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# Role of Kupffer cells in the survival after rat liver transplantation with long portal vein clamping times

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**Abstract** Applying the orthotopic rat liver transplantation (ORLT) model, postoperative survival has been shown to be mainly dependent on the portal vein clamping time (PVCT). It was hypothesized that prolonged intestinal congestion was responsible for the activation of Kupffer cells (KC) with overproduction of TNF, secondary to splanchnic endotoxin accumulation and release on reperfusion. The role of KCs was directly investigated in the context of long PVCTs by eliminating them (using liposome-encapsulated dichloromethylene diphosphonate), by preventing their activation (using a calcium channel blocker, nisoldipine) and by inhibiting TNF production (using thalidomide). Livers from different groups of rats were transplanted following 24-h cold preservation in the UW solution with long PVCTs (from 18–21 min). KCs depletion, preservation with nisoldipine and pretreatment with thalidomide significantly improved survival in conditions using long PVCTs. KC depletion and nisoldipine preservation had no effect on liver enzymes or pathological findings while lung injury was significantly improved. The present data confirm that, in the context of ORLT with long PVCTs, KCs are directly responsible for the systemic endotoxin-like shock syndrome and their effect is mediated through overproduction of TNF.

**Keywords** Kupffer cells · Rat liver transplantation

**Abbreviations** *KC* Kupffer cell · *MDP* Dichloromethylene diphosphonate · *ORLT* Orthotopic rat liver transplantation · *PVCT* Portal vein clamping time · *ROI* Reactive oxygen intermediate · *SEC* Sinusoidal endothelial cell · *TNF* Tumour necrosis factor α · *UW* University of Wisconsin

## Introduction

In human liver transplantation, preservation injury remains a major problem and "initial liver poor-function" still occurs in 5%–15% of patients [12, 22]. The underlying mechanism of cold ischemia-reperfusion injury culminating in "primary liver non-function" is still poorly understood. An increasing number of studies have shown that sinusoidal endothelial cells (SECs) are the cellular targets of cold ischemia-reperfusion injury [3, 5, 16, 28, 32], and it has been suggested that their alteration, leading to microcirculatory disturbances, is the primary cause of storage-related graft failure [4, 28, 32]. Kupffer cells, the resident macrophages of the liver, are known to produce a variety of biologically toxic mediators such as proteases, reactive oxygen intermediates (ROIs) and, particularly, tumour necrosis factor-α (TNF) [1, 10, 30], which have been strongly implicated in the pathogenesis of hepatic injury in various animal models [24, 33, 39]. It is currently hypothesized that Kupffer cell activation plays a causal role in cold ischemia-reperfusion injury, precipitating SEC death and

leading to "primary graft non-function" [2, 41, 43]. Kupffer cell activation has also been implicated in leukocyte and platelet adhesion to altered sinusoids, leading to further disturbance of liver micro-circulation [6, 8, 27]. In addition, Kupffer cell activation has been implicated in the systemic inflammatory response observed after transplantation, particularly with respect to lung injury [15]. Indeed, experimental studies on warm intestinal ischemia-reperfusion and orthotopic rat liver transplantation (ORLT) have indicated that gut-derived endotoxin and TNF secreted by activated Kupffer cells, may contribute to the pathogenesis of lung and graft complications observed after warm as well as cold ischemia-reperfusion of the liver [7, 15].

However, at odds with the current hypothesis, recent data using the ORLT model following extended cold ischemia-reperfusion showed that the severity of SEC dysfunction did not correlate with graft survival [47]. Moreover, our group [21] and others [36] have reported that the presence or absence of Kupffer cells did not modify the effect of 24-h cold ischemia-reperfusion on SEC and hepatocyte function in the isolated perfused rat liver (IPRL) model, neither on survival following ORLT [21]. The current hypothesis is based on experimental findings using mainly the ORLT model, first described by Lee et al. [25] and subsequently modified by Kamada and Calne [23]. Indeed, the ORLT model has received wide acceptance for the study of liver transplantation immunology and cold ischemia-reperfusion injury, particularly in evaluating the effects of various solutions and different biochemical strategies aimed at protecting the transplanted liver. A large number of these studies used a cold (4 °C) UW solution as the preservation solution and 24 h as the preservation time, conditions considered to be severely compromising for the grafted rat liver. Under these preservation conditions, reported survival rates varied widely from centre to centre, ranging from 0–100 % [7, 35, 37, 40, 41, 44]. This was attributed to differences in surgical skills and led to conflicting results and confusion in the overall understanding of the pathophysiological mechanisms involved and potential therapeutic interventions.

