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Results of the TOP Study: Prospectively Randomized Multicenter Trial of an Ex Vivo Tacrolimus Rinse Before Transplantation in EDC Livers

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Background. Organ shortage results in the transplantation of extended donor criteria (EDC) livers which is associated with increased ischemia-reperfusion injury (IRI). Experimental studies indicate that an organ rinse with the calcineurin inhibitor tacrolimus before implantation protects against IRI. The tacrolimus organ perfusion study was initiated to examine the effects of ex vivo tacrolimus perfusion on IRI in transplantation of EDC livers. **Methods.** A prospective randomized multicenter trial comparing ex vivo perfusion of marginal liver grafts (≥ 2 EDC according to Eurotransplant manual) with tacrolimus (20 ng/mL) or histidine-tryptophane-ketoglutarate solution (control) was carried out at 5 German liver transplant centers (Munich Ludwig-Maximilians University, Berlin, Heidelberg, Mainz, Regensburg) between October 2011 and July 2013. Primary endpoint was the maximum alanine transaminase (ALT) level within 48 hours after transplantation. Secondary endpoints were aspartate transaminase (AST), prothrombine ratio, and graft-patient survival within an observation period of 1 week. After an interim analysis, the study was terminated by the scientific committee after the treatment of 24 patients (tacrolimus $n = 11$, Control $n = 13$). **Results.** Tacrolimus rinse did not reduce postoperative ALT peaks compared with control ($P = 0.207$; tacrolimus: median, 812; range, 362-3403 vs control: median, 652; range, 147-2034). Moreover, ALT ($P = 0.100$), prothrombine ratio ($P = 0.553$), and bilirubin ($P = 0.815$) did not differ between the groups. AST was higher in patients treated with tacrolimus ($P = 0.011$). Survival was comparable in both groups ($P > 0.05$). **Conclusions.** Contrary to experimental findings, tacrolimus rinse failed to improve the primary endpoint of the study (ALT). Because 1 secondary endpoint (AST) was even higher in the intervention group, the study was terminated prematurely. Thus, tacrolimus rinse cannot be recommended in transplantation of EDC livers.

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The organ shortage represents one of the biggest challenges in transplantation medicine today and has resulted in an increasing acceptance of extended donor criteria (EDC) grafts

for liver transplantation.¹ In this respect, an increased ischemia-reperfusion injury (IRI) is associated with a diminished organ survival in these grafts.²

Thus, novel strategies have to be developed to attenuate IRI and improve the function and survival of EDC organs.

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Several models of experimental hepatic ischemia-reperfusion have revealed protective effects of tacrolimus preconditioning.^{3,4} The authors recently demonstrated a reduction of IRI after an ex vivo tacrolimus rinse in a model of experimental liver transplantation in rats.⁵ In the clinical arena, controversial data have been published on the effectiveness of a tacrolimus rinse before liver transplantation^{6,7}: within a phase 1 trial, St Peter et al⁶ showed a significant reduction of aminotransferase levels in the transplantation of non-EDC livers after a tacrolimus rinse. In contrast to these findings, Kristo et al⁷ recently failed to show a reduction of alanine transaminase (ALT) levels through such a treatment after transplantation of nonmarginal livers. Nevertheless, the authors demonstrated a distinct reduction of precursors of proinflammatory enzymes on RNA level after a tacrolimus rinse in those patients. However, as both of these studies investigated the transplantation of nonmarginal organs, their relevance to the setting of EDC organ transplantation is limited.

Therefore, the present prospectively randomized multicenter trial (tacrolimus organ perfusion [TOP] study; Trial register: EUDRA CT number: 2010-021333-31, ClinicalTrials

ID: NCT 01564095) included exclusively marginal livers with 2 or more EDC according to Eurotransplant's guidelines for marginal liver grafts. The aim of the TOP study was to determine whether an ex vivo rinse of such organs with tacrolimus results in a decreased IRI, thereby improving liver function and organ survival. The study outline has been published previously.⁸

MATERIALS AND METHODS

Study Design

The TOP study was designed as an investigator-initiated, prospectively randomized, multicenter trial according to German Medicines Act (Section 42b, Abs. 1 Arzneimittelgesetz German Pharmaceuticals Act). Patients were randomized into 2 groups within this placebo-controlled, nonblinded study:

- Ex vivo perfusion of marginal liver grafts with tacrolimus (20 ng/mL) solved in 1000 mL histidine-tryptophane-ketoglutarate (HTK) (tacrolimus + HTK)
- Ex vivo perfusion of marginal liver grafts with 1000 mL HTK (HTK-alone)

