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ORIGINAL ARTICLE

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Kupffer cells play an important role in the cytokine production and activation of nuclear factors of liver grafts from non-heart-beating donors

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Abstract In the non-heart-beating donor (NHBD) deterioration of microcirculation of the liver graft is strongly associated with secretion of cytokines and eicosanoids. In this study we investigated the excretion of cytokines, eicosanoids, and DNA binding activity of transcription factors in the grafts from NHBD and evaluated the effects of the elimination of Kupffer cells on them. The purpose of this study was to clarify the impact of Kupffer cells on transcription factor activity and the that of cytokine and eicosanoid production on reperfusion injury of liver grafts from NHBD. Wistar rats were allocated to four groups: (a) control group: livers were extracted under heart-beating conditions and perfused without cold storage, (b) heart beating (HB) group: livers extracted under heart-beating conditions were perfused after 6 h of cold storage, (c) non-heart-beating (NHB) group: livers extracted after cardiac arrest were perfused after cold storage, (d) Kupffer cell eliminated (KE) group: liposome-encapsulated dichloromethylene diphosphonate was intravenously administered to eliminate Kupffer cells before extraction, and the liver was perfused after cold storage. Cytokines and eicosanoids in perfusate were measured. DNA binding activity of nuclear factor κB, activating protein 1, and nuclear factor–interleukin 6 of tissue were investigated. Concentrations of interleukin 1β and thromboxane B_2 in the perfusate were significantly higher in NHB group, but they were completely suppressed in the KE group. A rise in binding activity of nuclear factor κB and activating protein 1 was not observed during cold storage in any groups, but these activities did increase remarkably after reperfusion. Significant buildup of those activities were recognized in the NHB group, and this phenomenon was inhibited in the KE group. The histological structures of the sinusoid in the KE group were well maintained, as with those of the control group. These results indicate that cytokines, eicosanoids, and the DNA binding activity of the transcripton factor are strongly associated with reperfusion injury, and Kupffer cells play an important role in this mechanism in grafts from NHBDs.

Keywords Non-heart-beating donor · Liver transplantation · Cytokine · Eicosanoid · Nuclear transcription factor · Kupffer cell

Introduction

Liver transplantation, which is established as a therapy for end-stage hepatic disease, has become a common surgical treatment all over the world. Due to technical progress and the development of immunosuppressive agents the outcome of liver transplantations has improved markedly, and the indications for transplantation have been extended. However, donor numbers do not increase in most countries, and graft shortage has become a major problem, especially in Europe and North America. Consequently the utilization of grafts from so-called marginal donors, which would previously have been classified as unsuitable for transplantation, are now being considered [20], and a special program has been devoted to the use of liver grafts from donors with cardiac arrest [8]. According to a prospective study, the number of donors would increase by 20–25% if donation of liver grafts from marginal donors were to be introduced [22]. At present, however, transplantation of liver grafts from cardiac arrest donors is rarely carried out because of the high incidence of primary graft nonfunction (PGN).

Ischemic reperfusion injury of the liver graft subjected to cold preservation is considered one of the main factors of PGN [10]. It has been reported that PGN is strongly associated with the involvement of the impaired sinusoidal endothelial and activated Kupffer cells [6, 7, 30, 31, 36]. We have already reported that active oxygen production, lipid peroxidation, and activation of both neutrophils and Kupffer cells are strongly associated with the mechanism of PGN [16, 23, 24, 25, 30, 31, 34, 35, 37]. Conditions such as an agonal state of hypoxemia, hypotension, abnormal electrolyte levels, and acidosis seem to induce serious biological reactions by the transplanted liver graft. The reactions caused by hypoxia and acidosis in NHBD are probably more complicated than those induced by simple cold storage and reperfusion injury [41]. Especially the increase in inflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin (IL) 1β, and IL-6 as well as the increment of DNA binding activity of transcription factors, such as nuclear factor κB (NF-κB) and activating protein 1 (AP-1) are strongly associated with the mechanism of graft injury [5, 26, 33].

The purpose of the present study was to investigate the mechanism of ischemic reperfusion injury in liver grafts in a cardiac arrest (NHBD) model focusing on inflammatory cytokines, eicosanoids, and the DNA binding activity of transcription factors. We furthermore wanted to clarify the role of Kupffer cells in ischemic reperfusion injury.

