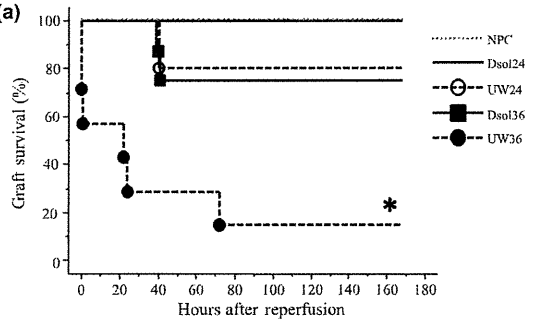


(b)

	Graft survival							
NPC	>7d	>7d	>7d	>7d	>7d	>7d		
Dsol24	>7d	>7d	>7d	>7d	>7d			
UW24	40 h	>7d	>7d	>7d	>7d			
Dsol36	39 h	41 h	>7d	>7d	>7d	>7d	>7d	>7d
UW36	0 h	0 h	1 h	22 h	24 h	3d	>7d	

Figure 1 Seven-day cardiac isograft survival. Cardiac grafts were preserved for 24 h (UW24: $n = 5$; Dsol24: $n = 5$) or 36 h (UW36: $n = 7$; Dsol36: $n = 8$) following syngeneic heterotopic transplantation. Grafts without preservation were used as a non-preservation control (NPC; $n = 6$). (a) Survival curve after reperfusion. (b) Survival time of individual hearts in each group after reperfusion. Dsol significantly improved 7-day graft survival after 36-h cold preservation. $^*P < 0.05$ by log-rank test, UW36 vs. Dsol36.

planimetry software (KEYENCE). The fibrotic area was expressed as the percentage of the total LV area.

Calpain and caspase 3 activation

To determine the levels of activation of calpain and caspase 3, calpain-specific cleavage of cytoskeleton-bound proteins (α-fodrin and talin) and cleavage of caspase 3 were assessed by a standard Western blot analysis [31,32]. The graft was homogenized with a glass-Teflon homogenizer in 4 ml/g of lysis buffer containing 25 mmol/l Tris–HCl, 150 mmol/l NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/l EDTA, and 1% protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA) at pH 7.6. The homogenate was centrifuged for 15 min at 14000× *g* and 4 °C. The protein concentration of the resulting supernatant was determined with a bicinchoninic acid assay (Thermo Scientific, Rockford, IL, USA). Then, the proteins were separated with standard SDS–PAGE techniques. After transfer to a PVDF membrane, the proteins were probed with mouse anti-α-fodrin mAb (1:1000; Biomol, Plymouth Meeting, PA, USA), mouse anti-talin mAb (1:200; Sigma), and rabbit anti-caspase 3 Ab (1:1000; Cell Signaling, Danvers, MA, USA). Then, IgG-horseradish peroxide-conjugated anti-mouse or anti-rabbit secondary antibody (1:2500–1:10000; Amersham Bioscience, Buckinghamshire, UK) was applied for chemiluminescence detection (Amersham Bioscience). α-tubulin was detected with rabbit mAb to α-tubulin (1:1000; Cell Signaling) as an internal control. The cleaved bands of α-fodrin and talin were then normalized by the respective intact bands. Cleaved bands of caspase 3 were normalized by α-tubulin. The values were finally expressed as a percentage of the value in the normal heart controls.

Cytosolic Ca^{2+} concentration *in vitro*

Cells expressing a FRET based Ca^{2+} indicator, Premo Cameleon Calcium Sensor™, were subjected to 24-h cold preservation in UW or Dsol. Cameleon was excited at 370 nm to produce fluorescence from CFP detected at 480 nm in the Ca^{2+} -unbound form. In the Ca^{2+} -bound form, FRET occurred from CFP to YFP, resulting in the production of additional fluorescence at 535 nm. The mean fluorescent intensity at 535 nm (MFI_{535}) was expressed as a percentage of the MFI_{535} before preservation.

Statistical analysis

Data were expressed as the mean ± standard deviation or mean ± standard error of the mean as annotated. Graft survival was plotted by the Kaplan–Meier method, and was applied to a log-rank test for comparisons. One-factor ANOVA followed by *post hoc* test was applied as appropriate. A value of $P < 0.05$ was considered statistically significant.

Results

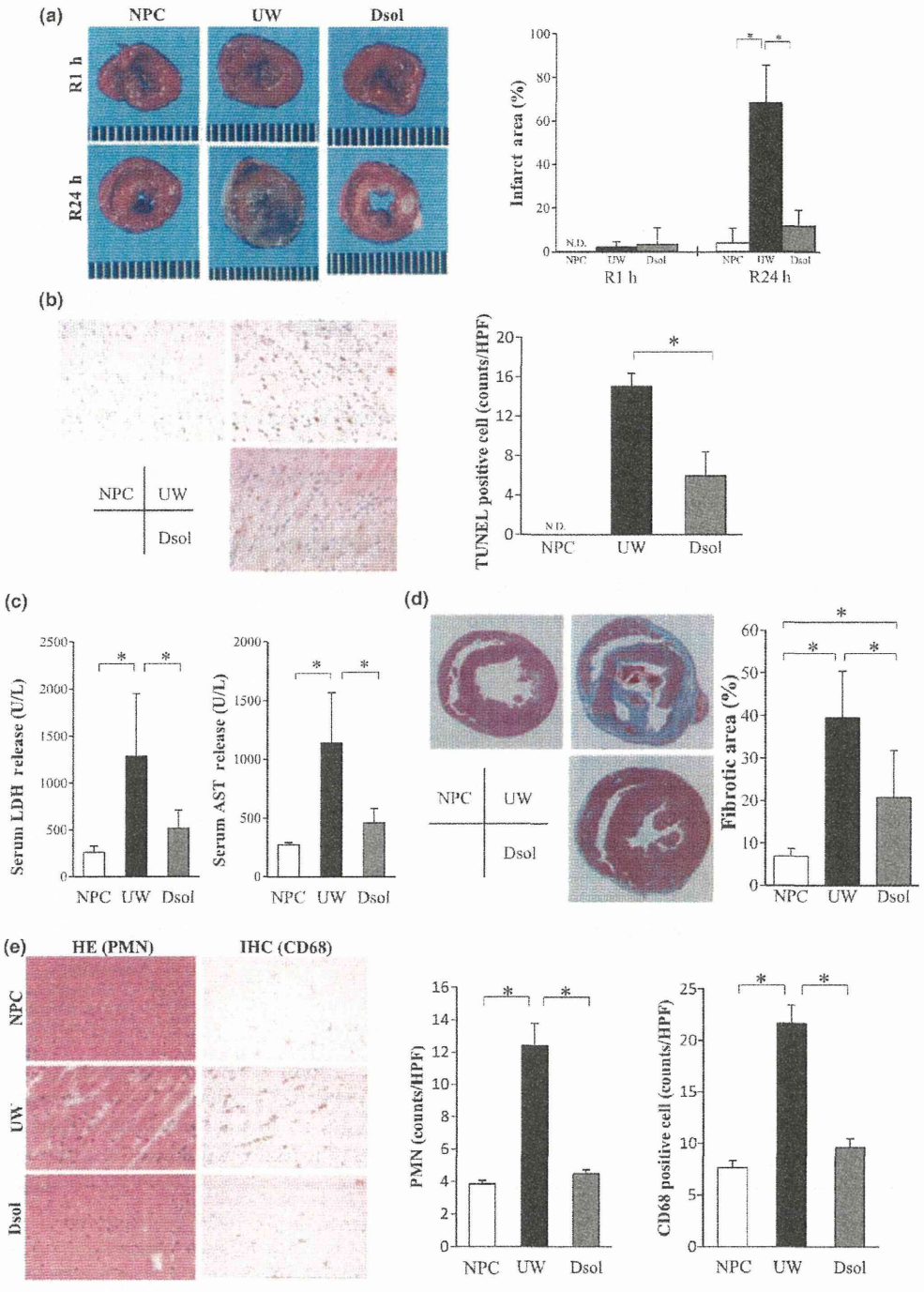
Dsol ameliorated graft survival

All hearts in the non-preservation control group (NPC) survived for 7 days (Fig. 1). In the 24-h cold preservation experiment, the rate of 7-day graft survival in the Dsol group was 100% (5/5), versus 80% (four of five) in the UW group. In the 36-h preservation experiment, the rate of 7-day graft survival was 75% (six of eight) in the Dsol group, whereas it was only 14% (one of seven) in the UW group ($P < 0.05$; Dsol36 vs. UW36).