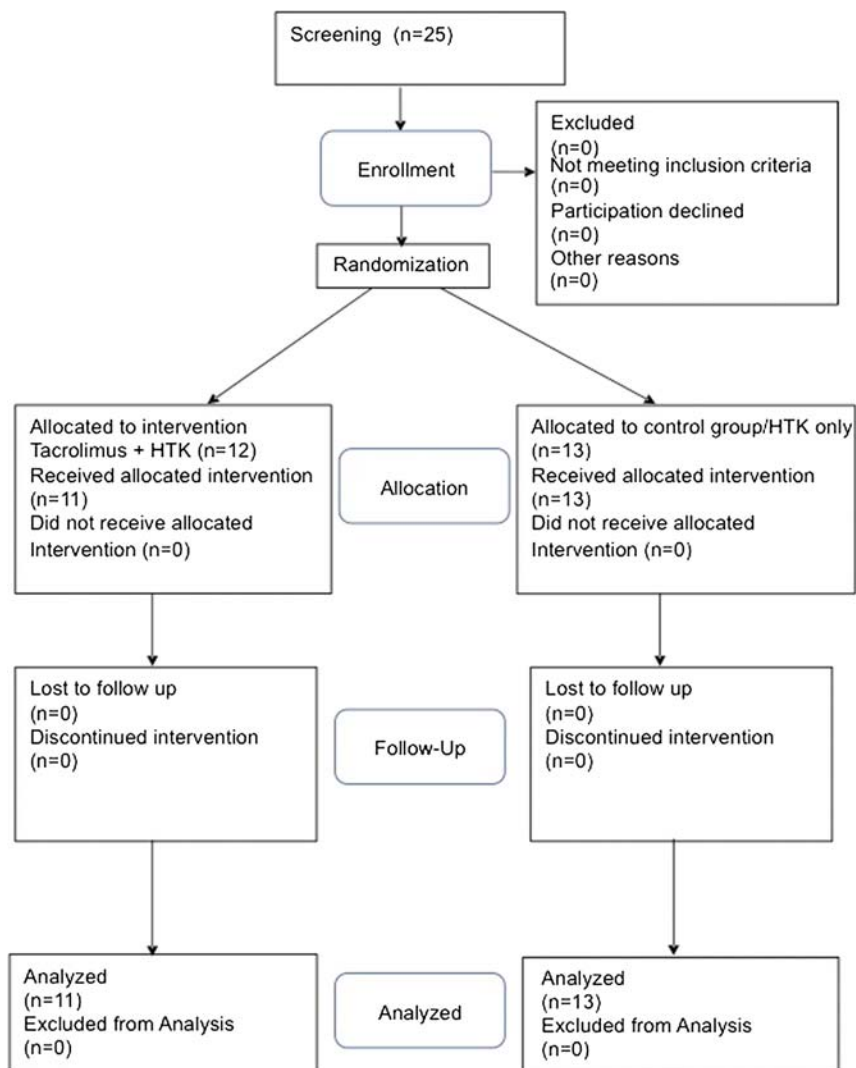


FIGURE 1. CONSORT flow diagram TOP study.

The observation period was 1 week. The inclusion period for this trial was between October 2011 and July 2013. Besides Munich Ludwig-Maximilians University as the initiating center, 4 German transplant departments participated in this trial: Berlin-Charité, Heidelberg, Mainz, and Regensburg.

Twenty-five patients were enrolled in the study from October 31, 2011 (first patient in) until July 9, 2013 (last patient out). One patient was included and randomized but not transplanted due to medical reasons. Eleven patients were treated with tacrolimus + HTK and 13 with HTK alone (Figure 1, CONSORT flow diagram). The study was terminated by the scientific committee in July 2013 after an interim analysis due to missing evidence of the effectiveness of the study medication (20-ng/mL tacrolimus in 1000-mL HTK) relative to the comparison group (1000-mL HTK) concerning the primary endpoint of the study, the maximum measured ALT values on the first 2 postoperative days. Moreover, potentially even harmful action was evident when analyzing another secondary endpoint of the study, postoperative aspartate transaminase (AST).

Protocol version 2.1 was approved by the local ethic committees of the ethics committee of the University of Munich. The study complied with the Declaration of Helsinki and Good Clinical Practice Guidelines. Informed consent was obtained from each patient in written form before randomization.

Inclusion and Exclusion Criteria

Recipients with end-stage chronic liver disease older than 18 years receiving their first organ transplant were evaluated for inclusion in this trial. Only patients receiving livers with 2 or more EDC following the definition of EDC by Eurotransplant⁹ (Table 1) were finally included in this trial. All recipient and donor inclusion and exclusion criteria are outlined in Table 1.

