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S. Okamoto
C.O. Corso
D. Nolte
W. Rascher
J. Thiery
Y. Yamaoka
K. Messmer

Impact of brain death on hormonal homeostasis and hepatic microcirculation of transplant organ donors

S. Okamoto (✉) · C. O. Corso · D. Nolte · K. Messmer
Institute for Surgical Research, Klinikum Grosshadern, University of Munich, Marchioninistrasse 15, D-81 366 Munich, Germany, Tel: + 49-89-7095-4355; Fax: + 49-89-7095-8897

J. Thiery
Institute for Clinical Chemistry, Klinikum Grosshadern, University of Munich, Marchioninistrasse 15, D-81 366 Munich, Germany

W. Rascher
Department of Pediatrics, University of Giessen, Feulgenstrasse 12, 35385 Giessen, Germany

S. Okamoto · M. Y. Yamaoka
Department of Gastroenterological Surgery, Kyoto University, Kawara-cho 54, Shogoin, Sakyo-ku, Kyoto, 606 Japan

Abstract To elucidate the pathophysiological mechanisms involved in the deterioration of hepatic graft viability in brain-dead organ donors, the impact of brain death on hepatic microcirculation was investigated with respect to hormonal homeostasis and graft viability. Rats were assigned to two groups: group I ($n = 6$) served as sham controls, and in group II ($n = 6$), brain death was induced through insufflation of an intracranial balloon. Mean arterial pressure was elevated significantly within 5 min after the induction of brain death and then decreased significantly to below the control value. Urine osmolality was significantly lower and serum osmolality significantly higher than the control values. Antidiuretic hormone level was significantly lower than the control value. Bile secretion also decreased significantly. Furthermore, in group II there were significantly higher numbers of nonperfused sinusoids (15.9% vs 6.2% in group I), and sinusoidal stagnant and postsinusoidal venular adherent leukocytes (53.9/lobule and 258.6/mm^2 versus 25.2/lobule and 124.8/mm^2 in group I, respectively). In summary, sinusoidal perfusion is compromised after brain death, possibly, in part, through an increased leukocyte activation and accumulation in the hepatic microvasculature, leading to the deterioration of hepatic function.

Key words Brain death · Organ donor · Liver transplantation · Graft viability · Hepatic microcirculation

Introduction

The shortage of donor organs is one of the central problems in liver transplantation [1, 14]. According to data from the United Network for Organ Sharing, the number of patients and also the number of deaths occurring while on the waiting list for liver transplantation have been continuously increasing over the past 8 years [18]. Therefore, in order to maximize the utility of potential grafts, it is important to understand the pathophysiological organ changes after brain death. The macrohemodynamic, hormonal and metabolic impairment of the brain-dead organ donor is often associated with the deterioration of graft viability, leading to organ exclusion or acceptance with a high risk for poor initial graft function [3, 7, 8, 11, 17]. The pathophysiological mechanisms for the deterioration of graft viability in brain-dead organ donors has not been fully elucidated. In particular, the impact of brain death on hepatic microcirculation, which has a close relation to graft viability, has not been clarified. In this study we therefore developed a novel experimental model and investigated the alterations of hormonal levels and hepatic microcirculation in brain-dead organ donors with respect to graft viability.

Table 1 Changes in mean arterial pressure, urine osmolality and bile secretion

	Baseline	3 min	5 min	10 min	1 h	2 h	3 h
Mean arterial pressure (mm Hg)							
Group I	107 ± 17	106 ± 16	106 ± 19	107 ± 18	109 ± 13	109 ± 11	106 ± 8
Group II	107 ± 17	181 ± 12*F	149 ± 26*F	91 ± 19	74 ± 16*F	63 ± 20*F	63 ± 20*F
Urine osmolality (mosmol/kg)							
Group I	953 ± 284				710 ± 284	774 ± 325	721 ± 286
Group II	1065 ± 192				409 ± 178*F	185 ± 72*F	165 ± 33*F
Bile secretion (µl/min/g liver)							
Group I	1.65 ± 0.26				1.64 ± 0.30	1.60 ± 0.28	1.56 ± 0.26
Group II	1.61 ± 0.31				1.30 ± 0.43F	1.26 ± 0.44F	1.16 ± 0.25*F

* $P < 0.05$ versus group I, F $P < 0.05$ vs baseline

Materials and methods

Male Sprague-Dawley rats (200–250 g) 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). After a tracheotomy they were mechanically ventilated. Pa_{CO2} and Pa_{O2} were maintained at 35–40 mmHg and at 100–130 mmHg, respectively. Lactate Ringer solution was infused intravenously at the rate of 2 ml/h per 100 g body weight. For the placement of an epidural balloon (500 µl) and electrodes for electroencephalogram (EEG) monitoring, the animals were craniotomized. This was followed by laparotomy for catheterization of the common bile duct and urinary bladder. Mean arterial pressure (MAP), bile flow and urine osmolality were monitored.

