lower level ($P < 0.01$, vs. group I). Also in group III arterial pressure was lower ($P < 0.01$, vs. group I). In group II, bile production was diminished ($P < 0.05$). Impaired sinusoidal perfusion ($P < 0.01$) and enhanced leukocyte endothelium interaction ($P < 0.05$) were documented in hepatic microvasculature. Electron microscopic analysis revealed vacuolization of hepatocytes; these changes were not observed in group III. Brain death induces specific changes of liver microcirculation, function and histomorphology. Independent of associated hypotension, brain death per se impairs donor liver graft quality.

**Keywords** Liver transplantation · Brain death · Organ donor · Hypotension · Hepatic microcirculation · Graft viability

**Abbreviations** *ADH* Antidiuretic hormone · *ALT* Alanine aminotransferase · *AST* Aspartate aminotransferase · *EEG* Electroencephalogram · *HR* Heart rate · *IVM* Intravital fluorescence microscopy · *MAP* Mean arterial pressure

## Introduction

In spite of the increase of clinical liver transplant programs across the United States and Europe, the limited number of available donor organs is one of the major restricting factors in liver transplantation [1, 25]. This has led to a dramatical increase of the number of patients and the number of deaths on the waiting list for liver transplantation [30]. In liver transplantation, the grafts from brain-dead organ donors often show impaired viability leading to discarding of the organ or its acceptance with high risk for poor initial graft function [3, 6, 8, 15, 16, 28]. To maximize the utility of the potential grafts, the use of liver grafts from marginal donors such as those of old age, receiving high dose of vasopressors or presenting with preexisting hypotension has been discussed [1, 34]. However, there is still controversy about the criteria for graft procurement [23]. Considering the

issue of donor shortage, it is important to understand the pathophysiological organ changes occurring upon brain death and to establish an optimal management of the brain-dead potential organ donors.

In a previous rat model of brain-dead organ donors, we have reported that impairment of the hepatic microcirculation, which plays a crucial role in graft viability, with enhanced leukocyte-endothelium interaction, is involved in the deterioration of graft quality [20, 21]. Among multifactorial changes such as systemic hemodynamic, hormonal and metabolic changes in brain-dead donors, hypotension is considered to be one of the determining factors of graft viability. However, its precise role for graft viability has not been elucidated. Even the grafts from normotensive or mildly hypotensive brain-dead donors may fall into primary non-function. The aim of this study is to test the hypothesis that impairment of hepatic microcirculation and deterioration in graft viability are not the result of hypotension itself but of brain death specific factors.

In a brain death model and by deliberately inducing hypotension, we compared sham, brain-dead and hypotensive rats as far as systemic hemodynamics, blood hormone and cytokine levels, adhesion molecule expression, hepatic microcirculation and histomorphology of the liver are concerned. We adopted continuous intravenous administration of sodium pentobarbital to induce hypotension in absence of brain death, considering the fact that high doses of barbiturates are known to decrease myocardial contractility, cardiac output and systemic vascular tone, as is known to occur in brain-dead donors [3, 6, 9].

## Materials and methods

### Animals and surgical interventions

The "Principles of laboratory animal care" (NIH publication No.86–23, revised 1985), as well as the current version of the German law on the Protection of Animals, were followed in this study. Twenty-one male Sprague-Dawley rats (Charles River Deutschland, Sulzfeld, Germany) weighing 200–250 g were used for the experiments. After overnight fasting, but with free access to tap water, the animals were anesthetized with pentobarbital sodium (60 mg/kg i.p. followed by 6 mg/kg per h i.v.) and atropine sulfate (0.5 mg/kg), tracheotomized and mechanically ventilated. $PaCO_2$ and $PaO_2$ were maintained at 35–40 mm Hg and at 100–130 mm Hg, respectively. The right carotid artery, the right jugular vein and the right femoral vein via right caudal epigastric vein were cannulated with polypropylene catheters (Polythene Tubing, Portex, Kent, UK) for continuous recording of mean arterial pressure (MAP) and heart rate (HR), fluid replacement with lactated Ringer solution at the rate of 2 ml/h per 100 g body weight, and later administration of sodium pentobarbital, respectively. Rectal temperature was continuously monitored and maintained at 36–37 °C by means of a heating pad. For the placement of an epidural balloon (500 µl) and electrodes for electroencephalogram (EEG) monitoring, the animals of all groups were craniotomized. This was followed by laparotomy and catheterization of the common bile duct and urinary bladder for monitoring of bile flow and urine osmolality. After dissection of its ligamentous attachments, the left lateral lobe of the liver was carefully exteriorized, positioned onto a special stage, and covered with a cover glass for intravital fluorescence microscopy (IVM).