Surgical Procedure/Perfusion Procedure

Tacrolimus was dissolved in 1000-mL HTK (concentration, 20 ng/mL) in the treatment group; in the control group,

the rinse solution consisted of 1000-mL HTK-only. The rinse was administered sequentially to the portal vein and the common hepatic artery (500 mL each) at the end of back-table preparation via a 12-gauge cannula from a height of 100 cm without additional pressure using polyvinyl chloride-free infusion sets (Braun Melsungen AG, Germany). The duration of the perfusion procedure was 16 minutes (median) versus 18.1 ± 7.3 minutes (mean \pm SD) and was similar between the groups ($P > 0.05$). Cava sparing liver transplantation was performed afterward. Immunosuppression and postoperative care were carried out according to center-specific standards.

Primary and Secondary Endpoints

The primary endpoint was the maximum ALT level (U/L) within the first 48 hours after liver transplantation.

Secondary endpoints were ALT and AST levels (U/L) and graft function (prothrombine ratio/quick (%), bilirubin (mg/dL) on postoperative days 1, 2, 4, and 7. Within the follow-up, organ and patient survival was monitored.

Donor and Recipient Characteristics

The following data were collected for each donor and recipient, respectively: height, bodyweight, body mass index (BMI)/graft steatosis, age and diagnosis. In addition, the duration of intensive care, cold ischemia time, donor risk index (DRI) (according to Feng et al¹⁰) and the number and type of EDC were captured (Tables 2 and 3).

Recipients' diagnoses were classified as follows: alcoholic cirrhosis, malignancy, viral hepatitis, primary biliary cirrhosis, and others. Based on the preoperative serum creatinine, bilirubin, and international normalized ratio levels, laboratory model for end-stage liver disease (MELD) scores were calculated as described previously.¹¹

Statistic Evaluation/Sample Size Calculation

The sample size estimation was based on previous findings published by St Peter et al⁶ in which nonmarginal grafts had

TABLE 1.

Inclusion/Exclusion criteria TOP study

Inclusion criteria	
Recipient	Donor
Chronic end-stage liver disease, age > 18 years, first organ transplant	<ul style="list-style-type: none"> • Donor age >65 years • Macrovesicular steatosis >40% (secured macroscopically or by biopsy) • BMI >30 • Na >165 mmol/L • Intensive care and mechanical ventilation >7 d • Cold ischemia time >13 h • AST >99 U/L • ALT >105 U/L • Bilirubin >3 mg/dL (>51 μmol/L) • Application of epinephrine
Exclusion criteria	
Recipient	Donor
<ul style="list-style-type: none"> • Multiorgan transplantation • High urgency listing • Extrahepatic tumor diseases • Pregnancy • Denial or withdrawal of consent by the patient or his relatives • Accommodation in an institution due to governmental or judicial authorities • Missing knowledge of German language, no understanding of information not guaranteed 	Hepatitis B or hepatitis C infection

TABLE 2.

Donor characteristics/EDC (according to eurotransplant manual): portion of donors meeting inclusion criteria (n [%]) versus absolute number (median, mean \pm SD) for each tacrolimus + HTK and HTK-only

