The Amide Local Anesthetic Ropivacaine Attenuates Acute Rejection After Allogeneic Mouse Lung Transplantation

Tatsuo Maeyashiki[1] · Jae-Hwi Jang[1] · Florian Janker[1] · Yoshito Yamada[1] · Ilhan Inci[1] · Walter Weder[1] ·
Tobias Piegeler[2] · Wolfgang Jungraithmayr[1,3]

Abstract

Purpose Acute allograft rejection after lung transplantation remains an unsolved hurdle. The pathogenesis includes an inflammatory response during and after transplantation. Ropivacaine, an amide-linked local anesthetic, has been shown to attenuate lung injury due to its anti-inflammatory effects. We hypothesized that the drug would also be able to attenuate acute rejection (AR) after allogeneic lung transplantation.

Methods Allogeneic, orthotopic, single left lung transplantation was performed between BALB/c (donors) and C57BL/6 (recipients) mice. Prior to explantation, lungs were flushed with normal saline with or without ropivacaine (final concentration 1 μM). Plasma levels of tumor necrosis factor-α and interleukins −6 and −10 were measured 3 h after transplantation by ELISA. Lung function was assessed on postoperative day five and transplanted lungs were analyzed using histology (AR), immunohistochemistry (infiltrating leukocytes) and Western blot (phosphorylation and expression of Src and caveolin-1).

Results Ropivacaine pre-treatment significantly reduced AR scores (median 3 [minimum–maximum 2–4] for control vs. 2 [1–2] for ropivacaine, $p < 0.001$) and plasma levels of tumor necrosis factor-α ($p = 0.01$) compared to control, whereas plasma concentrations of interleukin −6 ($p = 0.008$) and −10 ($p < 0.001$) were increased by ropivacaine. The number of T-lymphocytes infiltrating the transplanted lung was attenuated ($p = 0.02$), while no differences in macrophage or B-lymphocyte numbers could be observed after ropivacaine pre-treatment. Caveolin-1 phosphorylation in ropivacaine-treated lungs was diminished ($p = 0.004$).

Conclusions Pre-treatment of donor lungs with the local anesthetic ropivacaine diminished histological signs of AR after orthotopic left lung transplantation in mice, most likely due to reduced infiltration of T-lymphocytes into the graft.

Keywords Local anesthetics · Lung transplantation · Inflammation · Endothelium · T-lymphocytes

✉ Tobias Piegeler
tobias.piegeler@medizin.uni-leipzig.de

Extended author information available on the last page of the article

Introduction

Lung transplantation has been established as a surgical treatment for a number of terminal lung diseases [1]. Despite significant progress in surgical techniques and immunosuppressive therapies over the last decades, long-term survival of the recipients has not improved similarly, especially when compared to other solid organ transplantations [2]. Lung allograft rejection in humans is still a major issue in transplantation medicine. Although the overall rate of allograft rejection is declining, there is still a high rate of 50% of rejection, which remains clinically undetected but histological evident [3].

The pathogenesis of acute rejection (AR) is a response of the adaptive immune system of the recipient and involves key types of cells such as T-lymphocytes, macrophages and

B cells, which infiltrate into the newly transplanted organ and damage it [4]. The endothelial transmigration of these cells is at least in part the result of an increased permeability of the pulmonary endothelium due to ischemia/reperfusion (I/R) injury occurring during transplantation [5].

The amide-linked local anesthetic ropivacaine has been shown to protect and preserve the endothelium in vitro by an attenuation of inflammatory processes, such as the propagation of tumor necrosis factor α (TNF-α)-related signaling events [6]. Subsequently, ropivacaine has also been demonstrated to attenuate experimental acute lung injury as triggered by bacterial lipopolysaccharide with or without high-tidal ventilation in vivo [7, 8].

We therefore hypothesized that ropivacaine might also be able to attenuate AR in a mouse model of allogeneic left lung transplantation due to its ability to preserve endothelial barrier function.

Materials and Methods

Animals

The study was approved by the local animal care committee (License No. ZH103/2016). Specific pathogen-free male in-bred mice C57BL/6 (H2b) and BALB/c (H2d; Charles River Laboratories, Sulzfeld, Germany) received adequate care according to The Principles of Laboratory Animal Care (National Institutes of Health Publication No. 85-23, promulgated in 1985, most recently revised in 1996). Ten- to 14-week-old animals weighing 24–30 g were used. In this allogeneic model of left mouse lung transplantation, BALB/c mice served as organ donors, whereas C57BL/6 mice were used as transplant recipients.