In recent evaluations of the ORLT model after 24-h cold preservation in the UW solution [42], postoperative survival was mainly dependent on the portal vein clamping time (PVCT) during recipient surgery: 100 % survival was observed in rats operated with short PVCTs (less than 14 min), while only 20 % survived with long PVCTs (18–21 min). Under PVCTs longer than 18 min, animals died from an endotoxin-like shock, secondary to prolonged warm intestinal ischemia. Kupffer cell activation with overproduction of TNF was proposed as the cause of this syndrome, secondary to splanchnic endotoxin accumulation and release during intestinal congestion-reperfusion [42]. However, the direct role of KCs was not addressed to in that specific experimental conditions. Interestingly, in previous studies where neither KC-elimination nor nisoldipine improved animal survival, ORLT were performed using PVCTs shorter than 18 min [9, 21]. Whereas, in studies reporting a beneficial effect of nisoldipine [41] as well of other treatments aimed at decreasing Kupffer cell activation [13, 26], ORLTs were usually performed with PVCTs longer than 17 min.

We thus investigated the direct role of Kupffer cells in the context of ORLT with long PVCTs by eliminating them (using liposome-encapsulated dichloromethylene diphosphonate, MDP), by preventing their activation (using a calcium channel blocker, nisoldipine) and by inhibiting TNF production (using thalidomide).

## Materials and methods

Inbred male Lewis rats (Charles River, Canada) were purchased to exclude immunological interference. Rats weighing 275–300 g were used as liver donors, and rats weighing 300–325 g as recipients at transplantation. Animals were housed in a controlled environment with a 12 h light/dark cycle. Donor rats had access to water only 12 h before organ harvesting. In experiment 1 and 2, recipient rats had free access to normal rat chow until surgery. The experiments described in this report were conducted according to the Guide for the Care and Use of Laboratory Animals.

### Experiment 1: role of KCs in ORLT with long PVCTs

Rats were randomly assigned to liposome-encapsulated MDP ($n = 21$) or control ($n = 21$) groups and underwent transplantation after 24-h cold preservation in the UW solution (4 °C).

#### *KC elimination*

Liposome-encapsulated MDP was prepared using van Rooijen's technique [21, 46]. MDP was a courtesy of Boehringer Mannheim (Laval, Quebec, Canada). For control animals, empty liposomes were prepared in the same manner as described above but without MDP. Liposomes were resuspended in 4 ml PBS, and 2 ml were injected intravenously 40–42 h before liver harvesting. Using this technique, KCs usually disappear completely from MDP-treated rat livers within 24 h [21, 46].

#### *Surgical procedures*

ORLT was performed according to Kamada's cuff-technique [21, 23] under halothane and nitrous oxide anaesthesia. Briefly, before liver harvesting, 300 units of heparin were injected via the penile vein, and the donor liver was perfused directly in situ via the portal vein with 10 ml of cold (4 °C) UW solution (DuPont Merck Pharma, Mississauga, Ontario, Canada). The excised liver was placed in a bath of the cold preservation solution and was perfused with another 10 ml of cold UW solution through the portal vein. Following the cuff installation, livers were stored in the UW solution at 4 °C for 24 or 48 h. At the end of the storage period, livers were flushed with 10 ml of cold (4 °C) Ringer's lactate solution and transplanted into recipient animals with predetermined PVCTs.

The hepatic artery was not reconstructed since, as previously reported, its reconstruction does not modify changes in the hepatic microcirculation induced by ORLT on survival [18]. After surgery, the rat received 2 ml of 5% dextrose through the penile vein and 150 mg/kg ampicillin given intramuscularly. No further treatment was given. Animals had free access to normal rat chow and water after surgery.