	Tacrolimus + HTK		HTK-only		P [CI] (tacrolimus + HTK vs HTK-only)		Overall Median (mean \pm SD)
	Portion of donors meeting inclusion criterion, n (%)	Absolute number, median; (mean \pm SD)	Portion of donors meeting inclusion criterion, n (%)	Absolute number, median, (mean \pm SD)	Portion (fisher exact test)	Absolute number (Mann-Whitney U test)	
Donor age, >65 y	6/11 (54.5%)	57 (56.7 \pm 21.3)	3/13 (27.3%)	52 (52.4 \pm 12.5)	0.41 (0.51-2.9)	0.43	55 (54.4 \pm 16.8)
Graft steatosis >40%	1/11 (9.1%)	n.a.	1/13 (7.7%)	n.a.	0.45 (0.0-32.5)	n.a.	n.a.
Serum sodium >165 mmol/L	1/11 (9.1%)	147 (148.4 \pm 9.1)	0/13 (0%)	144 (143.2 \pm 6.9)	0.46 (0-33)	0.32	147 (145.4 \pm 8.3)
ICU stay, >7 d	5/11 (45.5%)	7 (6.8 \pm 5.2)	2/13 (15.4%)	4 (7.2 \pm 8.7)	0.36 (0.024-3.08)	0.93	4 (7.0 \pm 6.9)
BMI >30	4/11 (36.4%)	26.5 (28.1 \pm 6.3)	6/13 (46.2%)	29.5 (29.6 \pm 4.9)	1 [0.16-7.97]	0.56	28 (22 \pm 5.7)
Cold ischemia time >13 h	1/11 (0.1%)	10.1 (10.6 \pm 2.8)	2/13 (15.4%)	9.1 (10.4 \pm 2.9)	1 (0.0098-71.84)	1.00	10.1 (10.5 \pm 2.9)
AST >89 U/L	5/11 (45.5%)	50 (100.4 \pm 65.1)	4/13 (30.8%)	40 (91.6 \pm 125.2)	1 (0.21-0.47)	0.28	50 (96.8 \pm 92.8)
ALT >105 U/L	3/11 (27.3%)	38 (83.3 \pm 88.1)	4/13 (30.8%)	47 (115.5 \pm 165)	1 (0.15-10.64)	1.00	40 (97.6 \pm 123.6)
Bilirubin >3 mg/dL	0/11 (0%)	0.77 (0.79 \pm 0.55)	0/13 (0%)	0.51 (0.57 \pm 0.296)	0.46 (0-33)	0.44	0.74 (0.69 \pm 0.44)
Adrenaline	9/11 (81.8%)	n.a.	10/13 (76.9%)	n.a.	0.59 (0.0058-5.22)	n.a.	n.a.

Portions of extended criteria are compared between groups using Fisher exact test, absolute numbers by using Mann-Whitney U test.

ICU, intensive care unit; n.a., not available.

been transplanted. An effect size of approximately 0.7 was considered appropriate for the sample size calculation presuming higher postoperative ALT levels and a more pronounced reduction in marginal liver grafts.⁸ The power of the test was 80% at a significance level of 0.05. Therefore, sample size estimation (nQuery Advisor 6.1; Statistical Solutions, Saugus, MA) for 2 unpaired samples using the Wilcoxon rank-sum test with an expected dropout rate of 15% resulted in an estimated sample size of 86 patients (43 tacrolimus + HTK vs 43 HTK-only). The randomization was performed in blocks of variable length, stratified according to the transplant centers. The Hodges-Lehman estimator was used to estimate effects on the primary endpoint (ALT). Secondary endpoints were calculated using the multivariate rank-sum test by O'Brien. The portion of EDC in both groups was compared by Fisher exact test, the absolute numbers of these parameters were analyzed using the Mann-Whitney U test. The statistical analysis was performed using statistical software Predictive Analysis Software statistics 18.0.0 (SPSS Inc., Chicago, IL).

For all statistical tests, a test wise α -level of 5% was used. P values less than 0.05 were considered statistically significant.

RESULTS

Donor Characteristics

The number and type of EDC as a basis for patient inclusion did not differ between the study groups. In this respect, the average number of EDC (which represent the inclusion criteria to the study) was 2.75 in both groups. The most prevalent EDC were high donor age older than 65 years ($n = 9/24$), an intensive care unit stay longer than 7 days ($n = 7/24$), obesity (BMI > 30) ($n = 10/24$), and elevated liver enzymes (AST > 105 U/L) ($n = 9/24$). The portion of donors which met these inclusion criteria was similar in both groups (Table 2). These factors also did not differ in absolute numbers (median vs mean \pm SD; Table 2).

The donor risk index was not different whether grafts had been treated with tacrolimus or control/HTK (tacrolimus: median, 1.9; mean \pm SD, 2.0 \pm 0.4; control: median, 1.8; mean \pm SD, 1.8 \pm 0.3) ($P = 0.35$). When analyzing the donors' cause of death, 10 donors had died of cerebral hypoxia and 14 of an intracerebral bleeding. These diagnoses had the same portion in both groups.

Recipient Characteristics

Recipient characteristics did not differ between the 2 study groups. Relevant prognostic factors were comparable in patients randomized to tacrolimus or control (ie, laboratory MELD scores, BMI, age; $P > 0.05$) (Table 3). Fifteen patients were transplanted due to malignant diseases (hepatocellular carcinoma, $n = 14$; cholangiocellular carcinoma, $n = 1$) and 2 for a cryptogenic liver cirrhosis. The remaining indications were classified as follows: hepatitis C ($n = 1$), autoimmune hepatitis ($n = 1$), PSC ($n = 1$), and α 1-AT deficiency ($n = 1$).