Dsol decreased graft infarction, apoptosis, LDH and AST release

Graft infarction at 1 h after reperfusion (R1h) was not evident in all groups, and ranged from 0% to 3.4% of the total LV area. At R24h, the infarct area was 67.8% ± 16.5% of the total LV area in the UW group, whereas it was 11.7% ± 7.3% in the Dsol group ($P < 0.05$; Dsol vs. UW; Fig. 2a).

TUNEL-positive cells, i.e. apoptotic cardiomyocytes, were not found in the NPC group at R24h. The number of TUNEL-positive cardiomyocytes at R24h was signifi-



cantly smaller in the Dsol group (5.97 ± 2.44 counts/HPF) than in the UW group (15.1 ± 1.30 counts/HPF, Fig. 2b).

Serum LDH and AST levels in the UW group (1282 ± 667 and 1144 ± 427 IU/l, respectively) were significantly higher than those in the Dsol group (516 ± 195 and 463 ± 120 IU/l, respectively) at R24h (Fig. 2c).

Dsol reduced graft fibrosis

The fibrotic area at R7d was significantly larger in the UW group (39.5% ± 11.0%) than in the Dsol group (20.7% ± 11.1%) or NPC group (6.9% ± 1.8%, $P < 0.05$ for UW versus NPC and for UW versus Dsol, Fig. 2d).

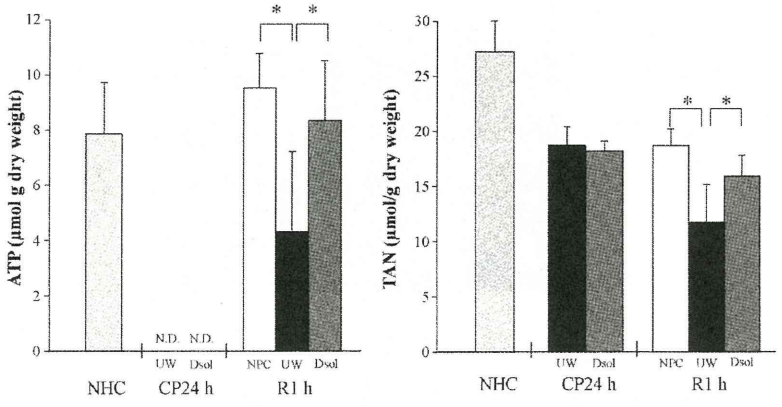


Figure 3 Graft ATP and total adenine nucleotide contents of the normal heart controls, after 24 h of cold preservation, and 1 h after reperfusion were measured by HPLC. Dsol was associated with significantly faster recovery of ATP and TAN content at 1 h after reperfusion. Values represent the mean ± SD, $n = 4$ each group, $^*P < 0.05$, Fischer's PLSD *post hoc* test. N.D., not detected; NHC, normal heart control; NPC, non-preservation control.

Dsol suppressed the infiltration of inflammatory cells

The number of polymorphonuclear neutrophils (PMNs) in the interstitium at R24h was significantly higher in the UW group (12.4 ± 1.37 counts/HPF) than in the Dsol group (4.5 ± 0.24 counts/HPF). The number of CD68-positive monocytes/macrophages at R24h was significantly higher in the UW group (21.7 ± 1.76 counts/HPF) than in the Dsol group (9.6 ± 0.87 counts/HPF, Fig. 2e).

Dsol improved the restoration of high energy phosphates after reperfusion

ATP content in the normal heart was 7.87 ± 1.86 (μmol/g dw), whereas ATP was not detected at the end of the 24-h cold preservation in either group. At R1h, it was significantly higher in the Dsol group (8.34 ± 2.16 μmol/g dw) than in the UW group (4.32 ± 2.90 μmol/g dw, Fig. 3). TAN was also significantly higher in the Dsol group (15.94 ± 1.89 μmol/g dw) than in the UW group (11.77 ± 3.39 μmol/g dw).

Dsol inhibited cold preservation-induced Ca^{2+} overload *in vitro*

After 24-h cold preservation, MFI_{535} increased to as much as 376% of the basal level in the UW group, whereas it increased to only 140% of the basal level in the Dsol group ($P < 0.0001$). Therefore, Dsol inhibited Ca^{2+} overload during cold preservation (Fig. 4a).

Dsol inhibited calpain and caspase-3 activation

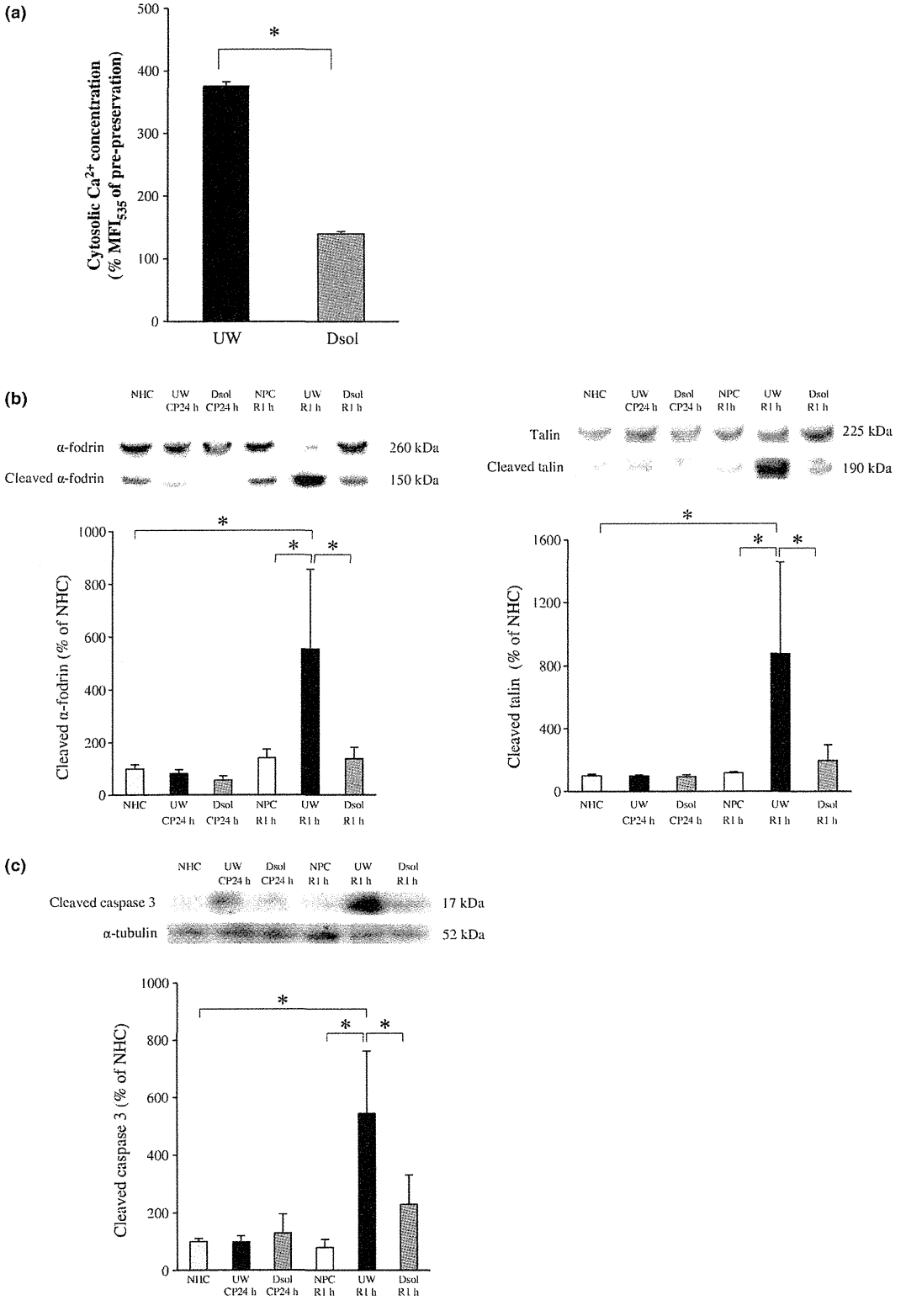
The calpain-specific substrates, talin and α-fodrin, were not cleaved at the end of the 24-h cold preservation period in either the UW or Dsol group (Fig. 4b). At R1h, they showed a significantly greater amount of cleavage in the UW group compared to the normal heart control group (NHC). Calpain-mediated cleavage was significantly suppressed in the Dsol group ($P < 0.05$ vs. UW, Fig. 4b).