For the survival study, twelve rats in each control and MDP-treated groups underwent ORLT and were observed daily for ten days. Results were expressed by comparing the ten-day survival rate for each group. The PVCT was timed between 18–21 min. These long PVCTs were chosen in order to be similar to the ones associated with the poor outcome after ORLT in our previous studies evaluating the importance of PVCTs on survival [42]. In nine other rats from each group, an indwelling intravenous catheter was placed in the infrahepatic inferior vena cava at the end of surgery. Two hours after surgery, blood samples (0.3 ml) were drawn from the catheter, followed by injection of 0.3 ml lactate Ringer's solution, and were kept for TNF determination. Nine hours after surgery, animals were anaesthetized with halothane to measure bile production for 20 min according to a technique previously described [19, 20]. Then, following blood sampling (for AST and LDH determination), animals were killed, and livers were flushed with 60 ml of oxygenated warm (37 °C) Krebs-Henseleit buffer (20 ml/min) through the portal vein. Lungs were also prepared by flushing the heart with 40 ml of the same buffer. The blocks of tissues from the right median lobe of the liver and the inferior lobe of the left lung were routinely sampled, fixed in 10% formalin and stained with hematoxylin/eosin for pathological evaluation. The graft viability was evaluated measuring bile production, plasma AST and LDH at 9 h after reperfusion.

Experiment 2: effect of nisoldipine on ORLT with long PVCTs

At the time of surgery, animals were randomly assigned to one of two experimental groups: a group ($n = 43$) with livers preserved for 24 h in a cold UW solution alone (control group) or a second group ($n = 43$) in which livers were perfused and preserved in a cold UW solution containing 1.4 μM nisoldipine, as proposed by Takei et al. [41], for 24 h (Nisoldipine group). In a first set of experiments used for survival study, each group was further divided into two subgroups according to the length of PVCT: an intermediate-PVCT group, where rats underwent transplantation with PVCTs between 15 and 17 min ($n = 16$ each); and a long-PVCT group, where livers were implanted with PVCTs between 18 and 21 min ($n = 16$ each). These PVCTs were chosen in order to be similar to the ones reported in previous studies evaluating the effect of nisoldipine on survival after ORLT and reporting conflicting results [13, 41, 44]. In eleven other rats operated with long-PVCTs, bile production, AST and LDH levels, as well as liver and lung biopsies, were obtained at killing time, 9 h after surgery, as described above.

Experiment 3: effect of thalidomide on ORLT with long PVCTs

Recipient animals were randomly assigned to thalidomide-treated ($n = 12$) or control ($n = 12$) groups. In both groups, recipient rats were fasted for 12 h before surgery, to ensure good absorption of thalidomide [11]. Thalidomide (100 mg/kg) (Research Biochemicals International, Natic, MA), prepared in vegetable oil (0.3mL), was given via gavage to recipient animals 4 h prior to expected reperfusion time, since peak concentrations after a single oral dose usually occur within 4 h and are maintained at the same levels for 12 h [11]. In these animals, the inhibitory effect of thalidomide on TNF synthesis was thus present at the time of ORLT, during the warm intestinal ischemia. Control animals received 0.3 ml vegetable oil alone. After 24-h cold preservation in the UW solution (4 °C), the ORLT was performed with long PVCTs (from 18–21 min), as described in experiments 1 and 2. These animals were only used for a survival study over ten days.

*TNF assay*

Plasma TNF was measured using commercially available rat TNF enzyme-linked immunosorbent assay kits (Factor-test X, Genzyme, Cambridge, MA). The detection limit of the assay was 10 pg/ml. In our laboratory, plasma TNF value in normal male Lewis rat was 14.7 ± 1.0 pg/ml (mean ± SEM, $n = 8$).

*Histology*

Liver- and lung biopsies were scored semiquantitatively according to Peng et al [34]. Briefly, coded liver biopsies were scored for periportal and pericentral necrosis on a 0–4 + scale on which 0 represented no necrosis and 1 +, 2 +, 3 +, and 4 + represented an involvement of about one quarter, one half, three quarters, and virtually all periportal and pericentral regions, respectively. Coded lung biopsies were also scored on a 0–4 + scale for interstitial infiltration of leukocytes, on which 0 was normal (no infiltration), 1 + was moderately increased interstitial cellularity without interstitial thickening, 2 + was increased cellularity with moderate interstitial thickening, 3 + was marked cellularity and thickening, and 4 + was interstitial infiltration and thickening with atelectasis. Ten randomly selected high-power fields were examined from each animal, and the group mean was calculated by averaging the mean score from each animal.