Primary Endpoint: Maximum ALT

Perfusion of marginal livers with tacrolimus resulted in no statistically significant effect on the maximum ALT values measured within the first 2 postoperative days after transplantation compared with HTK-only (tacrolimus + HTK:

TABLE 3.**Recipient characteristics: tacrolimus + HTK versus HTK-only is compared by using Mann-Whitney U test**

	Overall	Tacrolimus	Control	P (tacrolimus + HTK vs HTK-only)
Recipient age	60 (58.5 ± 7.7)	63 (59.8 ± 8.1)	55 (57.5 ± 6.5)	0.26
BMI	27.4 (27.2 ± 4.9)	25.3 (26.6 ± 4.7)	27.4 (27.7 ± 4.8)	0.52
Laboratory MELD	13 (14.3 ± 6.9)	13 (13.3 ± 4.9)	13 (15.2 ± 8.2)	0.69

median, 812 U/L; range, 362-3403 U/L vs HTK-only: median, 652 U/L; range, 147-2034 U/L) ($P = 0.207$). The Hodges-Lehman estimator for the 95% confidence interval for the median difference was (-178 to 1166).

On the first postoperative day, the maximum ALT was in median, 607 U/L (mean ± SD, 790.4 ± 714.4 U/L) in the tacrolimus group compared with 544 U/L (544.8 ± 326.9 U/L) in the control group, respectively ($P = 0.56$). On the second postoperative day, the maximum ALT levels were in median, 726 U/L (mean ± SD, 1010.5 ± 634.9 U/L) for tacrolimus versus median, 425 U/L (mean ± SD, 613.9 ± 439.8 U/L) for control ($P > 0.05$).

Secondary Endpoints

Alanine Transaminase

No statistically significant effect was evident when comparing tacrolimus + HTK versus HTK-only on postoperative days 1, 2, 4, and 7 ($P = 0.100$, multivariate rank sum test according to O'Brien). For example, the median ALT was 607 U/L (tacrolimus + HTK) versus 497 U/L (HTK-only) on day 1 and 726.5 U/L (tacrolimus + HTK) versus 400 U/L (HTK-only) on the second postoperative day (Figure 2).

Aspartate Transaminase

Patients whose grafts had been treated with tacrolimus showed increased systemic AST levels in the postoperative course. When comparing AST on postoperative days 1, 2, 4, 7, a P value of 0.011 was evident (multivariate rank sum test according to O'Brien). For illustration, on postoperative day 1, the maximum AST was 1196 U/L (median) in grafts treated with tacrolimus versus 802 U/L (control). On the second postoperative day, the median AST was 1030 U/L (tacrolimus) compared with control: 390 U/L (Figure 3).

Bilirubin

A tacrolimus rinse did not alter the postoperative bilirubin levels during the observation period of 1 week ($P = 0.815$). In grafts flushed with tacrolimus, the median bilirubin was 4 mg/dL versus 2.75 mg/dL (control) on the first postoperative day and 3.3 versus 3.05 mg/dL on the second postoperative day, respectively (Figure 4).

Prothrombin Ratio/Quick (%)

No statistically significant effect was evident when comparing tacrolimus and control on postoperative days 1, 2, 4, and 7 ($P = 0.553$). For example, the median of this parameter was 53.5 (tacrolimus) versus 57.5 (control) on postoperative day 1 and 55.5 versus 63 on postoperative day 2 (Figure 5).

Survival

Organ and patient survivals as well as the number of rejections were equal in both treatment arms ($p = 1.000$). No

study patient died or underwent retransplantation within the observation period of 7 days.

DISCUSSION

Extended donor criteria liver grafts exhibit an increased IRI during liver transplantation resulting in decreased graft function and survival.¹²⁻¹⁴ Due to the current shortage of organs from deceased donors and the declining number of liver transplantations in Germany (n = 1192 in 2010, n = 884 in 2013), these grafts are increasingly used for transplantation. In 2010, EDC organs made up more than 70% of all transplanted livers in Germany, and the proportion of liver grafts exhibiting 1 or more EDC increased from 29 % in 1997 to 73% in the year 2010 (data provided by Eurotransplant). Thus, new therapies have to be developed to reduce IRI in EDC organs.