Materials and methods

Experimental design

Male Wistar rats (Charles River, Tokyo, Japan) weighing 250–300 g were used. Under anesthesia with ether the livers was extracted and preserved with and without cold preservation. Livers were divided into the following four groups according to the surgical procedure and subsequent treatment [14]. (a) In the control group, the livers were extracted under heart-beating conditions and perfused without cold storage. (b) In the heart-beating (HB) group, the livers were extracted under heart-beating conditions and after 6 h of cold storage were perfused with University of Wisconsin (UW) solution. (c) In the non-heart-beating (NHB) group, livers were extracted after cardiac arrest and after 6 h of cold preservation were perfused. (d) In the Kupffer cell eliminated (KE) group, liposome-encapsulated dichloromethylene diphosphonate (5 ml/kg) was intravenously administered to eliminate Kupffer cells 42 h before hepatectomy. In this group, the livers were extracted after cardiac arrest and perfused after 6 h of cold storage. Each group consisted of 6 rats.

Experimental procedure

After heparinization, 50 mg/kg of Nembutal was intraperitoneally administered to induce general anesthesia. The abdomen was opened with a midline incision. After introduction of a silicone tube into the common bile duct, the sheath of a 12 gauge needle was inserted into the portal vein. The inferior vena cava was incised, and the portal blood was washed out with 10 ml of physiological saline at 4°C and UW solution under a pressure of approximately 10 cmH_2O. Then, the liver was preserved in UW solution at 4°C.

Induction of cardiac arrest

The diaphragm was incised to induce respiratory stop and cardiac arrest. The rat was left at room temperature for 30 min. Cardiac arrest was induced 10.0 ± 1.2 (mean $\pm$standard) min after the thoracotomy. Afterwards, the liver was washed out via the portal vein and extracted.

Elimination of Kupffer cells

Dichloromethylene diphosphonate was prepared in accordance with the method described by Van Rooijen [38]. In 20 ml methanol chloroform (1:1) 75 mg phosphatidylcholine (Sigma, St. Louis, Mo., USA) and 11 mg cholesterol (Sigma) were dissolved, and the mixture was dried in an evaporator under low pressure at 37°C. This procedure was repeated twice. A thin phosphatidylcholine and cholesterol membrane was formed in a flask. Of dichloromethylene diphosphonate (Boehringer-Mannheim, Laval, Quebec, Canada) 1.89 g was dissolved in phosphate-buffered solution (PBS), pH 7.4 at 23°C. The whitish thin membrane was dissolved with the mixed solution by shaking the flask for 1 h in a water bath at 68°C. Nitrogen gas was placed on the surface, and the solution was left at room temperature for 2 h. Then the solution was passed through a 0.6-μm filter. To remove dichloromethylene diphosphonate which was not encapsulated in the liposome by this procedure the solution was diluted with 100 ml PBS and centrifuged at 5500 rpm for 30 min. The supernatant was diluted with 20 ml PBS and centrifuged at 100,000 *g* for 30 min. After the supernatant was discarded, the pellet was suspended in 4 ml PBS. This final solution was administered intravenously to rats at a dose of 5 ml/kg body weight 42 h before hepatectomy. The effect of dichloromethylene diphosphonate on Kupffer cells has been reported previously, and

the validity was confirmed by immunohistological stain using ED2 antibody and colloid carbon phagocytosis [30].

Perfusion of the liver in vitro

The schema of the reperfusion system of the liver in vitro is shown in Fig. 1. The perfusion circuit was filled with Krebs-Henseleite bicarbonate buffer which was filtrated through a 0.2 μm filter. An oxygen and carbon dioxide gas mixture (95% O_2 + 5% CO_2) was bubbled into the buffer. The buffer was perfused into the portal vein of the liver at a pressure of 10 cmH_2O for 60 min. Liver specimens were taken under heart beating conditions, after cardiac arrest, during cold preservation (0, 6, 12, 24 h), and 60 min after perfusion. The specimens were freeze-clamped in liquid nitrogen. The frozen tissue sample was stored at –80°C until measurement of DNA binding activity of transcription factor.

Eicosanoids

Thromboxane (TX) B_2, 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) and leukotriene B_4 that were released into the perfusate from the liver were assessed by measuring eicosanoids concentration in the perfusate 60 min after reperfusion using commercially available, enzyme-linked immunosorbent assay kits (Cayman Chemical, Ann Arbor, Mich., USA).

Cytokines

Inflammatory cytokines, i.e., TNF, IL-1, and IL-6 that were released into the perfusate from the liver, were assessed by measuring cytokine concentration in the perfusate 60 min after reperfusion, using commercially available enzyme-linked immunosorbent assay kits (Biosource International, Camarillo, Calif., USA).