### Intravital fluorescence microscopy

Sodium fluorescein (2 µmol/kg; Merck, Darmstadt, Germany) and rhodamine-6G (0.1 µmol/kg; Merck) were administered intravenously as fluorescence markers for plasma and leukocytes, respectively. A modified Leitz Orthoplan microscope with a 100 W HBO mercury lamp attached to a Ploemo-Pak illuminator was used for epiillumination microscopy (Leitz, Wetzlar, Germany). Two different filters were used for the visualization of fluorescence; 450–490/ > 515 nm (excitation/emission) for sodium fluorescein and 530–560/ > 580 nm (excitation/emission) for rhodamine-6G, respectively. The observations were recorded by means of a CCD video camera (FK6990; Prospective Measurements, San Diego, CA, USA) and transferred to a video system for off-line analysis. 10–15 acini and 10–15 postsinusoidal venules, randomly selected, were observed. As parameters of hepatic microcirculation, the number of non-perfused sinusoids, sinusoidal stagnant and postsinusoidal venular adherent leukocytes were quantitatively evaluated.

### Experimental groups and protocol

After baseline IVM, 0.2 ml of blood was sampled for flow cytometric analysis (FACSORT® flow cytometer, Becton Dickinson, Heidelberg, Germany) of CD11b expression on polymorphonuclear cells at baseline. Then animals with an intracranial balloon were randomly assigned to three groups. The animals of group I, ($n = 7$) without balloon inflation were observed, for 3 h as controls. In the animals of group II, ($n = 7$) brain death was induced through inflation of the intracranial balloon [20] with saline (2 µl/g body weight) over the period of 5 min by means of a syringe pump (sp250i, World Precision Instruments, Sarasota, FL, USA). In the animals in group III ($n = 7$), instead of brain death induction, the infusion of sodium pentobarbital was started through the femoral vein catheter at the rate of 240 mg/kg per h i.v. followed by tapering to maintain MAP at 60 mmHg by means of syringe pump (sp250i, World Precision Instruments). In all the groups, total fluid replacement amounted to 2 ml/h per 100 g body weight.

Brain death was defined as a condition in which no electroencephalographic activity was observed; the complete loss of corneal reflex served as a further confirmation. The parameters, such as MAP, HR, urine osmolality and bile production, were measured before and until 3 h after the induction of brain death. At 3 h after induction of brain death, the second IVM was performed. At the end of the experiment, the infra-renal aorta was cannulated and arterial blood was sampled for the measurement of antidiuretic hormone (ADH), serum osmolality, aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin-6 (IL-6) and CD11b expression. Immediately after the blood sampling, the aorta was clamped just below the diaphragma and hepatic tissue was perfused-fixed with 20 ml of Karnovsky's solution (5% glutaraldehyde, 4% paraformaldehyde and phosphate buffer pH7.4) via aorta for electron microscopy.

The concentration of ADH was measured by radioimmunoassay. Urine and serum osmolality were determined by cryoscopic osmometer (OSMAT 030, Gonotec, Berlin, Germany). AST and ALT were measured by standard spectrophotometric analysis.