After a stabilization period of 30 min the left lateral lobe of the liver was carefully exteriorized, positioned onto a special stage and covered with a glass slide for intravital fluorescence microscopy (IVM) [10]. Sodium fluorescein (2 µmol/kg) and rhodamine-6G (0.1 µmol/kg) 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. Two different kinds of filters were adopted for the visualization of fluorescence: 450–490/ > 515 nm (excitation/emission) for sodium fluorescein and 530–560/ > 580 nm (excitation/emission) for rhodamine-6G. The observations were recorded using a CCD video camera and transferred to a video system for offline analysis. As indicators of hepatic microcirculation, sinusoidal perfusion, and sinusoidal stagnant and postsinusoidal venular adherent leukocytes were quantitatively evaluated.

After baseline IVM, the animals were randomly assigned to two groups. The animals in group I ($n = 6$) were observed for 3 h as controls. The animals in group II ($n = 6$) were subject to the same protocol as group I except that after the baseline IVM, brain death was induced through insufflation of the intracranial balloon. Brain death was defined as a condition in which no EEG activity was observed. Furthermore, the complete loss of corneal reflex served as further confirmation. The parameters 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 blood was sampled for the measurement of antidiuretic hormone (ADH), thyroxin (T_4), free thyroxin (f-T_4), triiodothyronine (T_3), free triiodothyronine (f-T_3), and serum osmolality.

Values are expressed as means ± SD. The statistical analysis was performed using the Mann-Whitney *U*-test and Wilcoxon's test. The level statistical significance was set at $P < 0.05$.

In this study current German law on the protection of animals was followed.

Results

EEG

In animals of group I, EEG activity was present during the whole course of the experiment. In animals of group II similar EEG activity was observed at baseline, which disappeared 2 to 3 min after the induction of brain death. At 3 h after induction of brain death EEG activity was completely absent and lack of a corneal reflex was also confirmed.

Mean arterial pressure

At baseline there was no significant difference in the MAP between the groups. In group I, the MAP remained constant over the total observation time. In contrast, in group II a significant rise in MAP, a so-called Cushing response, occurred within the first 5 min after the induction of brain death. This was followed by a steady fall to a significantly lower level compared with both baseline and group I (Table 1).

Urine and serum osmolality

The urine osmolality in group I remained over 700 mosmol/kg during the course of the experiment. In contrast, the urine osmolality in group II fell significantly below 200 mosmol/kg at 2 and 3 h after the induction of brain death (Table 1). However, serum osmolality was significantly higher in group II than in group I at 3 h after the induction of brain death (332 ± 16 mosmol/kg vs 314 ± 14 mosmol/kg in group I, $P < 0.05$).

Serum levels of ADH, T_4, f-T_4, T_3 and f-T_3

Serum levels of ADH were significantly lower in group II than in group I at 3 h after the induction of brain death. There were no significant differences in the se-

Table 2 Serum levels of antidiuretic hormone (ADH), thyroxin (T_4), free thyroxin (f–T_4), triiodothyronine (T_3), free-triiodothyronine (f-T_3) 3 h after the induction of brain death

	ADH (pg/ml)	T_4 (mg/dl)	f-T_4 (ng/dl)	T_3 (ng/ml)	f-T_3 (pg/ml)
Group I	83.9 ± 21.7	7.0 ± 1.3	1.8 ± 0.3	0.76 ± 0.08	1.5 ± 0.2
Group II	1.9 ± 1.7*	6.5 ± 1.5	1.6 ± 0.4	0.81 ± 0.15	1.8 ± 0.6