Extraction of nuclei from liver tissue

The nuclei were extracted according to the method described by Dignam et al. [11]. The following procedure was conducted at 4°C. The tissue was dissolved in 5 ml of a solution, consisting of 0.6% IGEPAL CA-630, i.e., a nonionic detergent, 150 mM NaCl, 10 mM hydroxyethylpiperazine ethanesulfonic acid (HEPES) pH 7.9, 1 mM ethylene diaminetetraacetate (EDTA), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). A pestle (Dounce tissue grinder, Wheaton, Millville, N.J., USA) was used to homogenize the sample approximately gently ten times. To remove uncrushed tissues the homogenate was centrifuged at 2000 rpm for 1 min. The supernatant was centrifuged at 5000 rpm for 5 min. The precipitated nuclei were introduced into a solution which consisted of 25% glycerol, 20 mM HEPES at pH 7.9, 420 mM NaCl, 1.2 M $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM PMSF, 2 mM benzamidine, 5 μg/ml pepstatin, 5 μg/ml leupeptin, and 5 μg/ml aprotinin, and the mixture was left on the ice for 20 min. The solution containing nuclei was centrifuged at 6,000 rpm, and the supernatant was collected. Then the sample was stored at –80°C. Nucleoprotein was quantified using a commercially available BCA protein assay kit (Pierce Chemical, Rockford, Ill., USA).

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay was carried out to investigate the DNA binding activity of NF-κB, AP-1, and NF IL-6 (C/EBP) of the liver specimens. Double-stranded oligonucleotides were prepared as follows: NF-κB: 5′-AGT TGA GGG GAC TTT CCC AGG C-3′, AP-1: 5′-CGC TTG ATG AGT CAG CCG GAA-3′ (Promega., Madison, Wis., USA), C/EBP: 5′-TGC AGA TTG CGC AAT CTG CA-3′ (Santa Cruz Biotechnology, Santa Cruz, Calif., USA), and T4 polynucleotide kinase (Promega). [^{32}P]ATP was incubated with these oligonucleotides at 37°C for 30 min for labeling and used as a probe. Then the sample was purified through Quick Spin G-25 column (Boehringer-Mannheim, Piscataway, N.J., USA). The liquid nuclear extract of 15 μg protein and the probe were incubated with the binding reaction buffer, which consisted of 10 mM Tris-Cl at pH 7.5, 1 mM $MgCl_2$, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM PMSF, 4% glycerol, and 0.1 mg/ml polydeoxyinosinic deoxycytidylic acid at room temperature for 20 min. For the supershift assay, before the addition of the probe, NF-κB was incubated with anti-p50, p65, and c-Rel antibodies (2 g/sample: Santa Cruz Biotechnology) at 4°C for 1 h. For the competition assay, before the addition of the probe, unlabeled oligonucleotide and mutant oligonucleotide were incubated at room temperature for 10 min. The sample was electrophoresed in 4% polyacrylamide gel with Tris-borate-EDTA buffer under 150 V for 2 h. After drying the gel was exposed to the film at –80°C.

Histological findings

Sixty minutes after perfusion the specimens of the liver were fixed in 10% neutral formalin, and hematoxylin and eosin staining was carried out.

Statistics

All values and figures are the mean ± standard deviation (SD). Statistical analysis was carried out using Student's t test. Differences at the level of $P < 0.05$ were considered statistically significant.

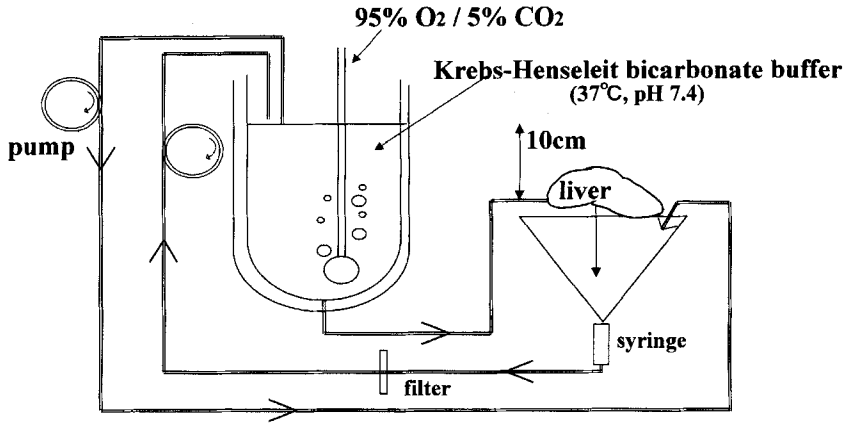

Fig. 1. Scheme of the perfusion system of the liver. The liver was perfused for 60 min under a pressure of 10 cmH_2O with 100 ml Krebs-Henseleit bicarbonate buffer (37°C, pH 7.4) saturated with 95% O_2 and 5% CO_2 mixture in a blood-free recirculating system