Surgical Technique

Orthotopic, single left lung transplantation between C57BL/6 and BALB/c mice was performed as previously described in detail [9, 10]. Induction of anesthesia was initiated and maintained with isoflurane. In brief, left lungs from BALB/c mice were prepared for transplantation and equipped with tubes for each structure (pulmonary artery 26 gauge, bronchus 20 gauge, pulmonary vein 22 gauge tube). Anesthetized recipients received the donor graft via a left thoracotomy in the 4th intercostal space. The anastomosis was completed by insertion of the preformed tube-equipped donor organ structures into the respective recipient artery, bronchus, and vein. The lung was then re-perfused and re-ventilated.

Intervention

In order to investigate the impact of ropivacaine, donor lungs were flushed prior to surgical removal with 3 ml of cooled (4 °C) normal saline with or without ropivacaine (Ropivacain-HCL Sintetica 2 mg/ml, Sintetica, Mendrisio, Switzerland) at a final concentration of 1 μM via transverse incision at the root of the pulmonary artery trunk with a pressure of 20 cmH$_2$O. Cold ischemia time was 1 h during which the fluid remained in the donor organs.

### Functional Analysis of Lung Transplants on Postoperative Day Five

An arterial blood sample was taken from the aorta for blood gas analysis (Epocal Inc, Ottawa, ON, Canada). The oxygenation index was calculated as the ratio of the arterial partial pressure of oxygen over the inspiratory oxygen fraction (P_aO_2/FiO_2). All animals were ventilated with an FiO$_2$ of 1.0. Mice were ventilated at a respiratory rate of 150 per minute, a peak airway pressure of 15 cmH$_2$O and a positive end-expiratory pressure (PEEP) of 2 cmH$_2$O for two minutes (Vent Elite small animal ventilator, Harvard Apparatus, Holliston, MA, USA). The right hilum structure was exposed and ligated. Peak airway pressure in the left lung was increased gradually up to 25 cmH$_2$O for 2 min and lung compliance (ml/cmH$_2$O) was measured.

Histology and Pathologic Grading

Parts of the transplanted lungs, which had been fixed in formalin, were subsequently embedded in paraffin. Sections of 4 μm thickness were cut and stained with hematoxylin and eosin (H&E). These sections were microscopically graded for rejection pathology (at 100× magnification, Leica DM6000 B microscope, Leica Microsystems, Heerbrugg, Switzerland) using the standard criteria guidelines proposed by the International Society for Heart and Lung Transplantation (Grades 1–4) [11] by three different investigators blinded for the corresponding treatment group.

Immunohistochemistry (IHC)