*Statistical analysis*

10-day survival rate was analysed using Fisher's exact probability test, and the survival curve was calculated according to the Kaplan-Meier method. For other analysis, when two or more groups were compared using unpaired $t$ tests, i.e. Student's $t$ test or $t$ test without assuming homogeneity of variances (separate-variances $t$ test), a Bonferroni correction was used to reduce the $\alpha$ level [14]. A $P$ value of $< 0.05$ was considered statistically significant. Results are expressed as mean ± SEM.

## Results

There were no differences between experimental- and control groups in terms of body weight ratios between donor and recipient, cold ischemia times, PVCTs, and recipient operation times ($P$ = NS, Table 1).

### Survival studies

Table 2 give the survival rate for the three experiments. Following 24-h cold preservation in a UW solution, 11 of 12 rats (91.7%) receiving MDP-treated livers survived for more than 10 days, while 6 of 12 control rats

**Table 1** Operative characteristics of different groups studied. Values are expressed as means ± SEM

| Group | No. | Donor-recipient weight ratio | Cold ischemia (h) | PVCT (min) | Operation time (min) |
|---|---|---|---|---|---|
| Experiment 1 | | | | | |
| MDP-treated | 21 | 0.92 ± 0.01 | 24.3 ± 0.03 | 19.3 ± 0.1 | 75.6 ± 0.9 |
| Control | 21 | 0.93 ± 0.01 | 24.3 ± 0.03 | 19.3 ± 0.1 | 75.2 ± 0.9 |
| Experiment 2 | | | | | |
| Intermediate PVCT | | | | | |
| Nisoldipine | 16 | 0.94 ± 0.01 | 24.3 ± 0.01 | 16.1 ± 0.2 | 83.9 ± 1.5 |
| Control | 16 | 0.93 ± 0.01 | 24.3 ± 0.01 | 16.1 ± 0.2 | 84.1 ± 1.1 |
| Long PVCT | | | | | |
| Nisoldipine | 25 | 0.94 ± 0.01 | 24.2 ± 0.03 | 19.2 ± 0.2 | 80.3 ± 1.7 |
| Control | 26 | 0.94 ± 0.01 | 24.2 ± 0.03 | 19.2 ± 0.2 | 79.9 ± 1.6 |
| Experiment 3 | | | | | |
| Thalidomide | 12 | 0.94 ± 0.01 | 24.1 ± 0.06 | 20.7 ± 0.1 | 82.7 ± 1.7 |
| Control | 12 | 0.94 ± 0.01 | 24.1 ± 0.06 | 20.7 ± 0.1 | 82.7 ± 1.4 |

**Table 2** Liver viability after ORLT with long PVCTs. Values are expressed as means ± SEM

| Group | No. | AST[a] (IU/l) | LDH[b] (IU/l) | Bile production[a] (µl/min) |
|---|---|---|---|---|
| Experiment 1 | | | | |
| MDP-treated | 9 | 4478 ± 426 | 19533 ± 2387 | 9.43 ± 1.21[b] |
| Control | 7 | 3836 ± 648 | 21014 ± 4823 | 5.29 ± 1.30 |
| Experiment 2 | | | | |
| Nisoldipine | 9 | 2156 ± 455 | 4433 ± 2182 | 9.43 ± 1.21[b] |
| Control | 7 | 3300 ± 581 | 11550 ± 4016 | 5.29 ± 1.30 |

[a] Nine h after surgery, blood was collected and bile production was measured for 20 min
[b] $P < 0.05$ vs. control group

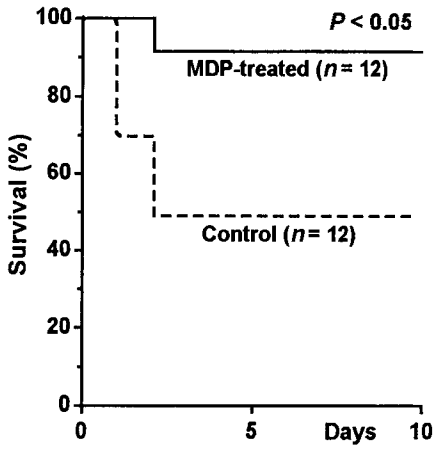


**Fig. 1** Effect of Kupffer cell elimination on transplant survival after extended cold ischemia. Donor Kupffer cells were eliminated prior to liver harvesting as described in *Materials and methods*. Livers from control or MDP-treated rats were flushed out with the UW solution (4 °C) and stored for 24 h, then orthotopically transplanted without rearterialization. Survival curves were calculated using Kaplan-Meier's methods. The PVCTs were controlled between 18 to 21 min for 24-h ischemic livers, and less than 14 min for 48-h ischemic livers. Between the experimental groups, ten-day survival rates differed significantly after 24-h storage ($P < 0.05$)

(50.0 %) survived after ORLT with long PVCTs; the ten-day survival rate was significantly better for the MDP-treated group than that of the control group ($p < 0.05$, Figure 1).