The activations of caspase 3 by cleavage were assessed. The active cleaved fragments of caspase 3 (17 kDa) were significantly increased at R1h in the UW group compared to the NHC group ($P < 0.05$, vs. NHC), whereas they were significantly suppressed in the Dsol group ($P < 0.05$, vs. UW, Fig. 4c).

Discussion

In the current study, we demonstrated that the novel organ preservation solution Dsol improved cardiac graft survival after 36-h cold preservation. After 24-h preservation, Dsol markedly suppressed necrosis and apoptosis as

Figure 2 Graft injury after 24-h cold preservation and reperfusion. (a) Graft infarction at 1 h and 24 h after reperfusion as determined by TTC staining. Representative TTC-stained sections from grafts (Upper, R1hr; Lower, R24hr) and infarct size as measured by planimetry ($n = 6$ each group). Each point on the scale represents 1 mm. (b) Apoptosis of cardiomyocytes after 24 h of reperfusion as determined by TUNEL staining. Representative TUNEL-stained sections and TUNEL-positive myocardial cell counts in each section are shown ($n = 6$ each group). TUNEL-positive nuclei appear dark brown. Magnification ×400. (c) Serum LDH and AST release at 24 h after reperfusion (NPC: $n = 6$; UW: $n = 5$; Dsol: $n = 5$). (d) Graft fibrosis at 7 days after reperfusion as determined by Masson's trichrome staining. The fibrotic area stains blue, and the viable area stains red. Representative sections (original magnification: ×20) are shown, and the fibrotic area was calculated (NPC: $n = 6$; UW: $n = 4$; Dsol; $n = 5$). (e) Histological and immunohistochemical examination of graft-infiltrating PMNs and monocytes after 24 h of preservation and 24 h after reperfusion. Representative photographs of HE staining and immunohistochemical staining by anti-CD68 antibody (magnification ×400). CD68-positive cells appear brown. PMNs and CD68-positive cells were counted in HE and IHC, respectively ($n = 6$ each group). Dsol diminished graft injury significantly, as revealed by the lower levels of infarction, apoptosis, serum LDH and AST release, graft fibrosis and infiltration of inflammatory cells after reperfusion. Data are presented as the mean ± SD, $^*P < 0.05$ by the Tukey–Kramer *post hoc* test. NPC, non-preservation control; N.D., not detected.



compared to UW solution. Dsol also enabled rapid restoration of the high energy phosphate that had been exhausted from the grafts during the preservation period. Dsol was clearly shown to prevent the elevation of cytosolic Ca^{2+} concentration during cold preservation *in vitro*, and inhibited Ca^{2+} -dependent activation of calpain and subsequent activation of caspases-3, compared to UW solution *in vivo*. These data clearly demonstrated the advantage of Dsol over UW, with the former showing excellent inhibition of cardiac graft injury after prolonged simple cold static preservation and subsequent cardiac transplantation in rats.

In previous reports using the same model, the graft function of the UW-preserved rat hearts after transplantation was recovered in the 12-h preservation group [33], whereas it was impaired in the 18-h preservation group [34]. Further, 24-h preservation in UW raised the possibility of graft loss due to the critical ischemia/reperfusion injury [5]. Infarction of grafts after prolonged cold preservation presents a risk of graft loss. To avoid graft loss in such cases, previous reports have suggested the importance of suppressing graft infarction to below 15% of the total area of individual grafts after reperfusion [35,36]. In the present study, Dsol suppressed the graft infarction in just 11% of total area of grafts, and prevented graft loss completely. On the other hand, UW induced 68% graft infarction and resulted in graft loss in 20% of grafts after 24-h cold preservation. In addition, the surviving grafts in the UW-preserved group tended to beat more weakly than the Dsol-preserved grafts. However, we could not evaluate graft function in this study because we employed a non-functional model. Functional assessment using a functional model remains a challenge for future studies. However, the present results do indicate that Dsol has a more powerful protective effect than UW solution, although this protective effect appeared more evident after 36-h preservation.

Necrosis at the center of the infarction and apoptosis around the necrotic area, the so-called area at risk (AAR), are closely related to graft survival and contractile function [37]. After prolonged cold preservation and reperfusion, cardiomyocytes fell into necrosis for various reasons, including hypercontracture, insufficient blood flow due to vascular failure, and activation of necrosis-inducing proteases [14,38,39]. In the present study, UW could not prevent necrotic cell death, as demonstrated by TTC staining, AST and LDH release, and eventual graft fibrosis, which was consistent with a previous report [35], whereas Dsol achieved nearly complete inhibition. Necrotic cardiomyocytes induced infiltration of inflammatory cells in the UW group but not in the Dsol group. These cells, in turn, damage viable cardiomyocytes by secreting inflammatory mediators [40]. Therefore, the prevention of necrosis also has important implications in terms of stopping this harmful cycle. Cardiomyocytes that manage to just avoid necrosis often fall into apoptotic cell death within the AAR [37]. We demonstrated that abundant TUNEL-positive apoptotic myocardia were found at the AAR in UW-preserved hearts, whereas they were significantly suppressed in the Dsol group. Dsol prevented cell death not only by preventing necrosis but also by preventing apoptosis.

Cytosolic Ca^{2+} overload during prolonged cold preservation and Ca^{2+} -dependent activation of calpain and caspases after reperfusion play a central role in cellular necrosis and/or apoptosis. Calpain is activated by cytosolic Ca^{2+} overload, and activated calpain, in turn, induces necrosis by cleavage of cytoskeletal proteins such as α-fodrin and talin [39]. Calpain also triggers apoptosis by caspase-12 activation [41], and Bid [42] and Bax cleavage [43], followed by caspase 3 activation. Among the many unique properties of D_2O, such as stabilization of the microtubules [18], actin cytoskeleton [19], plasma membrane [20], and membrane-bound proteins [21], we focused on the ability of D_2O to suppress the elevation of cytosolic Ca^{2+} concentration [17]. D_2O is reported to inhibit calcium influx via the plasma membrane L-type Ca^{2+} channel [44] as well as calcium efflux from the sarcoplasmic reticulum (SR) to the cytosol [45]. Our present *in vitro* study demonstrated that cytosolic Ca^{2+} concentration was elevated up to 3.8-fold after 24-h cold preservation in the UW group. Elevated cytosolic Ca^{2+} at the end of the cold preservation period in turn leads to the activation of Ca^{2+} -dependent proteases, and thereby protease-induced necrosis and apoptosis of cardiomyocytes after reperfusion. In this study, the major source of aug-

Figure 4 (a) The cytosolic Ca^{2+} concentration of H9c2 cardiomyocytes after 24-h cold preservation was assessed by using a Premo Cameleon Calcium Sensor™. After 24-h cold preservation, MFI_{535} increased to as much as 376% of the baseline level in the UW group, versus 140% of the baseline level in the Dsol group. Values represent the mean ± SD, $n = 6$ each group. $^*P < 0.0001$ by Fischer's PLSD *post hoc* test. (b and c) Western blotting analyses of calpain and caspase-3 activity in the cardiac grafts after 24 h of cold preservation and 1 h of reperfusion. (b) Activated calpain mediated the cleavage of α-fodrin and talin. Representative Western blots of cleavage of intact α-fodrin (260 kDa) to a cleaved fragment (150 kDa), and intact talin (225 kDa) to a cleaved fragment (190 kDa) are shown. Semi-quantitative analyses are shown below. (c) Representative Western blots of cleavage of caspase-3 to the active fragments of caspase-3 (17 kDa). The results of the semi-quantitative analyses are shown below. Dsol significantly inhibited calpain and caspase-3 activation after reperfusion. All values are expressed as the mean ± SD, $n = 3$, $^*P < 0.05$, Turkey–Kramer *post hoc* test. NHC, normal heart control; NPC, non-preservation control.

mented cytosolic Ca^{2+} during preservation should be the efflux from SR, because both UW and Dsol are Ca^{2+} -free solutions. The D_2O present in the Dsol could inhibit Ca^{2+} release from SR and suppressed the elevation of cytosolic Ca^{2+} concentration during cold preservation. Accordingly, Dsol dramatically suppressed the activation of these degradative Ca^{2+} -dependent proteases thereafter. This property of D_2O should be a key mechanism of the graft protection with Dsol.