IHC staining was performed with BondMax with Refine HRP-Kit DS9800 (Leica Biosystems, Muttenz, Switzerland) in accordance with the manufacturer's guidelines. Primary antibodies were directed against CD3 (RMAB005; Diagnostic Biosystems, Pleasanton, CA, USA), CD4 (4SM95; eBioscience, San Diego, CA, USA), F4/80 (T-1006; BMA Biomedicals, Augst, Switzerland), and rat CD45 (B220) (RA3-6B2; BD Pharmingen, Allschwil, Switzerland). The number of positive cells in the perivascular and
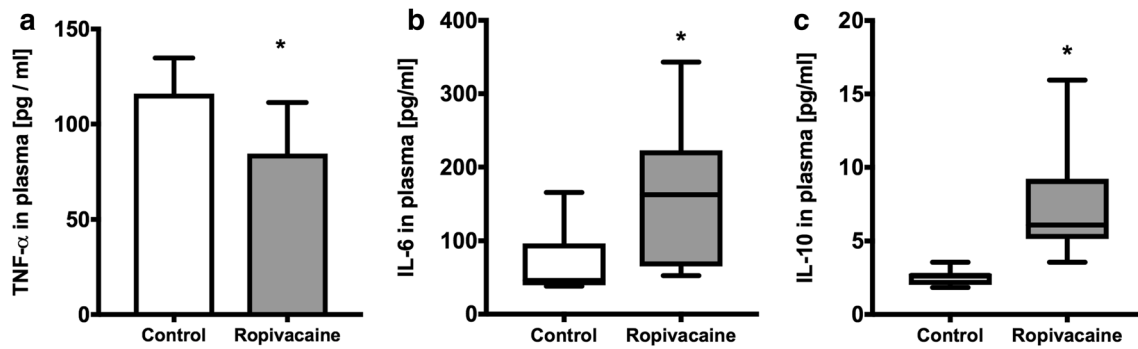
Fig. 1 Plasma concentrations of tumor necrosis factor α, interleukin-6 and interleukin-10 three hours after transplantation. Plasma concentrations of **a** tumor necrosis factor α (TNF-α), **b** interleukin-6 and **c** interleukin-10 in mice 3 h after allogeneic left lung transplantation from BALB/c (donor) to C57BL/6 (recipient) mice after flushing the lungs with either normal saline (control, white bars or boxplots) or ropivacaine (final concentration 1 μM, grey bars or boxplots) prior to transplantation. Data are presented as mean with standard deviation (**a**) or as median with whiskers indicating minimum and maximum (**b**, **c**). $N=9$ for both groups. *$p<0.05$ compared to control
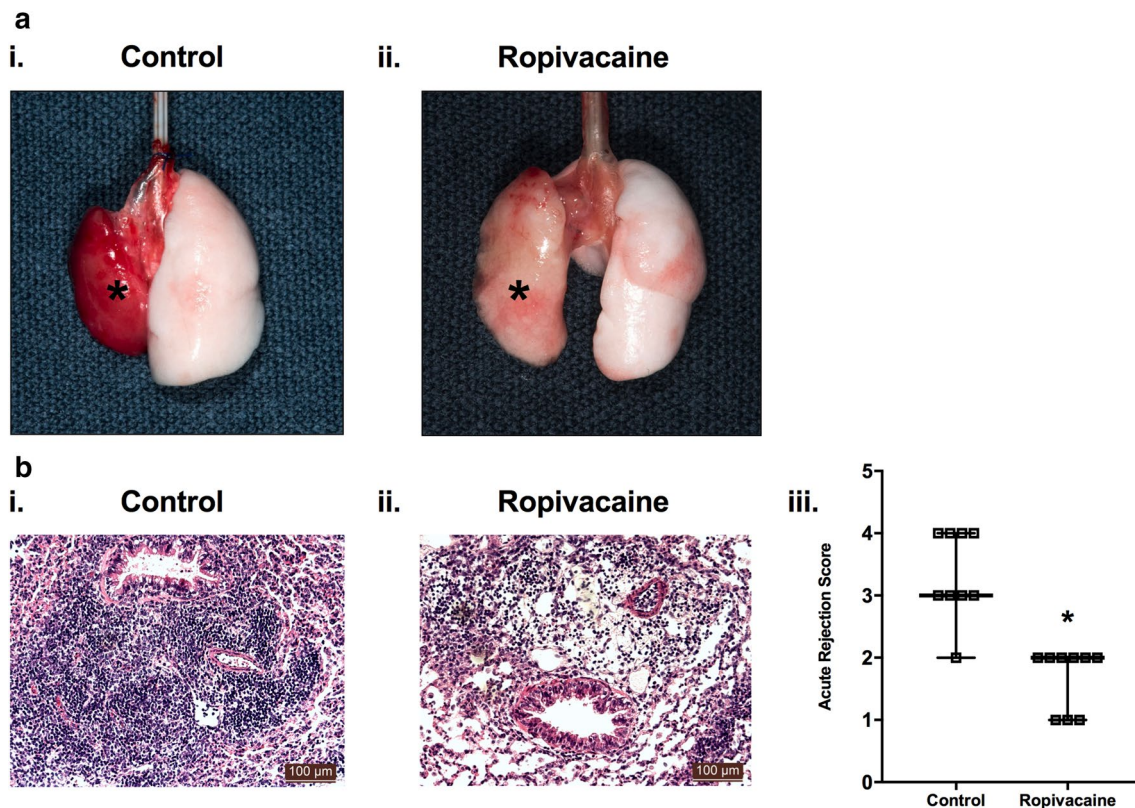
Fig. 2 Transplanted mouse lung allografts and microscopic AR on postoperative day five. **a** Representative macroscopic posterior views of lungs harvested on postoperative day five after allogeneic left lung transplantation from BALB/c (donor) to C57BL/6 (recipient) mice after flushing the lungs with either normal saline (**i** control) or ropivacaine (**ii** final concentration 1 μM) prior to transplantation. The transplanted lung is marked with an asterisk (*). **b** Representative microscopic image (magnification ×100) of left mouse lung slides stained with hematoxylin/eosin five days after allogeneic left lung transplantation from BALB/c (donor) to C57BL/6 (recipient) mice after flushing the lungs with either normal saline (**i** control) or ropivacaine (**ii** final concentration 1 μM) prior to transplantation. **iii** Quantification of AR using the AR score proposed by the International Society for Heart and Lung Transplantation (Grades 1–4, $N=9$ for both groups) [11]. Data are presented as median with whiskers indicating minimum and maximum. *$p<0.05$ compared to control
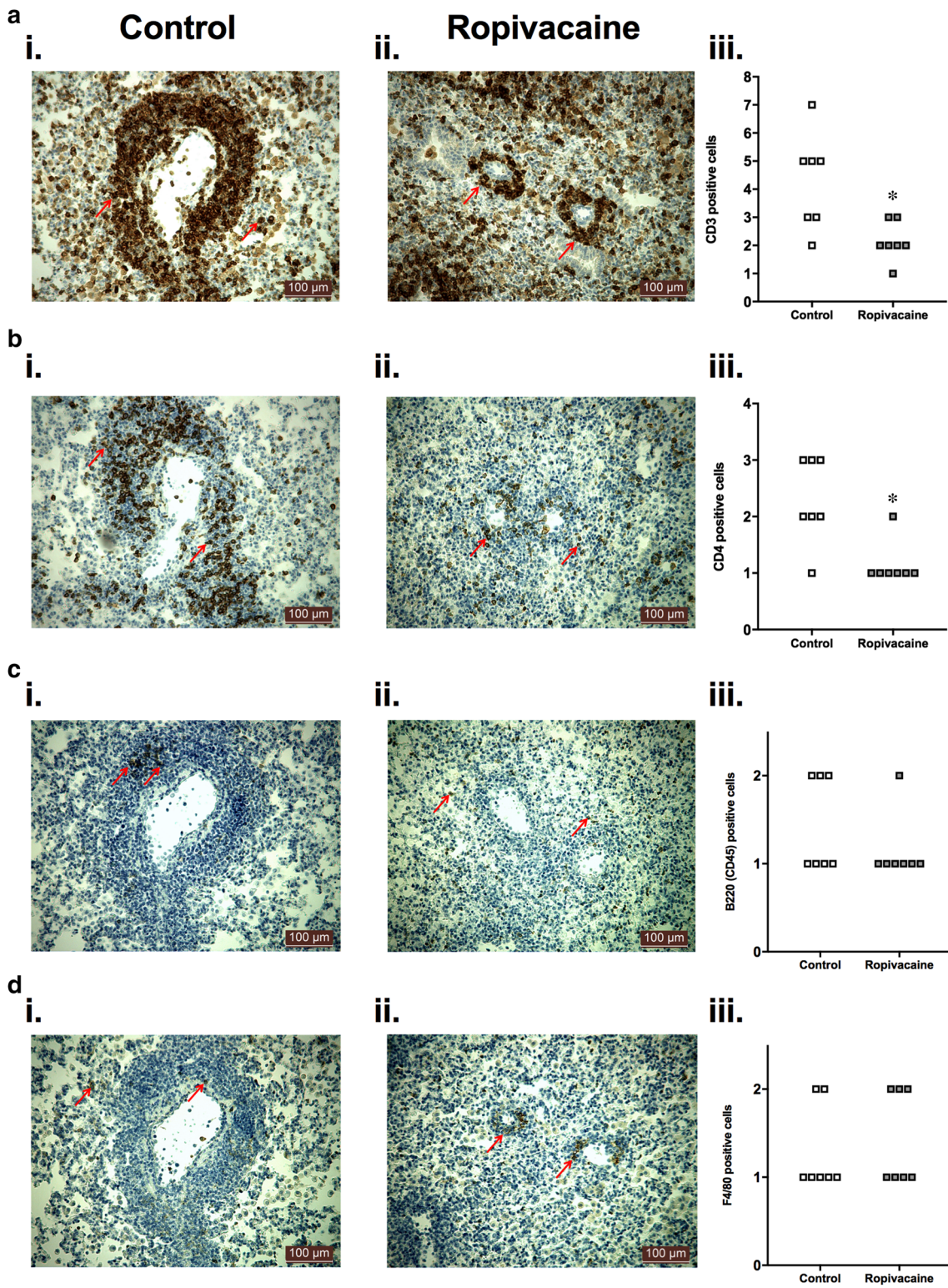
peribronchiolar areas was assessed. Vessels or airways with a diameter of 100 µm or more were selected for counting (five sites on each slide). Immunostained sections were assessed by three different investigators in a blinded fashion, who counted the cells in the corresponding sections manually.