Results

Eicosanoids in perfusate

The concentration of TXB_2 was significantly higher in the HB and NHB groups than in the other two groups; in the KE group it was completely suppressed, as was that of IL-1 (Table 1). The concentration of 6-keto $PGF_{1\alpha}$ was significantly higher in the HB group than in the other groups; in the NHB group it was reduced to the level in the control group. The ratio of TXB_2 to 6-keto $PGF_{1\alpha}$ was significantly higher in the NHB group than in the other groups; in the KE group it was completely suppressed (Table 1). Leukotriene B_4 was not detected in any of the groups (data were not shown).

Cytokines in perfusate

The concentration of IL-1β was significantly higher in the NHB group than in the other groups; in the KE group the production of IL-1β suppressed to the same level in the control group (Table 1). The values in the HB and NHB groups were slightly elevated, but there was no statistically significant difference between group. In the KE group, however, the production of TNFα was suppressed to the same level as in the control group (Table 1). IL-6 was not detected in any of the groups (data were not shown).

Transcriptions factor

No NF-κB activity was observed, in spite of cold preservation in each group. After 60 min of reperfusion activity in the NHB group was clearly higher than in the other groups. Activity in the KE group was lower than in the NHB group. In the competition and supershift assays of the nuclear extract from the NHB group, the band was abolished by 100-fold excess of unlabeled wild-type oligonucleotide (lane wt). In contrast, the mutated oligonucleotide had no effect on the band in the NHB group (lane mt). Supershift assay with antibodies against p50, p65, and c-Rel showed the identity of different NF-κB members. In the NHB group the p50/p50 homodimer was dominant (Fig. 2).

No AP-1 activity was observed in spite of cold preservation. Activity after 60 min of reperfusion was markedly higher in the NHB group than in the other groups. Activity in the KE group was lower than in the NHB group. In competition assays the band of NHB was abolished by 100-fold excess of unlabeled wild- type oligonucleotide (lane wt). In contrast, the mutated oligonucleotide had no effect on the band in the NHB group (lane mt; Fig. 3).

DNA binding activity of NF-IL6 was observed during cold preservation, and after 60 min of reperfusion the binding activity remained unchanged in all three groups. In the competition assay the band of NHB was abolished by 100-fold excess of unlabeled wild-type oligonucleotide (lane wt). In contrast, the mutated oligonucleotide had no effect on the band in the NHB group (lane mt; Fig. 4).

Histological findings

In the HB group the structure of the liver was well-maintained, although a slight disorder was recognized in the perisinusoidal area. In the NHB group the sinusoidal structure was remarkably deteriorated, and a hydropic change in parenchymal hepatocytes was observed. The histological structure was well maintained in both the control and KE groups (Fig. 5).

Discussion

The use of liver grafts from NHBD is still a matter of controversy in clinical transplantation. In some

Table 1. Cytokines and eicosanoids in perfusate 60 min after reperfusion (*HB* heart beating group: the liver, extracted under heart beating conditions, was perfused after 6 h of cold storage with solution, *NHB* non-heart-beating group: the liver extracted after cardiac arrest was perfused after 6 h of cold preservation, *KE* Kupffer cell eliminated group: liposome-encapsulated dichloromethylene diphosphonate was administered intravenously to eliminate Kupffer cells 24 h before extraction of the liver, and perfused after 6 h of cold storage)

Group	Control	HB	NHB	KE
Eicosanoid				
6-keto $PGF_{1\alpha}$ (pg/ml)	178 + 72	346 + 119*	150 + 30	120 + 50
TXB_2 (pg/ml)	81 + 26	2017 + 525**	2703 + 1380**	17 + 18
TXB_2/6-keto $PGF_{1\alpha}$ ratio	0.4 ± 0.1	4.3 ± 2.3	18.6 ± 9.6*	0.1 ± 0.1
Cytokine				
IL-1β (pg/ml)	34.2 + 13.0	32.8 + 12.2	61.4 + 14.7*	40.6 + 18.5
TNFα (pg/ml)	0.3 + 0.1	4.5 + 6.3	3.2 + 5.5	0.4 + 0.6