The hypothesis of the TOP study was that a single ex vivo tacrolimus rinse reduces IRI in transplantation of marginal liver grafts. In this respect, experimental data demonstrate a protective role of the calcineurin inhibitor tacrolimus on the hepatic IRI after warm and cold ischemic periods.^{5,15} For the present trial, an ex vivo tacrolimus rinse in EDC liver grafts has been chosen as study medication. This dosage form is based on previous experimental⁵ and clinical^{6,7} data indicating protective effects of a single ex vivo tacrolimus treatment in the cold liver graft: St Peter et al⁶ described a significant reduction of postoperative aminotransferase levels after a tacrolimus rinse in transplantation of normal,

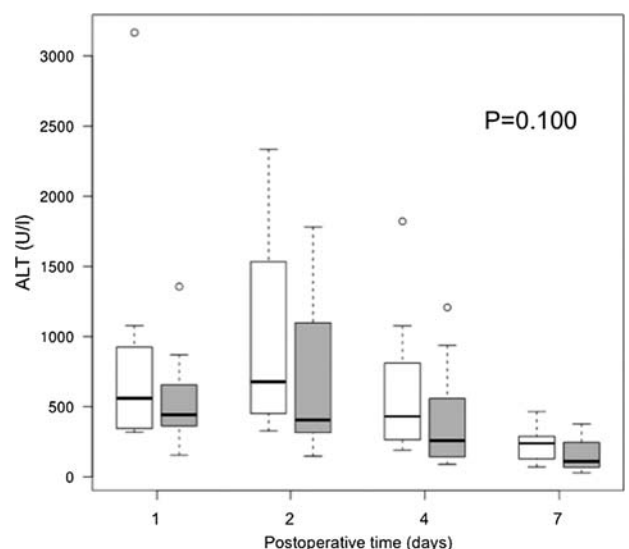


FIGURE 2. Serum ALT levels (U/L) on the first, second, fourth, and seventh postoperative days with respect to an ex vivo organ perfusion with tacrolimus (20 ng/ml) or with HTK-only. $P = 0.100$; multivariate rank sum test by O'Brien. Tacrolimus + HTK, white column; HTK-only, grey column.

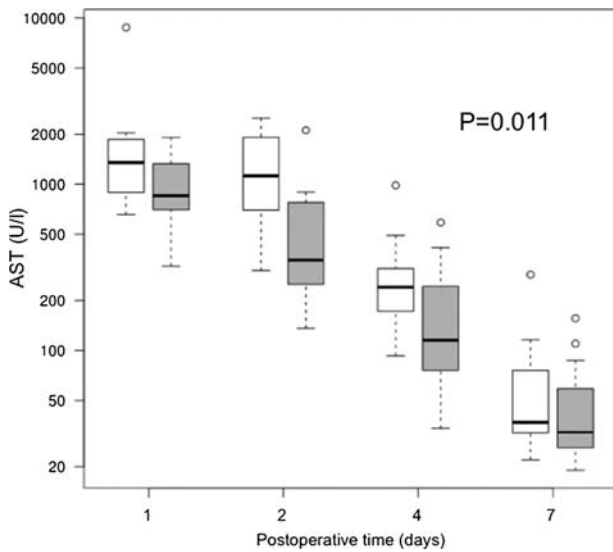


FIGURE 3. Serum AST levels (U/L) on the first, second, fourth and seventh postoperative days with respect to an ex vivo organ perfusion with tacrolimus (20 ng/ml) or with HTK-only. $P = 0.011$; multivariate rank sum test by O'Brien. Tacrolimus + HTK, white column; HTK-only, grey column.

nonmarginal liver grafts. The second trial by Kristo et al⁷ could not reproduce a clinical reduction of IRI but showed a decreased expression of inflammatory cytokines through such a treatment. In both studies, however, nonmarginal liver grafts were included. Thus, the results of these trials do not reflect the current clinical problem of organ shortage and marginal liver grafts.

With respect to the current study design it must be stated that the metabolism of the graft is certainly reduced during the ex vivo rinse treatment of the cold liver. As a calcineurin inhibitor, tacrolimus requires active T cells to develop its effect which are not present during the ex vivo perfusion. Nevertheless, tacrolimus may exert its therapeutic effects

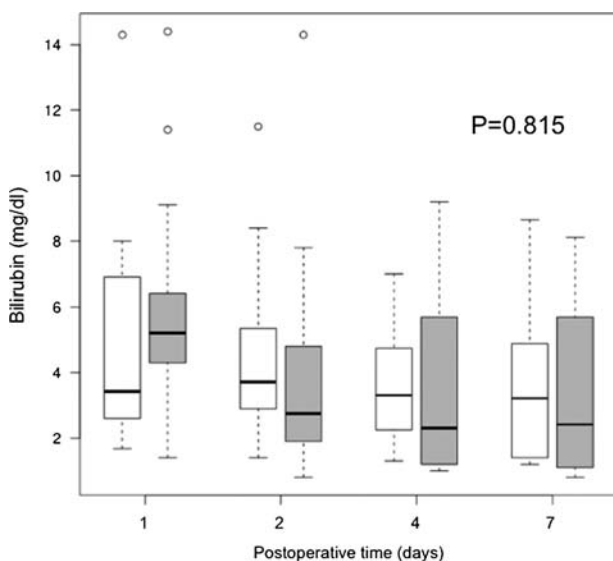


FIGURE 4. Serum bilirubin levels (mg/dL) on the first, second, fourth and seventh postoperative day with respect to an ex vivo organ perfusion with tacrolimus (20 ng/mL) or with HTK-only. $P = 0.815$; multivariate rank sum test by O'Brien. Tacrolimus + HTK, white column; HTK-only, grey column.