◀**Fig. 3** Immunohistochemical analysis of different subtypes of leukocytes in lung allografts on postoperative day five. **a** Representative immunohistochemistry sections of CD3-positive cells (brown, arrows indicating target cells) in lungs from control (**i**) or ropivacaine (**ii**) animals. (**iii**) Quantification of CD3-positive cells in sections from the control (white squares) or ropivacaine group (grey squares). Cell count was binned into categories as follows: 1=0–49 cells, 2=50–99 cells, 3=100–149, 4=150–199, 5=200–249 cells, 6=250–299, 7=300–349 cells. $N=7$ for both groups. *$p<0.05$ compared to control. **b** Representative immunohistochemistry sections of CD4-positive cells (brown, arrows indicating target cells) in lungs from control (**i**) or ropivacaine (**ii**) animals. (**iii**) Quantification of CD3-positive cells in sections from the control (white squares) or ropivacaine group (grey squares). Cell count was binned into categories as follows: 1=0–49 cells, 2=50–99 cells, 3=100–149, $N=7$ for both groups. *$p<0.05$ compared to control. **c** Representative immunohistochemistry sections of B220 (CD45, B-lymphocyte marker) positive cells (brown, arrows indicating target cells) in lungs from control (**i**) or ropivacaine (**ii**) animals. (**iii**) Quantification of B220-positive cells in sections from the control (white squares) or ropivacaine group (grey squares). Cell count was classified as category 1 with 5–9 cells present and as category 2 when 10–19 cells were present. $N=7$ for both groups. **d** Representative immunohistochemistry sections of F4/80 (murine macrophage marker) positive cells (brown, arrows indicating target cells) in lungs from control (**i**) or ropivacaine (**ii**) animals. (**iii**) Quantification of F4/80-positive cells in sections from the control (white squares) or ropivacaine group (grey squares). Cell count was classified as category 1 with 5–9 cells present and as category 2 when 10–19 cells were present. $N=7$ for both groups