*$P < 0.05$ versus all other groups,
**$P < 0.05$ versus KE group

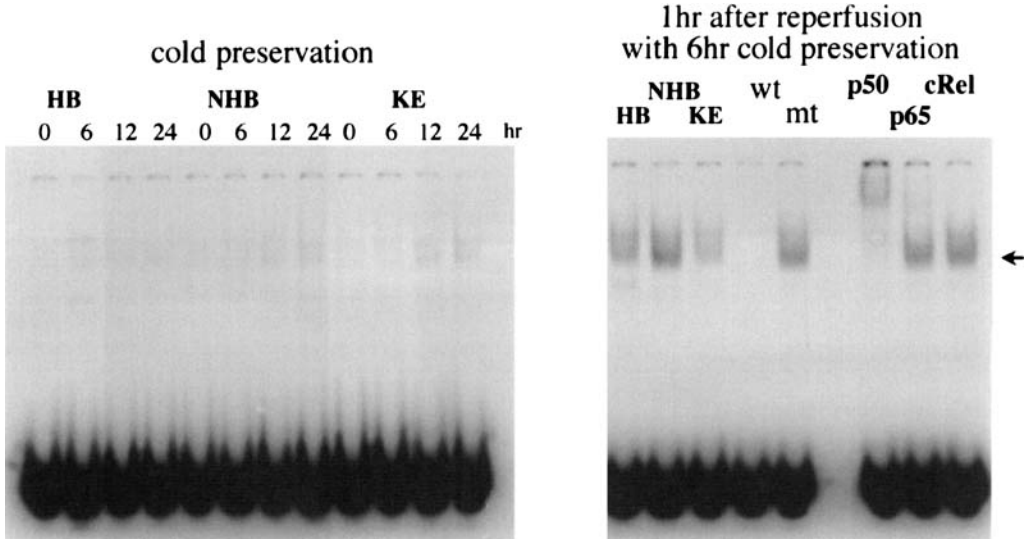

Fig. 2. Electrophoretic mobility assay of NF-κB activation. No activity was observed in spite of cold preservation in each group. Activity after 60 min of reperfusion was markedly higher in the NHB group and lower in the KE group than in other groups. In the competition and supershift assays of the nuclear extract from the NHB group the band was abolished by 100-fold excess of unlabeled wild-type oligonucleotide (*lane wt*). In contrast, the mutated oligonucleotide had no effect on the band in the NHB group (*lane mt*). Supershift assay with antibodies against p50, p65, and c-Rel showed the identity of different NF-κB members. In the NHB group the p50/p50 homodimer was dominant. *HB* Heart-beating donor with 6 h cold preservation; *NHB* non-heart-beating donor with 6 h of cold preservation; *KE* non-heart-beating donor with elimination of Kupffer cells and 6 h of cold preservation; *wt* wild type; *mt* mutated type

countries liver transplantation from NHBD is carried out in urgent cases as a trial, but transplantation results are still unsatisfactory. Such livers are usually not used in urgent cases, but in stable patients who are able to withstand the greater reperfusion injury of the liver from NHBD, according to the recent North American report. Injury of the liver graft from NHBD is associated with various cytokines released from activated Kupffer cells [26]. We have previously reported that Kupffer cells play an important role in reperfusion injury of the liver graft from NHBD after cold preservation [37]. In this study we found that IL-1β and TXB_2 concentrations are significantly higher in liver grafts from NHBD during reperfusion after cold preservation in NHBD liver graft than in with prostacyclin. We also observed that transcription factors, i.e., NF-κB and AP-1, were not activated during cold preservation, but were after reperfusion. The activation was enhanced especially in agonal NHBD. These phenomena were not induced by the elimination of Kupffer cells, and as a result reperfusion injury of sinusoidal microcirculation was effectively prevented. As this study suggests, the key to successful transplantation from NHBD is likely control of Kupffer cells function.

There are several models of NHBD, i.e., induction with respirator off, thoracotomy, and administration of KCl. Zhang et al. [41] reported that in the NHBD model induced by administration of KCl, arterial oxygen content and ability of ATP synthesis in mitochondria 30 min after induction were significantly higher than those in the NHBD model induced by the thoracotomy. Therefore in this study we chose the NHBD model induced by thoracotomy in which liver graft suffered from agonal conditions such as hypoxia.