In addition to cellular death, the energy state, which is established mainly by mitochondrial oxidative ATP production, is closely related to the cardiac kinetics after transplantation. Flameng *et al.* reported that the impairment of ATP restoration after reperfusion, even if the ATP content was maintained at the end of 24-h cold static preservation, causes cardiac contractile dysfunction after transplantation [12]. Although Dsol failed to preserve ATP content during cold preservation in the present study, rapid recovery of ATP content was clearly shown at 1 h after reperfusion. Meanwhile, UW failed to recover ATP synthesis, even though graft infarction was not evident.

Although the intracellular-type component and HES adopted by UW can potently prevent cellular swelling during cold preservation, they tend to induce graft infarction as a result of coronary endothelial injury [13,14]. Therefore, we adopted the extracellular-type component without HES for Dsol. In this respect, the concept of Dsol is similar to that of Celsior [46], which showed better preservation than UW within a relatively short period [47], but not after extended cold preservation [48,49]. The reasons for the potent protection by Dsol even after a prolonged period could be the modified impermeants and D_2O, which could compensate for the demerits of the extracellular-type composition. Modified impermeants such as mannitol and sucrose, which *per se* have cytoprotective [15] and anti-oxidative effects [16], could reduce organ swelling. Other properties of D_2O, in addition to the inhibition of Ca^{2+} -overload, such as stabilization of the microtubules [18], actin cytoskeleton [19], plasma membrane [20], and membrane-bound proteins [21], could help Dsol to inhibit graft injury.

In conclusion, Ca^{2+} overload initiated during cold preservation induces the activation of harmful proteases, and subsequent apoptosis and necrosis of cardiomyocytes after reperfusion, finally leading to graft loss. A novel organ preservation solution, Dsol, was shown to be superior to UW solution at inhibiting myocardial injury during extended cold preservation and subsequent syngeneic transplantation of rat hearts by inhibiting Ca^{2+} overload during cold preservation and subsequent activation of proteases. This solution could reduce the mortality of heart transplantation. Moreover, the protective effect of this solution could prolong the safe preservation time of cardiac grafts and increase the opportunities for organ distribution.

Authorship

KW, MF, KY and ST: designed the experiments. KW and MF: wrote the article. KW, MF, TK, GH, SS and DF: contributed to the acquisition of data and analysis. SH, TS, MT, TS and HF: provided expertise. MF and MS: provided new reagents. KW, MF, KY, TK and ST: interpreted the data.

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Professional Education and Hospital Development for Organ Donation

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ABSTRACT

Because of the strict Organ Transplantation Act, only 81 brain dead (BD) organ donations had been performed in Japan for 13 years since 1997. The Act was revised on July 17, 2010, allowing, organs to be donated after BD with consent from the family, if the subject had not denied organ donation previously. This act has lead to an expectation of a 6–7-fold increase in BD donation. The 82 organ procurement coordinators (OPC) in Japan include 32 belonging to the Japanese Organ Network (JOT) and the others to each administrative division. JOT has guideline manuals of standard roles and procedures of OPC during organ procurement from BD and cardiac death donors.

To manage the increased organ donations after the revision of the act, we have modified the education system. First, we modified the guideline manuals for OPC to correspond to the revised Transplant Act and governmental guidelines. Second, all OPC gathered in a meeting room to learn the new organ procurement system to deal with the revised Transplant Act and guidelines. Third, a special education program for 2 months was provided for the 10 newcomers. Last, the practical training in each donor case for newcomers was performed by older OPC.

Topics of the education program were the revised transplant act and guidelines, family approach to organ donation, BD diagnosis, donor evaluation and management, organ procurement and preservation, allocation system, hospital development and family care.

In the future, each OPC will be divided into special categories, such as the donor family OPC, the donor management OPC, and the operating room OPC. Therefore, we need to construct separate special education programs for each category.

TO DISCUSS PROFESSIONAL education and hospital development for organ donation, we need to develop the following programs in each country: organ transplant legislation, network for organ allocation and sharing, public awareness, basic education for medical students (physicians, nurses, and medical engineers, etc), and for transplant professionals. Since 1978, the donation of kidneys after cardiac death (DCD) has been legally accepted in Japan, if family consent was obtained. Small children have been able to donate their kidneys after cardiac death. The Japanese Organ Transplantation Act for brain dead (BD) donation was issued in October 1997. The act required a living written consent for BD and organ donation; it did not allow BD donation from children <15 years of age. For these reasons, only 81 BD organ donations were performed in Japan over 13 years after the Act was issued in October 1997.

Finally, the act was revised on July 17, 2010, to allow organ donation after BD with consent from the family,[1] if the patient has not rejected organ donation. By this renewal, we expected a 6 to 7-fold increase in BD donations.

There are 82 governmentally authorized organ procurement coordinators (OPC) in Japan: 32 OPC belong to The Japan Organ Network (JOT) and the others to each administrative division (prefectural OPC). About 400 donor hospitals hire in-hospital OPC individually. The JOT OPCs play a role in organ allocation and sharing, informed consent, and care of the donor family, donor evaluation and management, control of organ retrieval in the operating

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room, public awareness, and hospital development. The prefectural OPCs play a role in public awareness and hospital development in their district and assisting JOT OPC in organ donation and allocation. In hospitals, OPCs play a role in awareness of organ donation among the hospital staff and assisting authorized OPCs in donation processes.

The Department of Coordinators and the coordinator committee in The JOT play the main roles to educate these OPC. JOT has published guideline manuals for the standard roles and procedures of OPC during organ procurement in BD and DCD.

To manage increased organ donations after the revision of the act, we sought to modify the education system. First, we modified OPC guideline manuals to correspond with the revised Transplant Act and governmental guidelines. Second, all OPCs gathered in a room to learn the new organ procurement system, including the revised Transplant Act and the guidelines. Third, a special 2-month educational program was provided for 10 newcomers as well as practical training in individual donor cases by older OPCs.

EDUCATION OF THE JOT OPCs

For the rookie JOT OPCs, we provided a special 2-month education programs. The topics of the classroom lectures were an overview of transplant medicine, of the previous and revised organ Transplant Acts, organ transplant history in Japan and in the world, of the JOT and organ transplant network system, as well as the roles and tasks of OPO in organ donation (BD and DCD), the current status and problems in organ transplantation (heart, lung, liver, pancreas, and kidney), therapies for end-stage organ failure, process and roles of OPCs in BD organ donation under the revised act, donor evaluation and management, and family consent at BD donation and DCD. After 2 months of classroom lectures, they were assigned to the JOT branch for the on-job-the-training by older OPCs.

For the older JOT OPCs, we taught details of BD organ donation under the revised act, details of pediatric organ donation, such as pediatric emergency therapy, care of the pediatric family, and organ procurement in children. Simulations of family consent in special conditions, such as, how to address refusal of the person for organ donation, child abuse, consent of the child's family, and consent of priority organ donation to relatives.

The leader JOT OPCs were trained in how to supervise family consent and care, coordination of organ procurement surgery and control of the an OPC team in BD donation and DCD.

EDUCATION FOR THE PREFECTURAL OPCs

A 2-day seminar is held every February for the prefectural OPCs. The topics of the program are the processes of DCD and BD organ donations (initial action, family consent, donor evaluation), the roles of OPC, especially prefectural OPC in DCD and BD donation, the standard process of priority organ donation to relatives, information disclosure to the media, family consent at BD donation as well as case studies of DCD and BD donations.

Each JOT branch conducts its own classroom lectures 3–5 times a year. The topics include the current status of DCD and BD donation in each branch or in the special prefectures with case studies, how to proceed with pediatric organ donation, family care during and after donation, case studies of DCD and BD donations, public awareness, and hospital development.

EDUCATION FOR THE IN-HOSPITAL OPCs

The JOT OPCs and the prefectural OPCs conduct classroom lectures 1–3 times a year. The topics are legislation of organ transplantation/donation in Japan, the current status of DCD and BD organ donation in Japan, the standard process of DCD and BD organ donation (initial action, family consent, donor evaluation, etc), the roles of in-hospital OPCs in DCD and BD organ donations, information disclosure to Media and family consent at BD donation as well as case studies in DCD and BD donation.