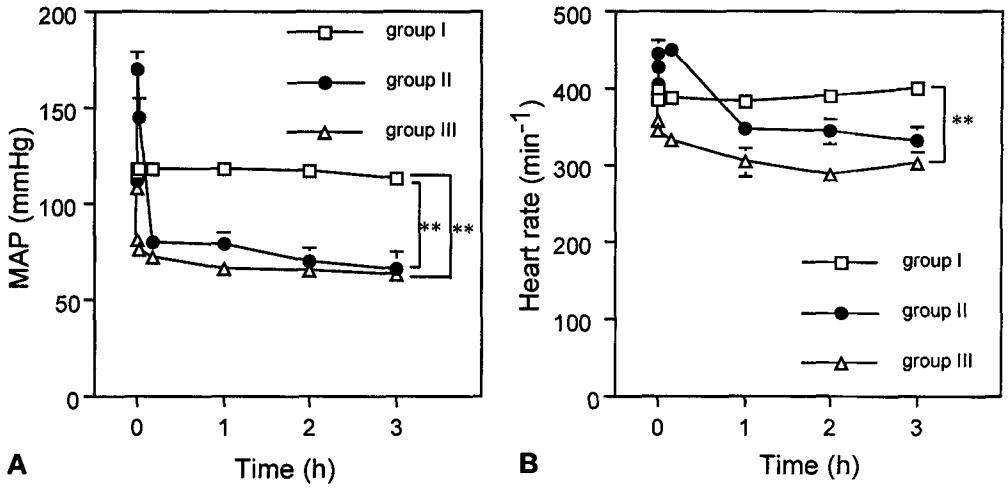



**Fig. 1** Mean arterial pressure (*MAP*) and heart rate (*HR*) (mean ± SE). *Open squares*, *closed circles* and *open triangles* stand for group I ($n = 7$), group II ($n = 7$) and group III ($n = 7$), respectively. ** $P < 0.01$, ANOVA

Flow cytometric analysis of surface antigen expression

Flow cytometric analysis was carried out on a FACSORT® flow cytometer (Becton Dickinson, Heidelberg, Germany). After electronic gating according to their characteristic volume-side scatter dot plots, granulocytes were selectively analyzed. Expression of CD11 b (α-chain of the β2-integrin MAC-1) was assessed by direct immunofluorescence of the monoclonal phyocerythrin-conjugated mouse anti-rat CD11 b monoclonal IgG-antibody (OX-42, PharMingen, San Diego, CA, USA). Fluorescence histograms were used to calculate mean fluorescence intensities.

Statistical analysis

The statistical analysis was performed using analysis of variance. The statistical significance was set at $P < 0.05$.

## Results

### Electroencephalogram (EEG)

In the animals of group I, EEG activity was present during the course of the whole experiment. In the animals of group II, a similar EEG activity was observed at the baseline, the activity disappeared 2–3 min after the induction of brain death. At 1, 2, and 3 h after induction of brain death, EEG activity was completely absent, and the lack of a corneal reflex was confirmed. In the animals of group III, EEG activity decreased with time after induction of hypotension, showing typical burst suppression at 3 h.

### Mean arterial pressure (MAP)

At baseline, there was no significant difference in the MAP among the groups. In group I, MAP remained constant over the total observation time. In group II, a steep rise in MAP, the so-called Cushing response, occurred within the first 5 min after the induction of brain death. This was followed by a fall to a significantly lower level, compared with group I, which was maintained from 10 min until 3 h after induction of brain death ($P < 0.01$). In group III, MAP was reduced significantly ($P < 0.01$, vs. group I), comparable with that of group II (Fig. 1A).

### Heart rate (HR)

The heart rate in group III was significantly lower, compared to group I and II ($P < 0.01$) (Fig. 1B).

### Urine and serum osmolality

The urine osmolality in groups I and III remained over 600 mosm/kg during the course of the experiment. In contrast, the urine osmolality in group II significantly fell below 200 mosm/kg at 2 and 3 h after the induction of brain death ($P < 0.01$) (Fig. 2). On the other hand, serum osmolality in group II was significantly higher, compared with group I and group III at 3 h after the induction of brain death ($P < 0.05$) (Fig. 3A).

### Serum concentration of ADH

Serum concentration of ADH was significantly lower in group II, compared with groups I and III at 3 h after brain death induction ($P < 0.01$) (Fig. 3B).