Statistical Analysis

Normal distribution was assessed using a Shapiro–Wilk test. Normally distributed data were analyzed using student's *t* test (TNF-α, compliance, oxygenation index) or one-way ANOVA with Bonferroni *post hoc* testing (Western blot densitometry) and are reported as mean ± standard deviation (SD). Not normally distributed data were compared using a Mann–Whitney U test (interleukin − 6 and − 10, AR score, cell count of CD3 and CD4 positive cells) and are reported as median with corresponding minimum and maximum. Categorical data of B220 and F4/80 positive cells were analyzed with Fisher's exact test. All analyses were performed using GraphPad Prism for Mac, version 7.0d (GraphPad Software, La Jolla, CA, USA). A *p* value < 0.05 was considered to be statistically significant.

Results

Ropivacaine Reduces Plasma Levels of TNF-α and Increases Plasma Levels of IL-6 and IL-10 3 h After Transplantation

Three hours after transplantation, mean plasma concentrations of TNF-α were 28% lower in the ropivacaine group than in the control group (84.5 ± 26.9 pg/ml for control vs. $116 \pm 18,7$ pg/ml for ropivacaine, $p=0.01$). Additionally,

plasma levels of IL-6 and IL-10 in the ropivacaine group were significantly higher (IL-6: median 46.0 (minimum–maximum 38.1–165.8) pg/ml for control vs. 162.7 (52.6–343) pg/ml for ropivacaine, $p=0.008$; IL-10: 2.66 (1.84–3.56) pg/ml for control vs. 6.01 (3.56–15.94) for ropivacaine, $p<0.01$, see all Fig. 1).

Ropivacaine Pre-treatment Attenuates AR Histologically

Allogeneic, orthotopic left-lung transplantation was performed using a major histocompatibility complex class I and II fully mismatch combination strain between BALB/c (donor) and C57BL/6 (recipient). After harvesting the transplanted lungs on postoperative day 5, the macroscopic appearance in the ropivacaine group appeared to show less hemorrhage and improved inflation in the ropivacaine group compared to the control group (representative pictures shown in Fig. 2a). Histology of control lung transplants showed severe AR pathology characterized by dense perivascular and peri-bronchiolar mononuclear infiltration in the interstitium and the ventilated areas (Fig. 2bi). In contrast, the ropivacaine group showed less perivascular mononuclear infiltration into the lung parenchyma. (Fig. 2bii). Quantification of these microscopic findings using the AR score proposed by the International Society for Heart and Lung Transplantation [11], revealed a significantly less severe rejection in the animals treated with ropivacaine (median 3 [minimum–maximum 2–4] for control vs. 2 [1–2] for ropivacaine, $p<0.001$, Fig. 2biii).