In experiment 2, the survival was identical for animals receiving livers preserved with or without nisoldipine when using intermediate PVCTs: 10 of 16 animals (62.5 %) survived in both groups over ten days ($P$ = NS, Figure 2A). However, when operated with long-PVCTs, 10 of 16 rats (62.5 %) receiving nisoldipine-treated livers survived more than 10 days, while only 4 of 16 animals (25.0 %) survived in the control group ($P < 0.05$, Figure 2B).

Finally, 9 of 12 thalidomide-pretreated animals (75.0 %) survived over 10 days, while only 3 of 12 control animals survived when ORLT was performed with long PVCTs; the survival rate was significantly different between the two groups ($P < 0.05$, Figure 3).

Although all animals recovered from the anaesthesia within 15 min after surgery, their clinical status deteriorated within 2–4 h after surgery. All non-survivors died within 48 h after transplantation, while survivors progressively improved during the same periods. Postmortem examination of non-survivors showed 2–3 ml ascites without frank blood and 1–2 ml serous pleural effusion. No thrombus in anastomosed vessels was observed.

## Plasma TNF levels after ORLT with or witout Kupffer cells

In experiment 1, a single sampling 2 h after vascularization was chosen, since the maximum TNF values were

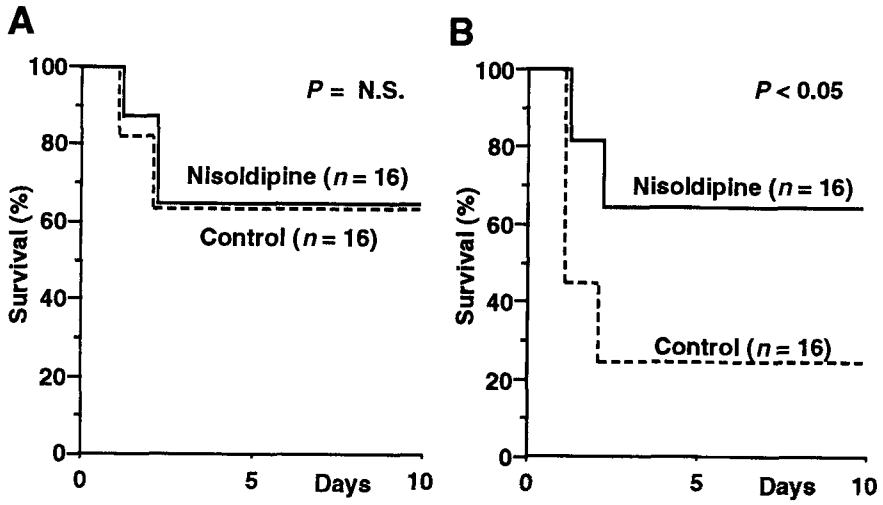


**Fig. 2 A, B** Effect of nisoldipine on transplant survival after 24-h cold ischemia. Rat livers were preserved with or without nisoldipine (1.4 μM) in the UW solution (4 °C) for 24 h. The livers were orthotopically transplanted with short PVCTs (15–17 min) (**A**), or long PVCTs (18–21 min) (**B**). Survival curves were calculated using Kaplan-Meier's methods. Between the experimental groups, ten-day survival rates differed significantly with long PVCTs ($P < 0.05$), but not with short PVCTs ($P$ = NS)