A simulation with classroom lectures of DCD or BD donation in each hospital was effective in the education of in-hospital OPCs as well as hospital development. To perform the simulation, the in-hospital OPC and medical staffs needed to establish their own organ donation system, to make their own guidelines and manuals of organ donation and to determine the roles of each hospital staff member in organ donation.

EDUCATION OF MEDICAL STAFF IN A DONOR HOSPITAL

The JOT OPCs and the prefectural OPCs conduct a classroom lecture for the medical staff in each donor hospital: physicians, nurses, medical engineers, and medical examiners. The topics are the organ transplantation/donation legislation in Japan, the current status of DCD and BD organ donation in Japan, the process of DCD and brain dead organ donation (initial action, family consent, donor evaluation, etc), and the roles of medical staff. Especially for physicians in the emergency department and in the intensive care unit as well as anesthesiologists, donor evaluation and management, multiple organ retrieval procedures in the operating room and donor management during surgery are important topics to increase the number of organs transplanted per donor and to improve their graft functions.[2] These lectures and simulations of organ donation are important for hospital development.

EDUCATION OF MEDICAL STAFF PERFORMING BD DETERMINATION IN A DONOR HOSPITAL

The JOT, the academic societies (Japan Neurosurgical Society, Japanese Association for acute medicine, Japanese Society of Emergency Pediatrics, etc), and the research groups supported by governmental grants conduct seminars

on BD determination for medical examiners, physicians and medical staffs in donor hospitals.

The topics are the definition of brain death, its determination, performance of the electroencephalogram, and options for organ donation.

HOSPITAL DEVELOPMENT

To increase organ donation, the recognition of importance of organ transplantation and donation by medical staffs in donor hospitals is important. Frequent visits to donor hospitals by OPCs are difficult, but represent the most effective method for hospital development. Frequent visits allow OPCs to find a key person for organ donation in each hospital, establishing a good relationship between OPOs and the medical staff. Moreover, seminars for the medical staff performing BD determinations or evaluating and managing donors until and during the organ procurement surgery are important for hospital development.

In the future, each OPC will be divided into special categories, such as those responsible to obtain informed consent and care donor families until the subject leaves the donor hospital, to evaluate organs and provide care until procurement surgery and to participate in organ procurement procedures.

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ELSEVIER

A Newly Developed Container for Safe, Easy, and Cost-effective Overnight Transportation of Tissues and Organs by Electrically Keeping Tissue or Organ Temperature at 3 to 6°C

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ABSTRACT

Background. As there is only one skin procurement organization in Japan the Japan Skin Bank Network (JSBN), all skin grafts procured in Japan are sent by a commercialized delivery system. Preliminarily, bottles containing saline were transported in a cardboard box using a so-called "cooled home delivery service" using a truck with a refrigerated cargo container. During transportation the temperature in the cardboard box increased to 18°C in summer and decreased to −5°C in winter. For these reasons, we investigated whether a newly developed container "Medi Cube" would be useful to transport skin grafts.

Objectives. Four bottles with a capacity of 300 mL containing 150 mL of saline in a Medi Cube container were transported from Osaka to the JSBN in Tokyo between 4 PM and 10 AM using a commercialized cooled home delivery service. Two bottles were transported in a Medi Cube container without phase change materials (PCM) in winter and summer, respectively. Another two bottles were transported in the Medi Cube with PCMs in winter. The temperatures inside saline, inside a transportation container, and outside the container, and air temperature were monitored continuously with a recordable thermometer.

Results. The temperatures inside saline and inside a Medi Cube container were maintained between 3 and 6°C, even when the temperature outside the container increased during parking. The temperature inside a Medi Cube container without PCM decreased to −3°C when the inside of the cargo container was overcooled in winter. However, the temperatures inside saline and inside a Medi Cube container with PCM were between 3 and 6°C, even when the temperature outside the container decreased to below 0°C in winter.

Conclusion. A Medi Cube container with PCM provided a safe, easy, and cost-effective method for overnight transportation of skin grafts.

IN Japan, only the the Japan Skin Bank Network (JSBN) performs frozen preservation and supplies skin. For example, when the skin sheets are procured from locations near Osaka, they are cooled temporarily at our bank overnight, and then transported using a commercial delivery system to the JSBN in Tokyo.[1] Although most Japanese delivery companies advertise that they can maintain the temperature of goods to

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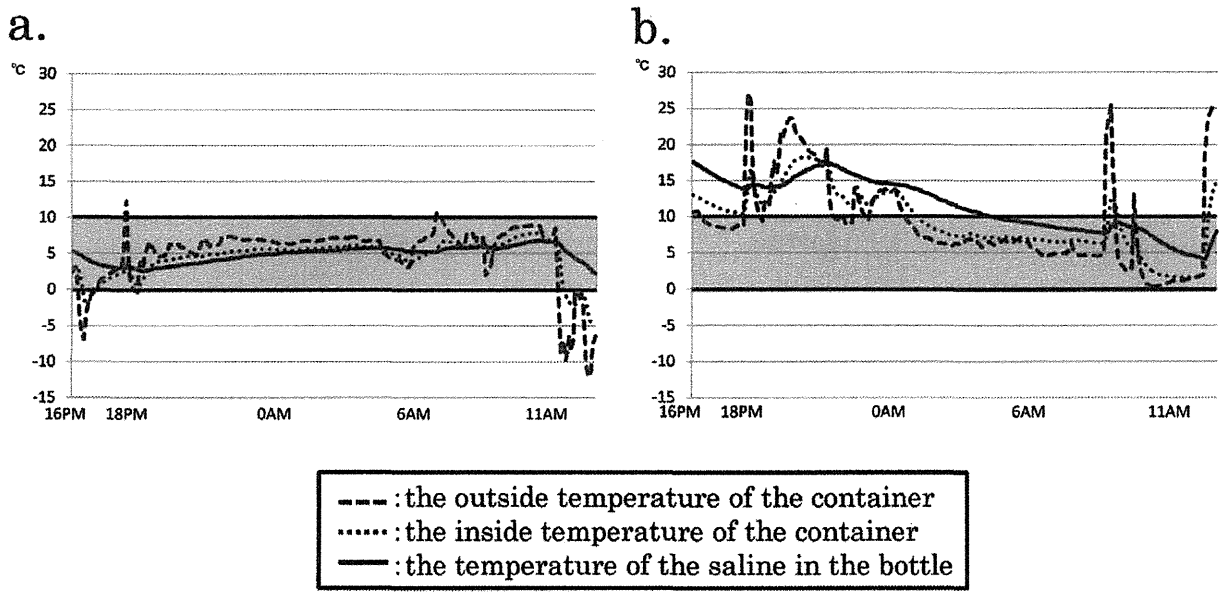

Fig 1. The results of preliminary experiment of transportation of the bottle using an usual cooled delivery system **(A)** transported in winter and **(B)** in summer.

between 0 and 10°C during transportation, no data were available whether this temperature was actually maintained. Thus, we investigated the temperature management state under tissue transportation.

A preservation bottle packed in a cardboard box was transported using the so-called "cooled home delivery service" from our bank in (Osaka) to the JSBN in (Tokyo). The distance between the two cities is approximately 560 km. The temperature in the bottle was influenced by the ambient temperature; it was not maintained between 0 and 10°C as stated by the company (Fig 1). Therefore, we sought to transport a bottle using a newly developed container, the Medi Cube, which is cooled by dry ice throughout the transport (Fig 2A). The container is divided into two sections (Fig 2B): the first for the load and the second, for dry ice. Cold air is supplied from the second to the first section by a fan turning automatically as needed. Required electric power is provided by a battery. In the present study, we investigated whether the newly developed Medi Cube container maintained the temperature of the