Ropivacaine Reduces Graft Infiltration of T-Lymphocytes

Immunohistochemical staining revealed a significant decrease in the number of CD3+ (Fig. 3a) and CD4 + positive (Fig. 3b) T-lymphocytes after treating the donor lungs with ropivacaine ($p=0.02$ for CD3+, $p=0.02$ for CD4+). No significant differences were detected regarding B220 + positive cells (CD45, B cell marker, $p=0.56$, Fig. 3c) or F4/80 + positive cells (murine macrophage marker, $p=1$, Fig. 3d).

Ropivacaine Does Not Significantly Improve Lung Function

There was a tendency toward a better oxygenation index in the ropivacaine group (246 ± 117 mmHg for control vs. 303 ± 164 mmHg for ropivacaine, Fig. 4a). However, this difference did not reach statistical significance ($p=0.43$). Similar results could be obtained for the compliance of the left lung, again with no significant differences (0.012 ± 0.001 ml/

Fig. 4 Functional data of mouse lung allografts on postoperative day five. Oxygenation index (**a**) and compliance (**b**) of left lungs on day five after allogeneic left lung transplantation from BALB/c (donor) to C57BL/6 (recipient) mice after flushing the lungs with either normal saline (control, white bars) or ropivacaine (final concentration 1 μM, grey bars) prior to transplantation. Data are presented as mean with standard deviation. $N = 9$ for both groups
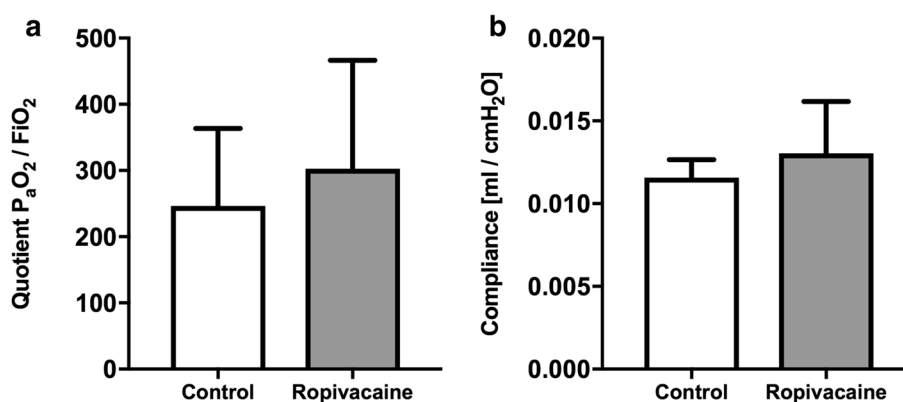
cmH_2O for control vs. 0.013 ± 0.003 ml/cmH_2O for ropivacaine, $p = 0.2$, Fig. 4b).

Ropivacaine Decreases the Phosphorylation of Caveolin-1 in Transplanted Lungs

Representative Western blots of lung homogenates probing for phosphorylation and expression of Src and caveolin-1 are shown in Fig. 5. Densitometry analysis revealed no significant impact on Src phosphorylation in both treatment groups ($p = 1$, Fig. 5a). However, a significant reduction in caveolin-1 phosphorylation by ropivacaine compared to control was observed ($p = 0.004$, Fig. 5b).

Additionally, expression of both proteins compared to baseline values obtained from untreated (non-transplanted) lungs were reduced as well (Fig. 5c), but the comparison of both treatment groups (control vs. ropivacaine) with each other did not show any statistically significant differences ($p = 1$ for both comparisons).

Discussion

The results of this study demonstrate beneficial effects of the amide-linked local anesthetic ropivacaine on AR 5 days after allogeneic lung transplantation in mice. Amide-linked local anesthetics such as ropivacaine and lidocaine have been demonstrated to possess profound anti-inflammatory properties [12–14]. As inflammatory signaling processes, leukocyte infiltration and subsequent damage to the transplanted organ are hallmarks of AR, we hypothesized that the drug would also be able to attenuate AR due to its anti-inflammatory effects, e.g., by preserving endothelial barrier function [6].