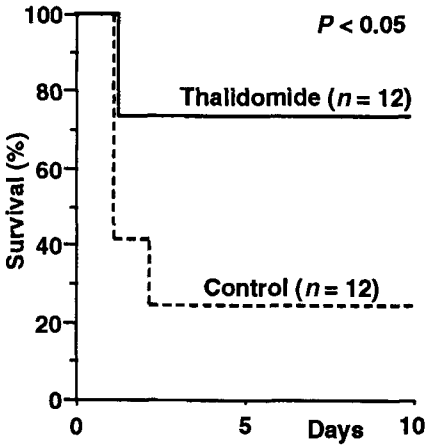


**Fig. 3** Effect of thalidomide on transplant survival after 24-h cold ischemia. Rat livers were preserved in the UW solution (4 °C) for 24 h. Recipient rats were pretreated with either thalidomide (100 mg/kg) or vehicle alone by gavage 4 h prior to reperfusion. Livers were orthotopically transplanted with long PVCTs (18–21 min). Survival curves were calculated using Kaplan-Meier's methods. Ten-day survival rates differed significantly ($P < 0.05$) between the experimental groups

obtained between 2 and 4 h after reperfusion in our previous study [9]. KC-elimination of donor liver significantly decreased plasma TNF levels 2 h after revascularization: 174.4 ± 53.8 pg/ml vs 53.8 ± 4.6 pg/ml ($P < 0.05$).

## Liver viability parameters after ORLT

No significant difference was found in plasma AST and LDH levels between control and MDP-treated groups nor between control and nisoldipine groups ($P$ = NS, Table 2). However, bile production significantly increased in MDP-treated ($P < 0.05$ ) and nisoldipine treated groups ($P < 0.05$) compared to their controls (Table 2). In these studies, two rats from experiment 1, and three animals from experiment 2 were excluded because they died before measuring bile production and blood-tissue sampling.

**Table 3** Liver and lung injury after ORLT with long PVCTs. Lungs and livers were fixed for histological examination 9 h after surgery. Liver necrosis and pulmonary leukocyte infiltration were scored on a 0–4 + scale as described in *Materials and methods*. Values are expressed as means ± SEM

| Group | No. | Liver | | Lung |
|---|---|---|---|---|
| | | Periportal | Pericentral | |
| Experiment 1 | | | | |
| MDP-treated | 9 | 1.1 ± 0.1 | 1.9 ± 0.2[c] | 1.8 ± 0.2[a] |
| Control | 7 | 1.2 ± 0.2 | 2.1 ± 0.2[c] | 2.8 ± 0.3 |
| Experiment 2 | | | | |
| Nisoldipine | 9 | 1.3 ± 0.3 | 2.1 ± 0.3 | 2.2 ± 0.2[b] |
| Control | 7 | 1.4 ± 0.2 | 2.4 ± 0.3[c] | 3.3 ± 0.2 |

[a] $P < 0.025$, [b] $P < 0.01$ vs. control group
[c] $P < 0.01$ vs. periportal areas

## Histology after 24-h ischemia-reperfusion

Hepatic necrosis was more conspicuous in pericentral than in periportal regions (Table 3). However, no significant difference was found in hepatic periportal and pericentral necrosis between control and MDP-treated groups, nor between control and nisoldipine-treated groups ($P$ = NS, Table 3).

Furthermore, pulmonary changes occurred within 9 h after surgery, characterized by accumulation of neu-

trophils and mononuclear cells in the alveolar septae, leading to septal thickening. These changes were similar to those reported previously after hepatic warm ischemia-reperfusion [17, 26] and after ORLT [39]. Both MDP and nisoldipine treatments decreased these pulmonary changes. When interstitial infiltration and thickening were scored on a 0 to 4 + scale, lung injury was significantly reduced by 35% and 33% with MDP ($P < 0.05$) and nisoldipine treatment ($P < 0.01$) respectively (Table 3).

## Discussion

The present study clearly shows that KC elimination from grafted liver definitely improved survival after ORLT following 24 h cold preservation in the UW solution, when surgery was performed with long PVCTs. However, this improvement was not associated with a decreased liver injury, since neither necrosis nor enzyme release were improved in rats receiving KC-eliminated livers, compared to control animals. Moreover, the liver injury observed with long PVCTs did not appear to be critical *per se*, since 92% of rats receiving KC-eliminated livers survived over ten days after transplantation, a survival rate comparable to the one observed, using the same preservation conditions, when rats were operated with PVCTs shorter than 14 min (100%) [42]. In a previous study, using intermediate PVCTs around 16 min, we also reported that the presence or absence of KCs did not modify survival after ORLT when using the same preservation conditions [21]. We have reported that the deleterious effects of long PVCTs on clinical outcome were associated with an overproduction of TNF and proposed that KCs were responsible for this overproduction following their activation by splanchnic endotoxin accumulation and its release during intestinal congestion-reperfusion [45]. The present study strongly supports this hypothesis by linking the beneficial effect of KC elimination with the splanchnic congestion during long PVCTs. In addition, the present study shows that elimination of KCs also improved one of the systemic manifestations of prolonged warm intestinal congestion, i.e. lung injury, also observed during endotoxin-like shock syndrome [8, 15].