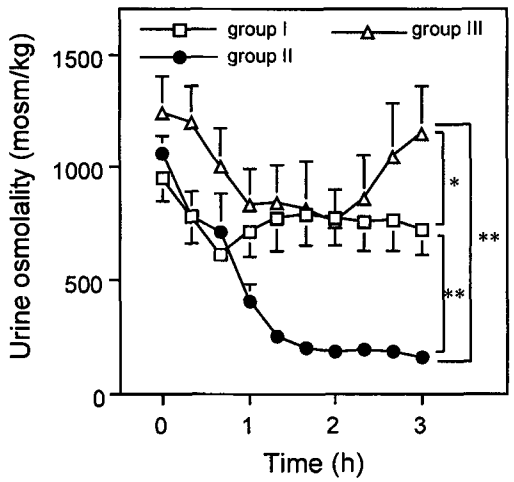



**Fig. 2** Urine osmolality (mean ± SE). *Open squares*, *closed circles* and *open triangles* stand for group I ($n = 7$), group II ($n = 7$) and group III ($n = 7$), respectively. * $P < 0.05$, ** $P < 0.01$, ANOVA

## Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Serum AST and ALT concentrations were 139 ± 11 (mean ± SE) U/l and 58 ± 8 U/l in group I, 155 ± 34 U/l and 74 ± 21 U/l in group II and 174 ± 16 U/l and 103 ± 16 U/l in group III, respectively. There were no significant differences in serum AST and ALT levels among the three groups.

## Serum IL-6 level

Serum IL-6 level at 3 h was 1636 ± 675 in group II, a value significantly higher, compared to those of groups I and III; 46 ± 10 and 265 ± 225, respectively ($P < 0.05$).

## CD11b expression on polymorphonuclear leukocytes

The expression of CD11b, α-chain of the β2-integrin MAC-1, on polymorphonuclear leukocytes at 3 h after induction of brain death was significantly higher in group II, 299 ± 26% vs. baseline, as compared to group I, 219 ± 38%, and group III, 211 ± 35% ($P < 0.05$).

## Bile production

Bile production, as a parameter of gross hepatic function, was significantly diminished in group II after induction of brain death, compared to group I and III ($P < 0.05$) (Fig. 4).

## Hepatic microcirculation

At baseline, there were no significant differences in the parameters of hepatic microcirculation among the three groups. At 3 h after induction of brain death, the numbers of non-perfused sinusoids and leukocytes stagnant in sinusoids were significantly higher in group II ($P < 0.01$ vs. group I and group III). In postsinusoidal venules, an elevated number of adherent leukocytes ($P < 0.05$ vs. group I) was observed in group II (Fig. 5).

## Electron microscopy

The electron microscopic analysis of the hepatic tissue displayed vacuoles in the hepatocytes in animals of group II. The vacuoles surrounded especially the sinusoids. The vacuolization of hepatocytes was more prominent in pericentral than in periportal area. The mitochondria were swollen or even disrupted, and the rough endoplasmic reticulum was disarranged. However, the microvilli were present. The hepatocytes of group I and III did not show morphological changes. In neither of

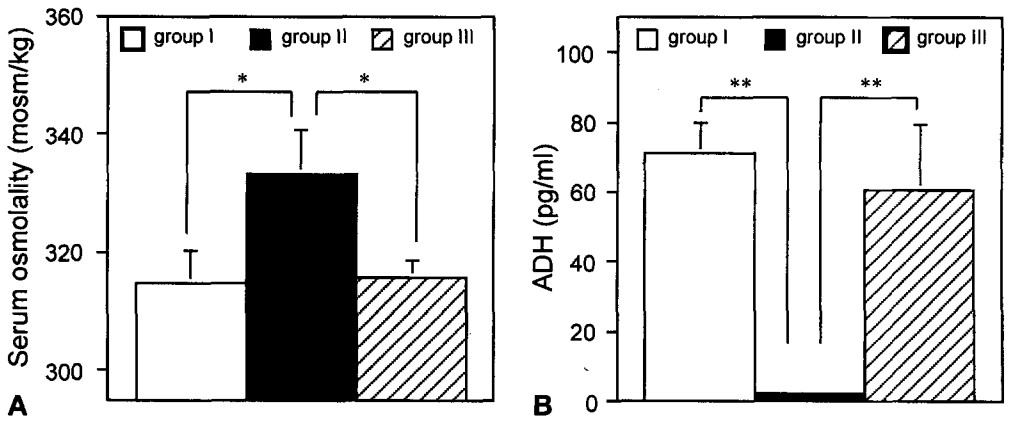



**Fig. 3** Serum osmolality and concentration of ADH. *Open*, *closed* and *striped bars* stand for group I ($n = 7$), group II ($n = 7$) and group III ($n = 7$), respectively. * $P < 0.05$, ** $P < 0.01$, ANOVA