According to previous studies on reperfusion injury of the liver after cold preservation, the parenchymal hepatocytes deteriorated as a result of the damage of sinusoidal endothelial cells [20]. The mechanism of this phenomenon has been explained as follows. ATP depletion [12, 19] and the increase in intracellular calcium activate various enzymes and affect the cellular structure and the permeability of cell membrane [13, 15, 21]. In addition, metabolites in the arachidonic acid cascade are also associated with the deterioration in sinusoidal endothelial cells and coagulopathy of sinusoid. TXA_2, which derives from the activation of phospholipase A_2, has strong effects of platelets aggregation and vasoconstriction [28] and induces the accumulation of neutrophils in sinusoid [18, 39, 40]. Moreover, TXA_2 impairs the microcirculation of the liver directly, and treatment by inhibition of TXA_2 production improves the microcirculation after reperfusion in the ischemic liver [32]. Post et al. [27] reported that the level of prostanoids in hepatic veins increased 100–500 times after reperfusion. TXA_2 and PGI_2 are unstable in the blood and metabolized to TXB_2 and 6-keto PGF_1, respectively. This study clearly indicated the increase in prostacyclin, which induced vasodilation in microcirculatory disturbance of the liver after reperfusion following cold preservation. We also showed that TX is significantly higher in the liver graft from NHBD than prostacyclin. Therefore, in view of the

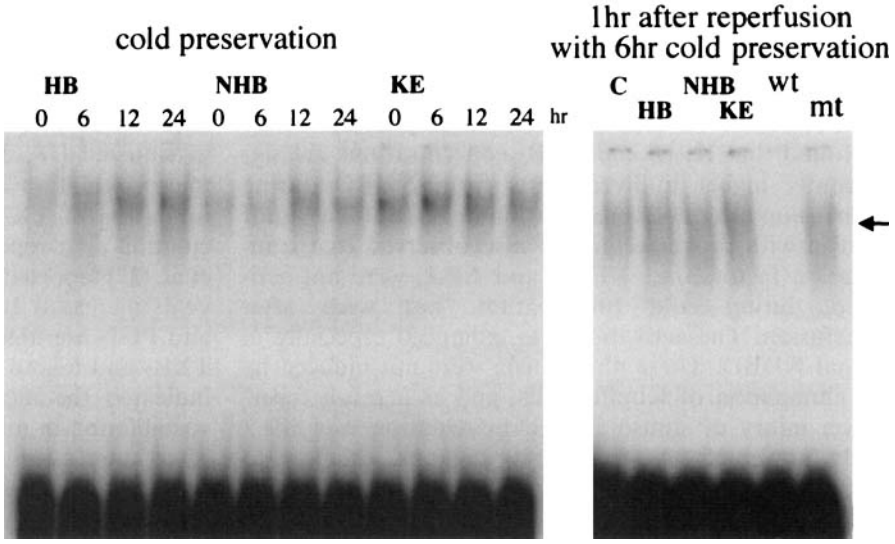

Fig. 3. Electrophoretic mobility assays for AP-1 activation. No activity was observed in spite of cold preservation in each group. Activity after 60 min of reperfusion was markedly higher in the NHB group and lower in the KE group than in other groups. In competition assays the band of NHB was abolished by 100-fold excess of unlabeled wild-type oligonucleotide (*lane wt*). In contrast, the mutated oligonucleotide had no effect on the band in the NHB group (*lane mt*). *HB* Heart-beating donor with 6 h cold preservation; *NHB* non-heart-beating donor with 6 h cold preservation; *KE* non-heart-beating donor with elimination of Kupffer cells and 6 h cold preservation; *wt* wild type; *mt* mutated type

arachidonic acid metabolism, the agonal condition of the NHBD donor has severe adverse effects on the sinusoidal microcirculation in the liver, as opposed to the heart beating donor. In addition, we demonstrated that Kupffer cells play important roles in activation of the arachidonic acid metabolism in the graft from NHBD.

Arachidonic acid, which is a component of the cell membrane, is a substrate metabolized through the arachidonic acid cascade. The rate-limiting enzymes involved in this cascade are divided into cyclo-oxygenases (COX) and lipoxygenases. COX metabolizes arachidonic acid into PGG_2. PGG_2 is metabolized from PGH_2, PGE_2, and PGD_2 to PGF_2. In addition to this process, there are two other metabolic processes, and the final metabolites are PGI_2 and TXA_2. Caughey et al. [9] reported that the productions of IL-1β and TNFα are inhibited by TXA_2 in human monocytes, and suggested activation of the arachidonic acid cascade by signal transduction. Two COX isozymes have been identified, i.e., COX-1 and COX-2. COX-1 is a constitutive protein in almost all the cells. On the other hand, COX-2 is an inducible protein. NF-κB, AP-1, and NF IL-6 exist in