Naidu and colleagues previously demonstrated that TNF-α release from pulmonary macrophages 4 h after reperfusion might be critical for the development of lung I/R injury [15]. Therefore, the reduced plasma levels of TNF-α found in the ropivacaine animals 3 h after transplantation

in the current study support the hypothesis that ropivacaine might be able to blunt I/R injury occurring during the process of transplantation and subsequently preserve endothelial barrier function [4, 16, 17].

IL-6 is considered to be a primarily pro-inflammatory and IL-10 an anti-inflammatory cytokine [18]. However, IL-6 is also known to be able to promote pro- *and* anti-inflammatory responses depending on the type of injury and the organ in which it is examined, e.g., by directly increasing IL-10 levels [19]. It is also known that lidocaine, another common amide-linked local anesthetic, might be able to decrease the release of pulmonary TNF-α and IL-1β after an inflammatory stimulus, while at the same time leaving the secretion of IL-6 unaffected [20]. The increase in IL-10 after treatment with ropivacaine might therefore again serve as a surrogate marker for the anti-inflammatory properties of the drug. Additionally, the results of the current study are at least able to show that the raised IL-6 levels did not seem to have a negative impact on the outcome regarding AR of the ropivacaine-treated transplants.

The number of CD3 + and CD4 + positive T-lymphocytes was significantly reduced in the lung allografts pre-treated with ropivacaine. It is well known that the immune response during AR and graft failure is at least in part due to T-lymphocyte activation [4, 21, 22]. Therefore, the reduced number of CD3 + and CD4 + positive cells might not only be able to serve as a surrogate measure for the attenuated AR in the ropivacaine group but might also be part of the explanation of this observed effect.