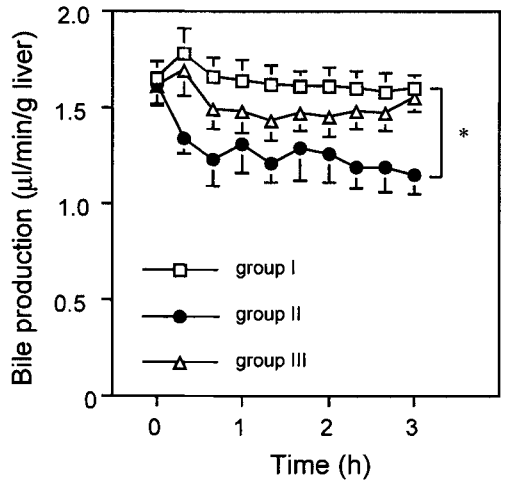


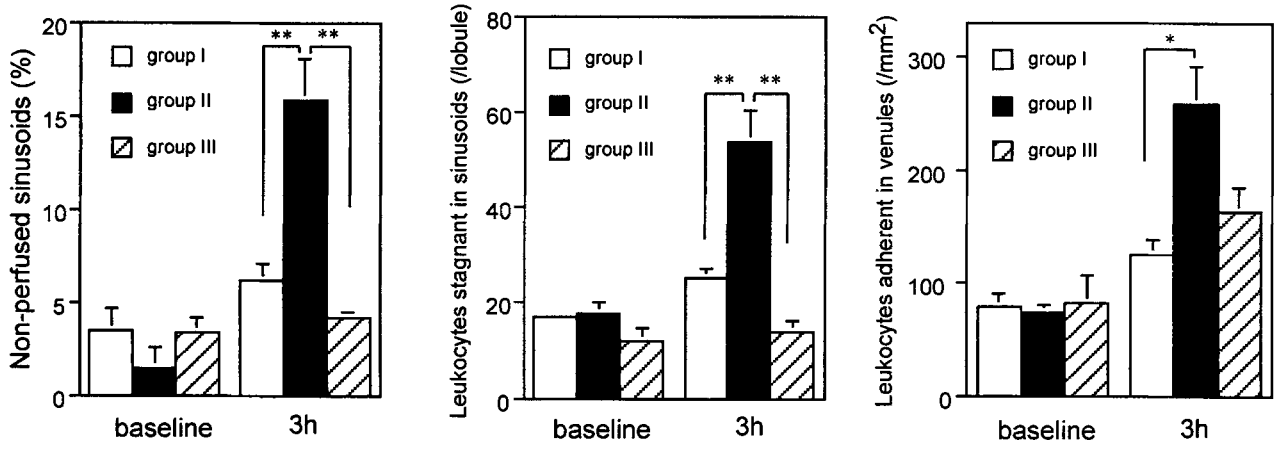

**Fig. 4** Bile production. *Open squares*, *closed circles* and *open triangles* stand for group I ($n = 7$), group II ($n = 7$) and group III ($n = 7$), respectively. * $P < 0.05$, ANOVA

the three groups was the space of Disse enlarged or the endothelial lining cells swollen (Fig. 6)