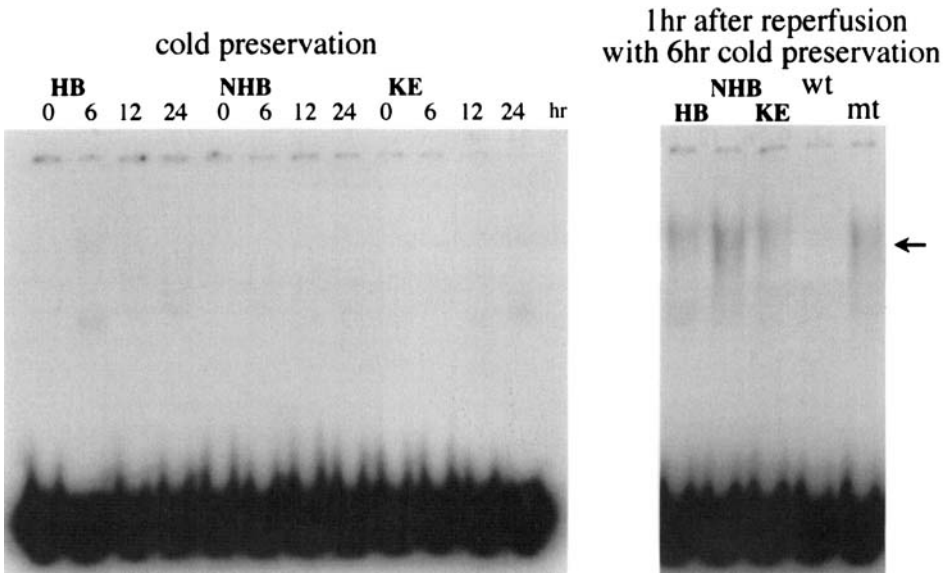

Fig. 4. Electrophoretic mobility assays for NF-IL6 activation. DNA-binding activity was observed during cold preservation and after 60 min of reperfusion the binding activity remained unchanged in the three groups. In the competition assay the band of NHB was abolished by 100-fold excess of unlabeled wild-type oligonucleotide (*lane wt*). In contrast, the mutated oligonucleotide had no effect on the band in the NHB group (*lane mt*). *HB* Heart-beating donor with 6 h cold preservation; *NHB* non-heart-beating donor with 6 h cold preservation; *KE* non-heart-beating donor with elimination of Kupffer cells and 6 h cold preservation; *wt* wild type; *mt* mutated type

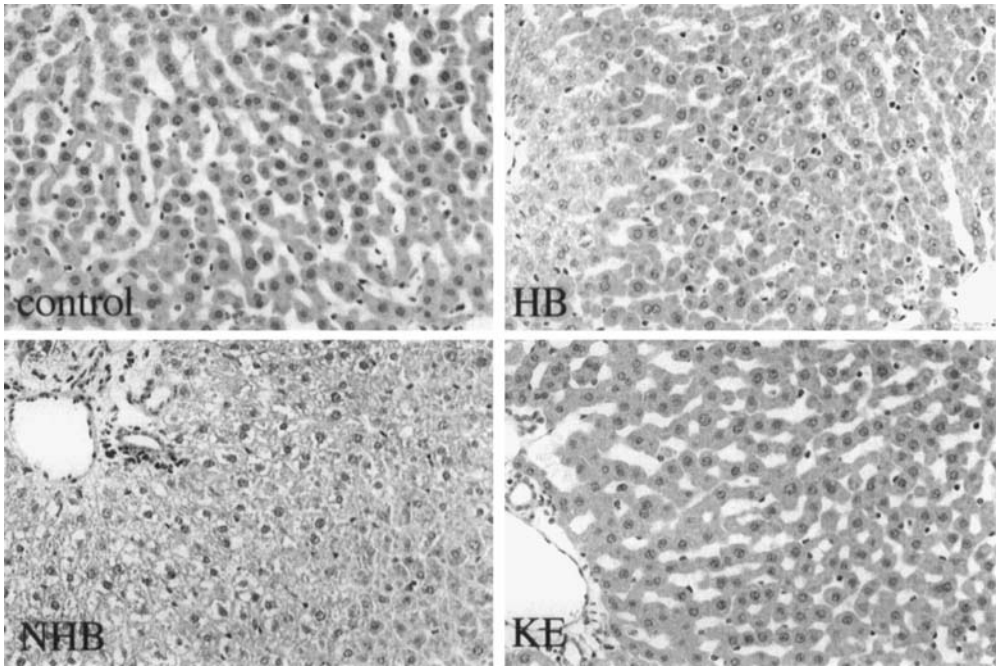

Fig. 5. Morphological findings in the livers 60 min after reperfusion. In the NHB group sinusoidal spaces became narrow, and hydropic changes were observed in hepatocytes. In the KE group sinusoidal structures and hepatocytes were well preserved, as in the control group. *Control* Heart-beating donor without preservation; *HB* heart-beating donor with 6 h cold preservation; *NHB* non-heart-beating donor with 6 h cold preservation; *KE* non-heart-beating donor with elimination of Kupffer cells and 6 h cold preservation. Hematoxylin and eosin stain, ×100