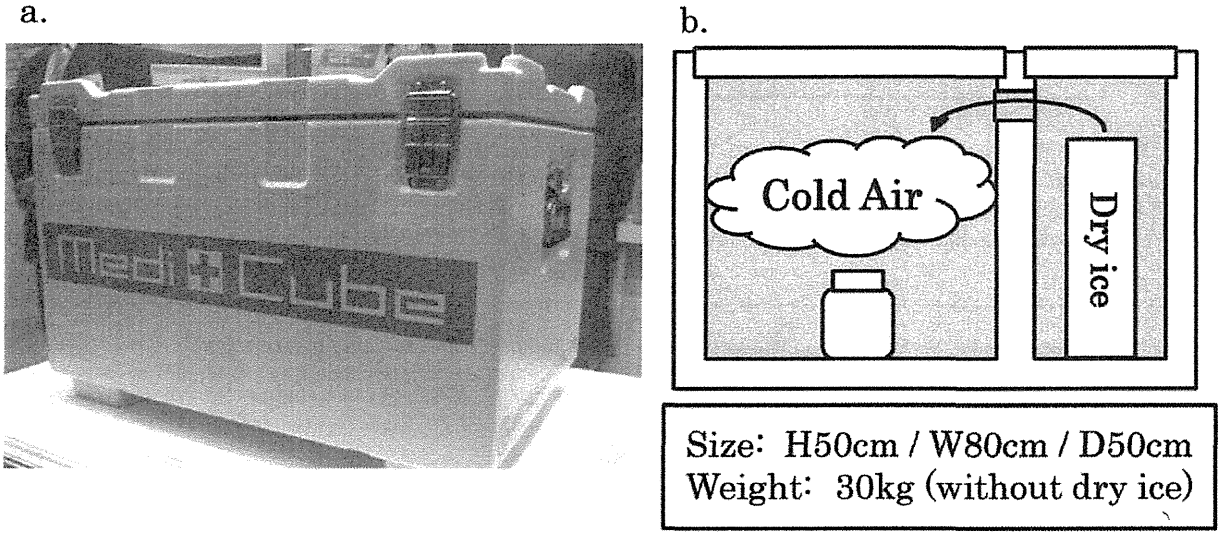

Fig 2. A "Medi Cube" container. **(A)** The container and **(B)** a sectional view of the container.

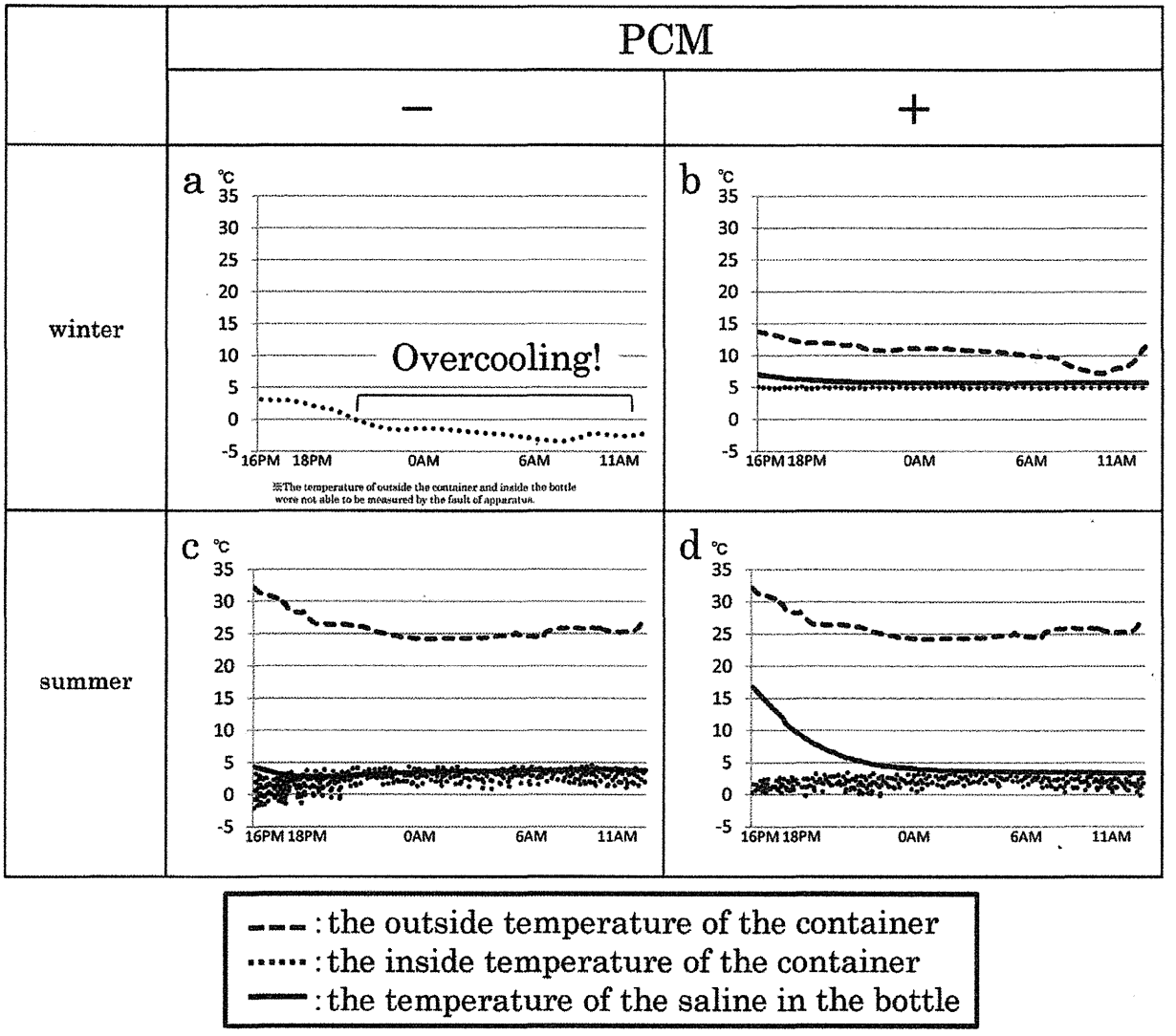

Fig 3. The outside and inside temperatures of the "Medi Cube" container and the temperature of the saline in the bottle during overnight transportation in winter **(A)** and summer **(B)** without phase change materials (PCM) and those **(C)** and **(D)** with PCM.

skin grafts at between 0 and 4°C to transport them safely from Osaka to Tokyo.

MATERIALS AND METHODS

Four bottles with a capacity of 300 mL containing 150 mL of saline in a Medi Cube container were transported from Osaka to the JSBN in Tokyo between 4 PM and 10 AM by a commercial cooled home delivery service. Two bottles were transported in a Medi Cube container in winter and two in summer. Another two bottles were transported in a Medi Cube with phase change materials (PCMs) to avoid overcooling of loads in winter and summer.

The terminal of a recorded type thermometer was inserted in the saline in the bottle. Simultaneously, a recording thermometer was attached inside and outside the Medi Cube. To trace the location of the Medi Cube during transportation, a ground positioning system was also packed in the container.

RESULTS

During winter transportation, the inside of the Medi Cube was overcooled to freezing temperature (Fig 3A). The preservation solution in the bottle might freeze at that time. When PCM was packed with a bottle, the inside temperature of Medi Cube was maintained at approximately 5°C without overcooling, and the temperature of the saline in the bottle was maintained at approximately 6°C throughout the transport period, even in winter (Fig 3B).

Even during summer, the inside temperature of a Medi Cube temporarily was below freezing however, the temperature of the saline in the preservation bottle was maintained at between 3 and 4°C (Fig 3C). When PCM was packed in a Medi Cube with a bottle during summer transportation,

the inside of the Medi Cube was maintained at approximately 5°C without overcooling, and the temperature of the saline in the preservation bottle, at approximately 6°C throughout the transport (Fig 3D).

DISCUSSION

From our preliminary study, conventional tissue conveyance did not maintain the temperature inside and outside of the container at approximately 5°C. Therefore, it is not safe for human tissue to be transported using the usual delivery system. We need to develop a safe, cost-effective transport system for human tissue.

We sought to use a special delivery system, a Medi Cube, to maintain the temperature of the load at approximately 5°C. However, as shown in the present study, the inside temperature of the container overcooled to freezing temperature during winter and even during summer. Therefore, we examined a safe, cost-effective method to prevent overcooling during transportation using a special material PCM, to prevent overcooling. In the present study, PCM maintained the temperature of the saline in the bottle at approximately 5°C throughout the transportation period during both winter and summer. Moreover, there are also data that the Medi Cube continued cooling for 72 hours (data not shown).

In conclusion, a Medi Cube container with PCM provided safe, easy, and cost-effective overnight transportation of skin grafts.