## Discussion

Brain-dead organ donors often show systemic hemodynamic disturbances and hormonal impairment. Novitzky et al. have demonstrated the temporary release of catecholamines and myocardial damage during and following brain-death, associated with a rapid depletion of plasma free triiodothyronine (T3) and thyroxine (T4), cortisol, insulin and ADH in a baboon model [15]. Myocardial dysfunction and loss of systemic vascular tone are characteristic features of systemic hemodynamic changes, leading to hypotension [3, 6]. In both experimental and clinical brain death, characteristical changes in hormonal homeostasis are reduction of the serum level of ADH and thyroid hormones [2, 5, 15, 16, 33]. Diabetes insipidus with polyuria and dehydration derives from the lowering of the ADH level, leading to hypovolemia and, possibly, hypoperfuison of the organs. Low T3 syndrome (sick euthyroid syndrome) characterizes the changes in levels of thyroid hormones with a shift from aerobic to anaerobic metabolism [17], which has been also observed under various types of hypotension [10, 19, 32]. These factors mentioned above are considered to be responsible for the deterioration of graft viability [6, 15, 16]. The brain death model used in this study [20] adequately simulated the changes of systemic hemodynamics, serum ADH level, serum- and urine osmolality encountered in brain-dead organ donors, and showed significantly impaired microvascular perfusion, function and histomorphology of the liver. As for thyroid hormone levels, no significant changes were observed in our previous study at 3 h after brain death induction [21], which may be explained by different half-lives of the hormones, 5–15 min for ADH [12], 7–8 days for T4 and one day for T3 [24]. On the contrary, in the hypotension group (group III), despite the similar reduction of mean arterial pressure achieved by intravenous pentobarbital, there was neither significant impairment of hepatic microvascular perfusion nor function, while histomorphological signs of impairment of hepatocytes were absent. Comparison of these two groups leads us to the conclusion that hypotension is not the major cause for the changes of hepatic microcirculation and function encountered in brain-dead organ donors. Although some authors have used hypotensive animals to simulate the brain-dead organ donor, these do not represent a suitable model. Therefore, the brain-death model should be used when studying liver grafts from brain-dead donors. When adopting healthy animals as graft donors for experimental liver transplan-

**Fig. 5** Hepatic microcirculation. *Open*, *closed* and *striped bars* stand for group I ($n = 7$), group II ($n = 7$) and group III ($n = 7$), respectively. * $P < 0.05$, ** $P < 0.01$, ANOVA



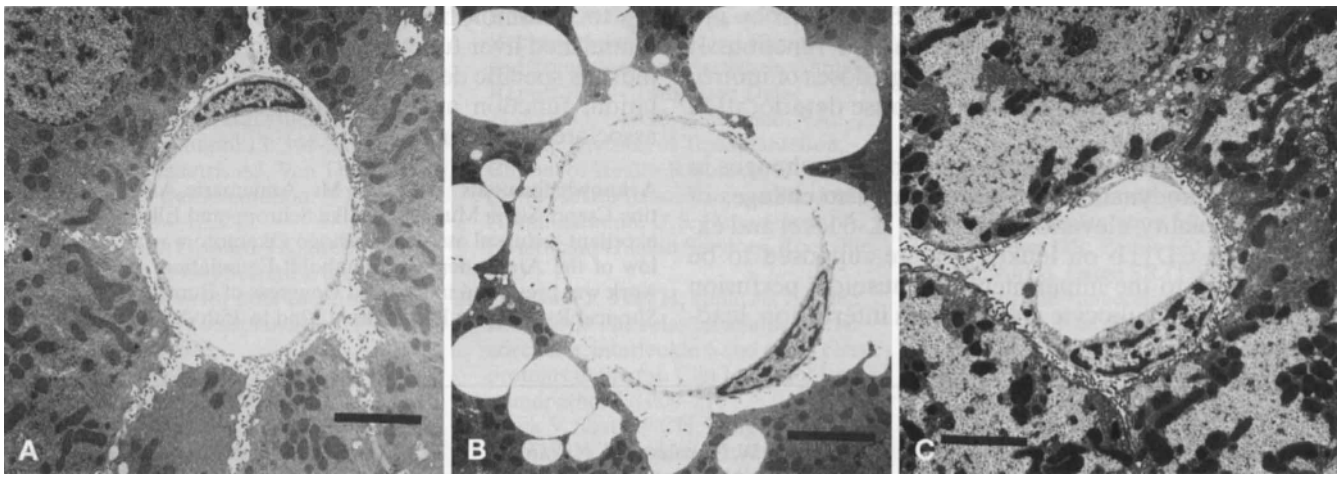



**Fig. 6** Electron microscopy of hepatic tissue: In animals of group I (**A**) and III (**C**) no damages to hepatocytes were observed. Animals of group II (**B**) showed vacuolization of hepatocytes. There was neither widening of the spaces of Disse nor swelling of sinusoidal endothelial cells in the three groups. *Black bars* in each picture stand for 5 μm

tation, it should be kept in mind that hepatic microcirculation and function have already been impaired due to the brain death of the non-living donor.