* $P < 0.05$ vs group I

Table 3 Changes in hepatic microcirculation

	Baseline	3 h
Nonperfused sinusoid (%)		
Group I	3.5 ± 3.3	6.2 ± 2.3[F]
Group II	1.5 ± 2.6	15.9 ± 5.4*[F]
Sinusoidal stagnant leukocytes (*n*/lobule)		
Group I	17.1 ± 2.6	25.2 ± 4.6
Group II	18.8 ± 4.9	53.9 ± 15.6*[F]
Postsinusoidal venular adherent leukocytes (n/mm^2)		
Group I	78.7 ± 28.3	124.8 ± 32.5[F]
Group II	74.7 ± 16.4	258.6 ± 83.7*[F]

* $P < 0.05$ vs group I, [F] $P < 0.05$ vs baseline

rum levels of T_4, f-T_4, T_3 and f-T_3 between the groups at 3 h after the induction of brain death (Table 2).

Bile secretion

At baseline there was no significant difference in bile secretion between the groups. At 3 h after the induction of brain death, bile secretion was significantly diminished in group II as compared with both baseline and group I (Table 1).

Hepatic microcirculation

At baseline there were no significant differences in the number of nonperfused sinusoids and stagnant leukocytes in sinusoids between the groups. At 3 h after the induction of brain death the numbers of nonperfused sinusoids, sinusoidal stagnant leukocytes and postsinusoidal venular adherent leukocytes were significantly higher in group II than in group I (Table 3).

Discussion

It is well known that macrohemodynamic and hormonal impairment occurs after brain death. Macrohemodynamic changes include myocardial dysfunction and loss of systemic vascular tonus, leading to hypotension [3, 7]. Concerning hormonal homeostasis, decreases in serum levels of ADH and thyroid hormones have been reported to occur after experimental and clinical brain death [2, 6, 11]. The decrease in ADH level leads to diabetes insipidus with polyuria and dehydration. The changes in thyroid hormone levels are characterized by low T_3 syndrome (sick euthyroid syndrome) with a shift from aerobic to anaerobic metabolism [12]. The factors described above are considered to be responsible for the deterioration of graft viability [7, 11]. The brain death model used in this study simulated well the changes in macrohemodynamics, serum ADH levels, and serum and urine osmolality encountered in brain-dead organ donors. There was no significant difference in thyroid hormone levels between the groups at 3 h after the induction of brain death. Since it has been found in the brain death model of the dog that T_3 and T_4 levels significantly fall for the first time at 7 h after induction of brain death [6], our observation time may have been too short to detect a decrease in these hormones. These differences in response of the hormone levels may be due to different half-lives of the hormones: 5–15 min for ADH [9], 7–8 days for T_4 and 1 day for T_3 [13].

Bile secretion is an energy-dependent process, providing information on global hepatic function and is considered to be one of the markers of hepatic function and viability [4, 15]. The major determinant of bile flow is the rate of secretion of bile salts across the hepatocyte canalicular membrane [5], which is an active ATP-dependent process [16]. Therefore, the reduction in bile secretion after brain death observed in our model is considered to indicate impairment of hepatic function and viability.

Our model revealed for the first time the changes in hepatic microvascular perfusion under the condition of brain death. Sinusoidal perfusion was compromised and an activation of leukocyte-endothelium interaction in hepatic sinusoids and postsinusoidal venules was observed after brain death, associated with compromised liver function. Perfusion failure of hepatic sinusoids may be attributable to (1) a reduction of perfusion pressure, (2) intravascular hemoconcentration, (3) leukocyte accumulation in hepatic vasculature and/or (4) elevation of microvascular resistance due to swelling of endothelial cells [19]. However, to the extent of the role of each of these factors in sinusoidal perfusion failure after brain death awaits further investigation. Furthermore, the sinusoidal perfusion failure associated with deteriorated hepatic function or the activation of leukocyte-endothelium interaction itself in brain-dead organ donors may play some role in ischemia/reperfusion injury during the ensuing cold preservation and implantation of the liver graft.

In conclusion, a novel model has been established which allows hormonal as well as hepatic microcircula-

tory analysis after brain death in the rat. The results indicate that ADH homeostasis and hepatic microcirculation are compromised within a period of 3 h after induction of brain death, leading to compromised hepatic function. The changes in hepatic microcirculation are characterized by sinusoidal perfusion failure and activation of leukocyte-endothelium interaction in sinusoids and postsinusoidal venules.

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