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ELSEVIER

Modification of the Education System for Organ Procurement Coordinators in Japan After the Revision of the Japanese Organ Transplantation Act

S. Konaka, O. Kato, J. Ashikari, and N. Fukushima

ABSTRACT

Background. From October 1997 to July 2010, only 86 brain-dead (BD) organ donations were obtained and no organs were retrieved from children under 15 years of age because of the strict Japan Organ Transplantation Act. The Act was revised on July 17, 2010, allowing organs to be donated after BD with family consent.

Objective. To manage the increased donations after the revision, the Japan Organ Transplant Network (JOT) employed 10 organ procurement coordinators (OPCs) and modified its education systems. We retrospectively reviewed the modified education programs to evaluate whether they were effective and whether the processes of organ donation were promptly performed after the revision of the Act.

Methods. The modifications of education program were: changing OPC to guideline manuals to correspond to the revised Transplant Act; OPCs were taught the new organ procurement system; and a special education program was provided for the 10 newcomers for 2 months.

Results. After 12 months of the revision, 58 BD organ donations were accomplished, whereas they had averaged 6.6 in a year before the revision. Two pediatric BD organ donations were accomplished without problem. One priority organ donation to a relative was performed uneventfully. After applying the modified education program, skilled JOT OPCs and leader JOT OPCs increased.

Conclusions. To manage increased organ donations after the revision of the Act, the educational system was modified and 58 brain dead organ donations were performed safely.

Only 86 brain-dead organ donations had been performed since the Japanese Organ Transplant Law was issued in October 1997, because only persons who had written consent for the procedure could donate their organs after brain death. Moreover, no children under 15 years of age were allowed to donate their organs, because a written living consent of children younger than 15 years of age was not valid. For this reason, donations after cardiac death (DCD) had comprised >90% of cadaver organ donations in Japan. The Japanese Organ Transplantation Act was revised on July 17, 2010.[1] In this law, organs could be donated after brain death with consent from the family if the subject had not denied organ donation.

There were 72 organ procurement coordinators (OPCs) in Japan, including 21 OPCs belonging to the Japan Organ Transplant Network (JOT) and the others to each administrative prefecture. The Department of Coordinators in the JOT plays the main role to educate these OPCs. JOT wrote manuals of standard roles and procedures for OPCs during organ procurement from a brain-dead donor and a donor after cardiac death. With this Act, we expected a 4- or 5-fold increase in brain-dead organ donation.

To manage increased the organ donations after the revision of the Act, JOT employed 10 OPCs and modified the educational systems. In the present study, modified education pro-

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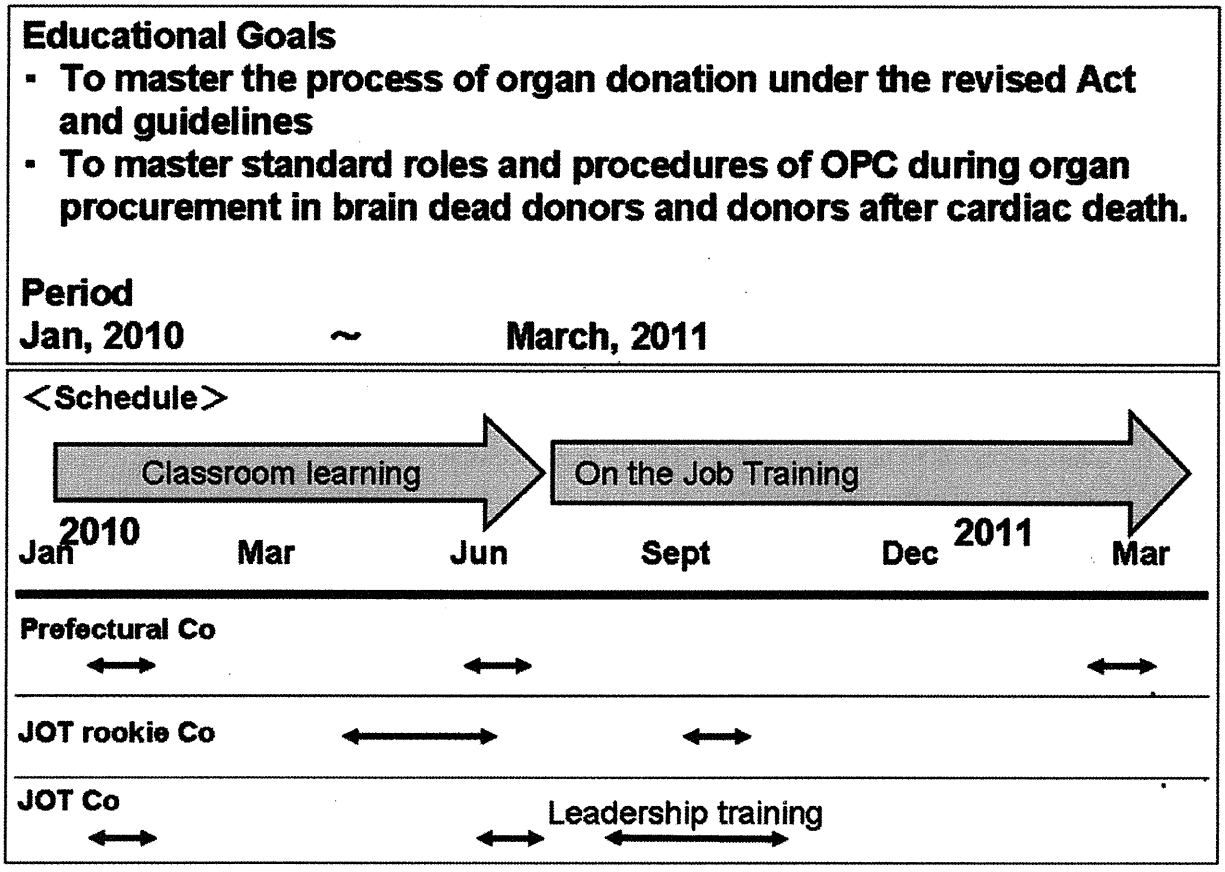

Fig 1. Education schedule for organ procurement coordinators.

grams to OPCs were retrospectively reviewed to evaluate whether they were effective to educate OPCs and whether the processes of organ donation were promptly performed after the revision of the Act.

Table 1. Education for Prefectural Organ Procurement Coordinators (OPCs)

1st classroom lecture
- Process of brain-dead organ donation (initial action, family consent, donor evaluation, etc)
- Standard process of priority organ donation to relatives
- Case studies (brain-dead donation)

2nd classroom lecture
- Process and roles of OPC in brain-dead organ donation under the revised Act
- Process and roles of OPC in pediatric organ donation
- Information disclosure to media
- Family consent at brain-dead donation

3rd classroom lecture
- Current status of brain-dead donation in Japan, with case studies
- How to proceed with pediatric organ donation
- Family care during and after donation
- Family consent at brain-dead donation

METHODS

Modifications of the education program included: first, the OPC guideline manuals corresponding to the revised Transplant Act were changed; second, all OPCs were taught the new system to deal with the revised Transplant Act; and third, special education programs for 2 months were provided for the 10 newcomers. The

Table 2. Education for Rookie Japan Organ Transplant Network (JOT) Organ Procurement Coordinators (OPCs)

1st classroom lecture: 2 months
- Overview of transplantation medicine
- Organ Transplant Act (previous vs revised)
- History of organ transplantation
- What is JOT and the JOT System
- The roles and tasks of OPCs
- Tasks of OPCs at organ donation (brain-dead and after cardiac death)
- Organ transplantation (heart, lung, liver, pancreas, and kidney)
- Therapies for organ failure
- Role plays of tasks of OPCs at organ donation

2nd classroom lecture
- Process and roles of OPCs at brain-dead organ donation under the revised Act
- Donor evaluation and management
- Family consent at brain-dead donation

Table 3. Education for Already Hired Japan Organ Transplant Network Organ Procurement Coordinators (OPC)s

Advanced classroom lectures
- Brain-dead organ donation under the revised Act
- Pediatric organ donation
  Pediatric emergency room, care of pediatric family, pediatric organ procurement
- Simulation of family consent
  - How to deny any refusal of the person for organ donation
  - How to deny child abuse
  - How to confirm consent of pediatric family
  - How to confirm consent of priority organ donation to relatives

Leadership training
- How to supervise family consent and care
- Coordination of organ procurement surgery
- How to control an OPC team at brain-dead and after-cardiac-death donation

practical training in each donor case by elder OPCs was also effective for newcomers.