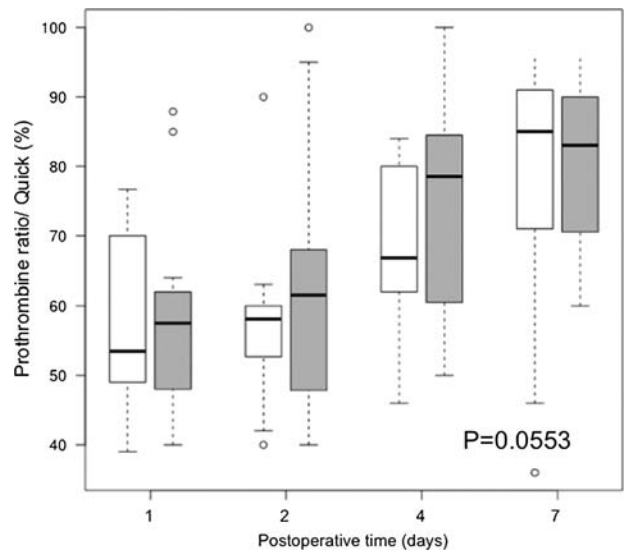


FIGURE 5. Serum ALT levels (U/L) on the first, second, fourth, and seventh postoperative days with respect to an ex vivo organ perfusion with tacrolimus (20 ng/mL) or with HTK-only. Tacrolimus + HTK, white column; HTK-only, grey column.

throughout the gradual warming of the graft during reperfusion. Furthermore, a local treatment is supposed to supply higher concentrations of tacrolimus compared with a systemic treatment of the recipient. In an experimental setting, the authors were also able to demonstrate a relevant decline of the tacrolimus concentration in the perfusate after passing the ischemic graft.⁵

In an attempt to provide protective action of tacrolimus from the beginning of reperfusion, most of the previous experimental models have performed a systemic donor preconditioning.^{16,17} Despite their promising results, the clinical implementation of these models is problematic due to the organ allocation policy of Eurotransplant generating a confusing group of recipients. Whether a systemic recipient treatment before organ implantation would improve the effectivity of tacrolimus should be considered in further studies.

The hepatic IRI is triggered by innate immune activation and is characterized by proinflammatory cytokines, neutrophil infiltration, and diminished microcirculation. In this respect, potential mechanisms for the effects of tacrolimus on the hepatic IRI have been described, including an impact on inflammatory processes, a decrease of neutrophil infiltration and apoptosis as well as an improved microcirculation.^{4,16,18-21} Because tacrolimus has its effects primarily through a downregulation of IL-2 and a consecutively diminished activation of T cells, this pathway may also account for the postulated action of a tacrolimus rinse besides the well-known anti-inflammatory properties of this substance. In this respect, Khandoga et al have reported a novel concept for the development of IRI demonstrating a pivotal of T cells in those pathophysiological processes.²²⁻²⁴

In the TOP study, an organ rinse with tacrolimus dissolved in HTK was compared with HTK-only (control group). This study design was also chosen because the rinse procedure itself may have protective effects on ischemia-reperfusion in liver transplantation. In this respect, experimental and clinical studies demonstrate that perfusion with Carolina Rinse or warm Ringer lactate exerts protective effects in terms of

graft damage and survival.^{25,26} In contrast to these findings, a recent prospective trial by Heise²⁷ incorporating 264 patients failed to show protective effects when performing an ex situ perfusion of the hepatic artery with HTK solution.

The inclusion criteria (Table 1) chosen for this trial were based on their association with decreased graft function and survival. The study proposal has been published previously.⁸

The prevalence of 2 or more EDC was the central inclusion criterion because some authors describe that even transplantation of grafts from extremely extended criteria donors may not negatively influence the long-term outcome as long as only a single-donor risk factor is present (ie, donor age >70 years).²⁸ Instead, certain combinations of donor and recipient factors seem to be of higher prognostic value than the existence of single risk factors. For instance, a high donor age and hepatitis C in the recipient²⁹ or the combination of a worsening MELD score (D-MELD) and the presence of ≥ 2 EDC in organ donors¹⁴ may decrease survival after liver transplantation. Therefore, the TOP study only included marginal liver grafts exhibiting 2 or more EDC.