Bile production is an energy dependent process, providing information on overall hepatic function and is considered to be one of the markers of the hepatic function and viability [4, 27]. Bile flow reflects the cellular content of ATP, and its recovery rate on reoxygenation decreases with increasing duration of the anoxic period [11]. Therefore, the reduction in bile flow after brain death observed in our experiments is considered to indicate impairment of hepatic function and viability, as a consequence of microvascular perfusion failure.

In this study, no differences in serum AST and ALT levels among the groups have been observed despite the significant differences in bile secretion, hepatic microvascular perfusion, and histomorphology. It would appear that such relatively mild damages of hepatocytes as vacuolization are mostly reversible phenomena and do not significantly affect the serum AST and ALT concentrations. Constancy of serum AST, ALT levels and arterial ketone body ratio [22] have also been reported in a brain death model of the dog [13].

The increased expression of adhesion molecule CD11b,α-chain of the β2-integrin MAC-1, on the surface of granulocytes in group II at 3 h after brain death, compared to group I and group III, indicates activation of polymorphonuclear cells and suggests its impact on the enhancement of leukocyte endothelium interaction. It has been reported that in brain-dead organ donors, IL-6 level is increased [2], as was observed in our study. The release of IL-6 has the potential to induce expression of adhesion molecules and activation of leukocyte. Recently, increased expression of IL-1, IL-6, TNF-α and IFN-γ and an increase in the interstitial leukocytes infiltration in kidney have been demonstrated in brain-dead rats [29].

The characteristical changes of the hepatic microcirculation under the condition of brain death were impaired sinusoidal perfusion and enhanced leukocyte endothelial cell interaction, resulting in increased accumulation of leukocytes in sinusoids and postsinusoidal venules, as was seen in group II, but not in group III. These changes of hepatic microvascular perfusion were associated with compromised liver function.

The electron microscopic analysis revealed that the first target for histomorphological damage following brain death is not the sinusoidal lining cell but the hepatocyte, the damage being characterized by vacuoles predominantly located in the vicinity of sinusoids. The vacuolization is considered a sign of hypoxia and may possibly be a first state in the development of fatty liver. It should be noted that these damages to the hepatocyte and hepatic microcirculation are present already before procurement of the hepatic graft from brain-dead donors, they will be intensified by injury of the sinusoidal lining cell during hypothermic storage [14]. These sequential damages are considered to be critical determinants of graft viability and initial graft function. Furthermore, the sinusoidal perfusion failure associated with impaired hepatic function, or the activation of leukocyte-endothelium interaction in the brain-dead organ donor itself may become further enhanced during the ensuing cold preservation and implantation of the liver graft.

During Cushing response, sympathetic discharge causes a redistribution of splanchinic organ blood flow. There is also evidence that catecholamines stimulate IL-6 secretion and modulate leukocyte-endothelium interaction [7, 26, 31]. Thus, the release of catecholamines

may cause increased secretion of IL-6 and thereby impairment of hepatic microcirculation and function. It should be noted in this context that high doses of inotropic support in the brain-dead donor cause deterioration of graft viability [18].

In brain-dead organ donors, not only the changes in systemic hemodynamic parameters but also changes of serum osmolality, elevation of plasma IL-6 level and expression of CD11b on leukocytes are supposed to be contributory to the impairment of sinusoidal perfusion with enhanced leukocyte endothelium interaction, leading to histomorphological changes of hepatocytes and diminished liver function. We conclude that brain death induces specific deleterious changes of liver microcirculation, function and histomorphology independent of associated hypotension.

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