Educational goals were to master the process of organ donation under the revised Act and guidelines, as well as the standard roles and procedures of OPCs during organ procurement from brain-dead and after-cardiac-death donors. The education schedule of each category of OPC is shown in Fig 1. Educational classroom programs of prefecture OPCs and rookie JOT OPCs are presented in Tables 1 and 2. Regarding non-rookie JOT OPCs, a different educational program was applied, as presented in Table 3. After classroom education, we performed on the job training.

RESULTS

At 12 months after the revision, 58 brain-dead organ donations were performed, whereas only 6.6 had been achieved annually before the revision. Moreover 5.5 organs were transplanted per donor (OTPD), which was almost 2-fold greater than that in other developed countries. Two pediatric brain-dead organ donations were done without problem. One priority organ DCD to relatives was performed uneventfully.

After applying the modified education program, skilled JOT OPCs, who could perform all processes of brain-dead organ donation, increased in number. Moreover, there was an increase in leader JOT OPCs, who could manage a team of OPCs during brain-dead organ donation (Fig 2).

DISCUSSION

By modifying the educational programs of OPCs, skilled OPCs increased in number, In addition to brain-dead organ

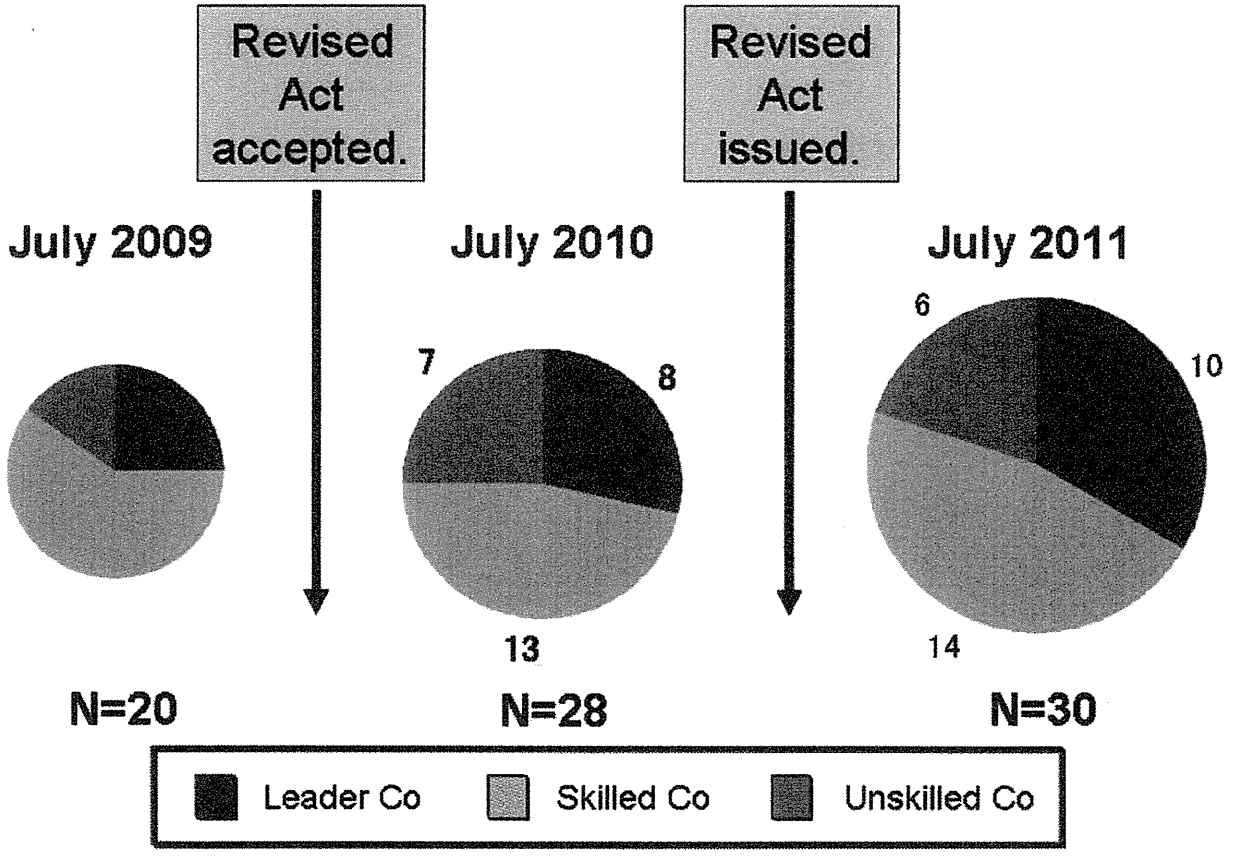

Fig 2. Changes in proportion of OPC skill.

donations in a year increasing from 6.6 to 58, the necessary processes were performed promptly, even in pediatric cases and priority relatives cases.

Because organs can be donated after brain death with consent from their family by the new Act, it is more important for OPCs to identify whether the family really wants to donate the organs. Because the donor shortage has been severe in Japan, special strategies have been established to maximize organ availability.[2] Therefore, education in organ evaluation and donor management are important. We need to continuously modify the education program. A new textbook for OPCs will be published soon by members of the JOT as well as transplantation and emergency surgeons.

In the future, OPCs will be divided into special categories, such as: donor family OPC to obtain informed consent for organ donation and to care for donor families until the donor leaves the donor hospital; a donor management OPC to evaluate donor organs and manage the donor until procurement surgery; and an operation room OPC to manage the organ procurement surgery. Therefore, we need to develop special education programs for each category in the near future.

In summary, to manage the increased organ donations after the Act, we modified the education system. A total of 58 brain dead organ donations were performed safely, whereas the average annual number of brain dead organ donations had been only 6.6 before the revision. OPCs will be divided into special categories, such as family care, donor management, and operation room. Therefore, we need to develop special education programs for each category in the near future.

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ELSEVIER

A Potent Anti-angiogenic Factor, Vasohibin-1, Ameliorates Experimental Bronchiolitis Obliterans

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ABSTRACT

Background. Bronchiolitis obliterans (BO) is a major cause of morbidity and mortality after lung transplantation. BO is pathologically characterized by neovascularized fibro-obliteration of the allograft airway. A recent study has shown that aberrant angiogenesis during fibro-obliteration contributes to the pathogenesis of BO. Vasohibin-1 (VASH1) has been isolated as a vascular endothelial growth factor–inducible gene in endothelial cells (ECs) that inhibits migration and proliferation of ECs and exhibits anti-angiogenic activity in vivo.

Purpose. This study examines whether VASH1 inhibits fibro-obliteration of the allograft in a murine intrapulmonary tracheal transplantation model.

Method. Tracheal allografts of BALB/c mouse were transplanted into the left lung of recipient C57BL/6J mouse. We performed gene transfer to the recipient lungs using an adenovirus vector encoding human VASH1 (Ad-VASH1) or beta- garactosidase (Ad-LacZ) as the control. Tracheal allografts were harvested and pathological on days 21 and 28.

Result. Ad-VASH1 treatment reduced the vascular area on day 21 (4.6% versus 13.0%, $P = .037$) and day 28 (5.4% versus 13.4%, $P = .022$) compared with the control group. This was accompanied by significantly inhibited luminal obliteration of the tracheal allografts in the animals transferred with Ad-VASH1 compared with the control (69% versus 93%, $P = .028$) on day 21. We were not able to observe this effect on day 28 (92% versus 97%, $P = .48$).

Conclusion. Transgene expression of VASH1 in the recipient lung significantly attenuated luminal obliteration of the tracheal allograft; this was associated with significantly reduced aberrant angiogenesis in the fibro-obliterative tissue in a murine model intrapulmonary tracheal transplantation.

BRONCHIOLITIS obliterans (BO) is a major obstacle to the long-term survival of lung transplant recipients. The clinical manifestation of BO, bronchiolitis obliterans syndrome (BOS), develops in 50% of all lung transplant recipients and accounts for 30% of recipient death at 5 years after transplantation.[1] Although the mechanisms involved in the etiology of BO are not fully understood, it is generally accepted that it develops as a result of persistent immunological and inflammatory insults to the allograft airways that cause epithelial injury, granulation tissue formation, and, ultimately, fibro-obliteration of the airways.

Angiogenesis is the formation of new blood vessels; it plays a central role in the progression of various chronic inflammatory diseases including diabetic retinopathy, rheu-

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