the promoter region of COX-2, and COX-2 is induced by IL-1 and NF-κB [1]. Although we did not examine COX-2 expression, the results of our study, i.e., increment of eicosanoid production and inflammatory cytokines and enhancement of transcription factor activity, suggest that cold preservation/reperfusion injury in the graft from NHBD is associated with COX-2 induction in the liver graft.

Many studies have been carried out recently on the mechanism of biological reactions, including acute-phase inflammation. Attention has been drawn especially to transcription factors, which bind to the promoter of gene expression and induce transcription activity. NF-κB was the first transcription factor identified as a nucleoprotein that binds to the enhancer of immunoglobulin k chain genes and was regarded as a B cell specific transcription factor [29]. Subsequent studies demonstrated that NF-κB is a transcription factor which controls the activities of various cytokines, i.e., IL-1, IL-2, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor, TNFα, COX-2, chemokine, adhesion molecules such as E-selectin, intercellular adhesion molecule 1, vascular cell adhesion molecule, inducible nitric oxide synthase, Fas ligand, IL-2 receptor, and gene expression, as well as human immunodeficiency virus and cytomegalovirus self-replication. NF-κB plays an important role in preventing apoptosis induced by TNFα [2]. There has been a report that massive liver-cell death was induced in NF-κB knockout mice, and this indicates the necessity of NF-κB in the liver [3]. NF-κB was induced when reperfusion disturbance occurred in the liver transplantation [4, 42, 43]. However, NF-κB activation is reported to have a protective effect on liver grafts from heart beating donors [5]. However, no study has investigated the mechanism of reperfusion injury in the liver grafts from cardiac arrest donors, and this study is the first report clearly indicating a relationship between TNFα, NF-κB activity, and Kupffer cells in reperfusion injury of the liver under agonal conditions.

NF-κB is composed of heterodimers p65 and p50. We conducted a supershift assay of NF-κB and found that NF-κB, which was induced by preservation/reperfusion injury, consisted of p50 homodimer. KBF-1 is identified as a transcription factor of the p50 homodimer which binds to the near NF-κB consensus site of DNA existing in the promoter region of MHC class I gene [17]. Therefore KBF-1 is likely to have a function similar to that of NF-κB [17]. It remains to be clarified whether the enhancement of the activity of transcription factors directly causes the deterioration of the liver graft after reperfusion. Therefore further studies are necessary to clarify the true mechanism.

As immediate early genes, there are AP-1, signal transducers and activators transcription-3, NF IL-6 (C/EBP), and cyclic AMP response element binding protein as well as NF-κB. These components are strongly associated with signal transduction of acute-phase inflammatory reactions. This study identified an absence of NF-κB and AP-1 activities during cold preservation despite agonal conditions, but their activities increased during reperfusion after cold preservation. Significant buildup of NF-κB and AP-1 activities were recognized especially in the liver specimens from the cardiac arrest model under agonal conditions. However, this

phenomenon was not induced by Kupffer elimination. These results indicate that inhibition of the enhancement of NF-κB and AP-1 activities was strongly associated with prevention of preservation/reperfusion injury in the liver graft with an agonal condition, and that this activation of NF-κB binding and AP-1 was induced by inflammatory cytokines, such as TNFα and IL-1β produced by Kupffer cells.

In conclusion, our study clearly indicates enhancement of TX production and inflammatory cytokines and that these changes in chemical mediators are strongly associated with sinusoidal microcirculatory disturbance if there are Kupffer cells in the liver. This ischemic/reperfusion study of the liver from an NHBD agonal rat clarified that the activities of transcription factors, i.e., NF-κB and AP-1 are increased after reperfusion, and that the elimination of Kupffer cells prevents the rise in these transcription factors and microcirculatory disturbances. In clinical transplantation the use of liver grafts from NHBD still remains a controversial issue; however, the control of Kupffer cell function must be the key to success for transplantation from NHBD.

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