Nisoldipine, which blocks the L-type high-voltage-activated calcium channels, has been reported to have a protective effect on survival after ORLT with prolonged cold preservation [41, 42], most probably by preventing Kupffer cell activation. However conflicting data have been reported on the protective effect of nisoldipine [9, 44]. Takei et al [41] first reported that storage of livers in nisoldipine-containing solution diminished KC phagocyte function and lung injury after ORLT and improved the post-operative survival. The PVCTs used in their experimental model was long, ranging from 17–25 min. In the two studies where a beneficial effect of nisoldipine was not reported, PVCTs were in the intermediate range (about 16 min) [9, 44]. However, in these studies, the number of animals was not well identified or too small [9, 41, 44]. In the present study including a large number of animals, we used two different ranges of PVCTs as used in previous conflicting studies: PVCTs between 15–17 min (intermediate PVCT group), or between 18–21 min (long PVCT group). Nisoldipine definitely improved animal survival, but only in rats operated with long PVCTs. However, as found with KC depletion, no decreases in hepatic enzyme release or in liver necrosis were found, compared to controls. Again, nisoldipine markedly diminished lung injury 9 h after surgery with long PVCTs. Therefore, improved survival with nisoldipine after ORLT with long PVCTs, may well be due to the prevention of KC activation normally observed with prolonged warm intestinal ischemia. Unfortunately, plasma levels of TNF were not measured in these experiments. This effect is probably not due to the direct protection of livers from the cold ischemia-reperfusion itself as from KC depletion. Indeed, in the *ex vivo* isolated perfused rat liver, nisoldipine did not have a protective effect on SEC and hepatocyte function following 24-h cold preservation-reperfusion [9].

The beneficial effects of KC depletion and nisoldipine on bile secretion are more difficult to interpret if no improvement of other liver viability parameters were found. However hemodynamic changes secondary to the endotoxin-like syndrome may well be responsible for the alteration of bile secretion, changes that should be partially prevented by both treatments.

To further explore the role of TNF in survival after ORLT with long PVCTs, we examined the effect of thalidomide. Thalidomide, a derivative of glutamic acid, selectively inhibits TNF production by stimulated human peripheral mononuclear cells [38]. Its unique inhibitory action on TNF is exerted by enhancing messenger RNA degradation. This inhibition is selective, and other cytokines are unaffected [31]. Again as found with KC depletion and nisoldipine, pretreatment of recipient animals with thalidomide significantly improved the survival rate of rats operated with long PVCTs. The negative role of endogenous TNF was further supported in a recent study where plasma TNF levels were significantly higher in non-survivors than survivors after ORLT with long PVCTs [42].

In conclusion, the present data clearly show the direct responsibility of KCs in the endotoxin-like shock encountered using the ORLT model when surgery is performed with a prolonged warm intestinal ischemia. With long PVCTs, excessive stimulation of Kcs occurs with an overproduction of TNF, and probably also nitric oxide and reactive oxygen radicals which lead to lethal multiorgan failure, as found in endotoxemia, with hy-

potension and reduction of regional blood flows. In such conditions, liver dysfunction is a secondary phenomenon and probably not the main cause of high mortality after ORLT. Indeed, in our laboratory, a marked increase in plasma nitric oxide levels was found 12 h after transplantation in rats, but only in rats operated with long PVCTs following 24 hour cold preservation in the UW solution [42].

Therefore, the effect of treatment aimed at improving survival after ORLT and extended cold ischemia in UW solution, may well be due to its action on the endotoxin-Kupffer cell relationship activated by prolonged warm intestinal ischemia, and not to hepato-protection from the cold ischemia-reperfusion injury itself. The present findings may also be important in human liver transplantation, particularly in patients with fulminant hepatic failure when operated on before the development of spontaneous portosystemic collaterals, decompressing the splanchnic venous return during portal vein clamping. In these clinical conditions, the occurrence of pulmonary insufficiency and/or multiorgan failure might well depend on the duration of portal vein clamping at the time of transplantation, particularly when a portal venous bypass is not performed, as is the case in most North-American centres.

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