The design of the TOP study resulted in a clinically relevant reduction of graft quality to prove therapeutic effects of the study medication. In this respect, the median DRI was 1.9 in all organ donors whose grafts had been used for the TOP study. The average number of EDC did not differ between the study groups. Although the DRI represents a rating system for graft quality developed specifically for the United States,¹⁰ it is also a valid marker for graft quality within the Eurotransplant allocation system.³⁰ The relevance of the DRI is further emphasized by a previous analysis conducted by our group which included 448 patients from our institutional liver transplant database, which showed a DRI of 1.25 or greater to be a highly prognostic marker in multivariate analysis with a hazard rate ratio of 3.2.³¹

The prognostic relevance of the hepatic IRI and other nonimmunological pathomechanisms, however, is the subject of ongoing discussion.³²⁻³⁴

Acute effects of IRI include the generation of reactive oxygen species and the release of inflammatory cytokines which result in microcirculatory disturbance causing graft damage and potentially graft loss.³⁵ Ischemia-reperfusion injury also correlates with the initial organ function.^{36,37} Therefore, clinical markers of hepatic IRI (ALT, AST) were chosen as endpoints in the TOP study. Although IRI is a diagnosis mainly made by histologic staining,³⁸ the primary endpoint of the study, ALT, correlates well with intrahepatic changes characteristic for reperfusion associated liver damage.³⁹

Moreover, IRI may be associated with chronic alterations of the graft. In this respect, some authors suggest an interaction between nonimmunological and immunological factors thereby influencing the long-term outcome of liver grafts: O'Leary et al^{40,41} have shown a correlation between organ damage and the generation of donor specific HLA-antibodies.

In the present study, a tacrolimus rinse at a concentration of 20 ng/mL did not reduce postoperative ALT levels. Even a trend toward slightly higher levels of ALT seems to be evident in grafts treated with tacrolimus, and this observation reaches statistical significance in AST in the postoperative course ($P = 0.011$). Nevertheless, based on the present results suggesting no therapeutic effect also if larger numbers of patients had been recruited or even harmful action of a tacrolimus

rinse the TOP study was terminated in July 2013 according to a decision of the scientific committee.

Conflicting data on ALT and AST levels after tacrolimus rinse before liver transplantation have been published.^{6,7} Similar to our results, Kristo et al⁷ reported no effect of tacrolimus on AST and ALT levels in nonmarginal organs. On the molecular level, a reduction of precursors of inflammatory markers using highly sensitive gene chip array analysis after a tacrolimus rinse with 20 ng/mL in liver transplantation was evident. These findings argue against a hepatotoxic effect of tacrolimus.

Altogether, previous data suggest that tacrolimus could also exert protective effects in marginal liver grafts which exhibit an increased IRI.

To analyze potential effects of a rinse treatment in marginal liver grafts itself, the results of the present study were compared with placebo using the liver transplant database of our hospital. For this purpose, 24 recent consecutive transplantations of livers exhibiting 2 or more EDC were analyzed. Preoperative ALT/AST values did not differ between study patients and placebo ($P > 0.05$). When analyzing postoperative ALT/AST values (days 1, 2, 4, 7), no differences were evident between study patients that had received an ex situ rinse and placebo (Mann-Whitney U test, $P > 0.05$).

In summary, the study medication failed to decrease IRI and partially even increased graft damage in transplantation of marginal livers. Only a small number of patients ($n = 24$) has been included in this study, which limits its significance. Nevertheless, missing evidence of the effectiveness of the study medication must be presumed based on the present data. Whether varying the dosage of tacrolimus in the rinse solution or new approaches (ie, adding tacrolimus to a continuous machine perfusion) will increase its effectiveness should be addressed in future trials.

CONCLUSIONS

Critical organ shortage contributes to decreased graft quality which is associated with an increase in IRI as well as reduced survival after liver transplantation. Experimental and clinical data indicate a protective role of a tacrolimus rinse in healthy, nonmarginal liver grafts. The aim of the TOP study was to reduce hepatic IRI with a single ex vivo tacrolimus rinse before reperfusion in EDC liver grafts. The present data do not indicate a protective role of tacrolimus rinse of marginal organs in liver transplantation rather demonstrating harmful effects. Thus, ex vivo tacrolimus rinse is contraindicated as an approach to decrease IRI after transplantation of marginal livers.

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