There was a trend toward a better functionality of the transplanted organ after treating the graft with ropivacaine as assessed by the oxygenation index and the compliance of the transplanted lung. Unfortunately, these results did not reach statistical significance. This is in accordance with previously reported findings that an attenuation of AR does not necessarily have an impact on the functionality of the transplanted organ on postoperative day 5 in this particular model [16]. However, the occurrence of AR has been linked to the development of chronic rejection, which is known to
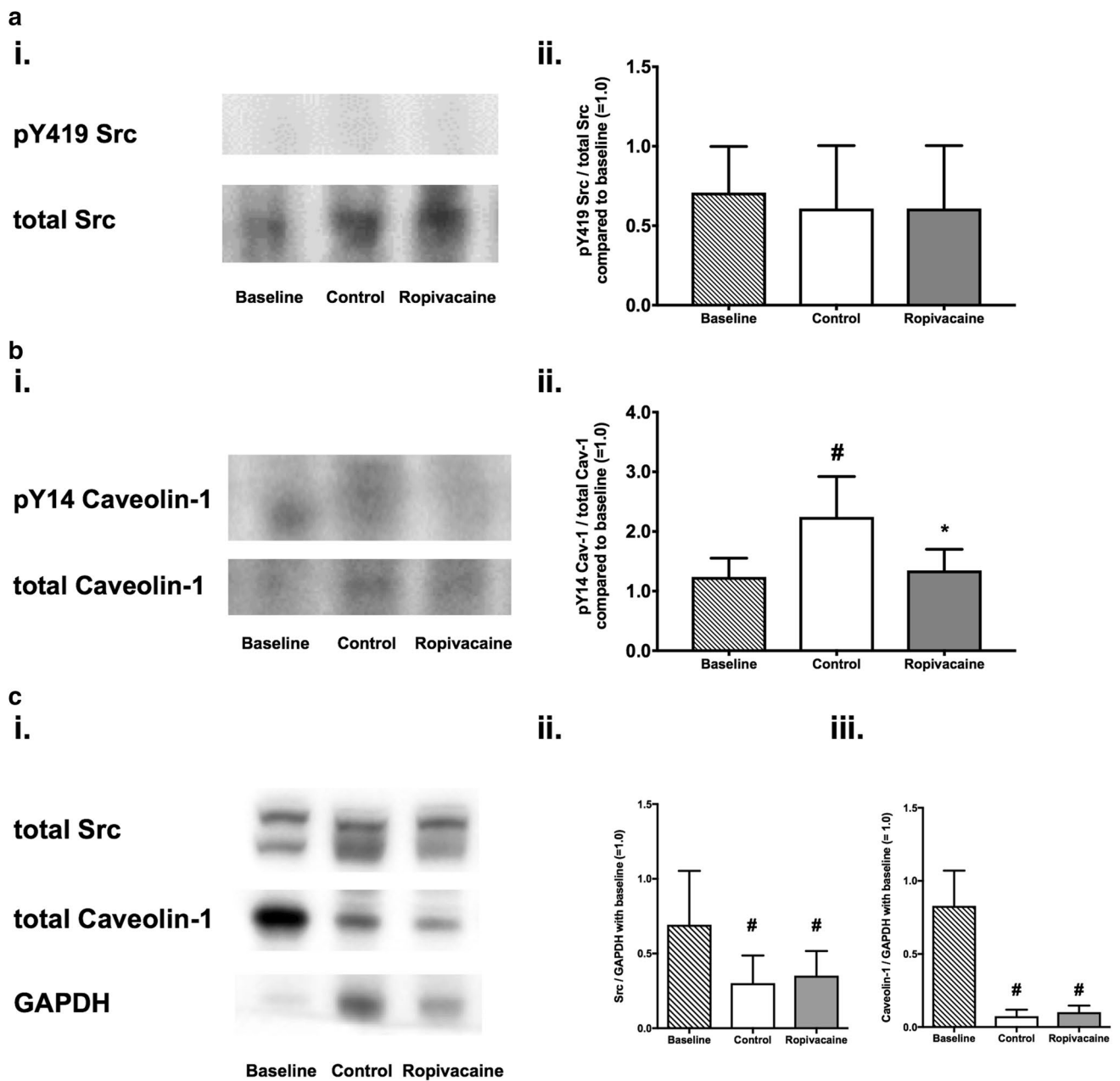
Fig. 5 Src tyrosine protein kinase and caveolin-1 in mouse lung allografts on postoperative day five. **a** (**i**) Representative Western blots of Src tyrosine kinase, phosphorylated at tyrosine 419 (pY419 Src) or the total amount of the enzyme (total Src) in lungs 5 days after allogeneic left lung transplantation in mice after flushing the lungs with either normal saline (control) or ropivacaine prior to transplantation. Baseline values were obtained from animals without any treatment. (**ii**) Quantitative analysis of densitometry of Western blots showing the ratio of analysis pY419 Src over total Src in lungs treated as explained above (baseline: striped bars, control: white bars, ropivacaine: grey bars). Data are presented as mean with standard deviation. $N = 4$ for all groups. **b** (**i**) Representative Western blots of caveolin-1, phosphorylated at tyrosine 14 (pY14 Caveolin-1) or the total amount of the protein (total Caveolin-1) in lungs 5 days after allogeneic left lung transplantation in mice after flushing the lungs with either normal saline (control) or ropivacaine prior to transplantation. Baseline values were obtained from animals without any treatment.

(**ii**) Quantitative analysis of densitometry of Western blots showing the ratio of analysis pY14 Caveolin-1 over total Caveolin-1 in lungs treated as explained above (baseline: striped bars, control: white bars, ropivacaine: grey bars). Data are presented as mean with standard deviation. $N = 9$ for control and ropivacaine, $N = 4$ for baseline. $\#p < 0.05$ vs. baseline, $*p < 0.05$ versus control. **c** (**i**) Representative Western blots of Src tyrosine kinase (Src), caveolin-1 and GAPDH in BALB/c lungs 5 days after allogeneic left lung transplantation from BALB/c (donor) to C57BL/6 (recipient) after flushing the lungs with either normal saline (control) or ropivacaine prior to transplantation. Baseline values were obtained from animals without any treatment. Quantitative analysis of densitometry of Western blots showing the ratio of analysis Src over GAPDH (**ii**) or caveolin-1 over GAPDH (**iii**) in BALB/c lungs treated as explained above (baseline: striped bars, control: white bars, ropivacaine: grey bars). $N = 9$ for control and ropivacaine, $N = 4$ for baseline. Data are presented as mean with standard deviation. $\#p < 0.05$ versus baseline

be one of the most important life-limiting factors after lung transplantation [3, 23, 24]. Thus, a significant attenuation of AR as shown in the current study might have a strong impact on the patients' overall outcome and survival.

Previous data suggested a potential mechanism involving Src tyrosine protein kinase and its primary target caveolin-1, by which ropivacaine might exert its anti-inflammatory properties in lung endothelial cells, thus preserving endothelial barrier function [6, 7, 25]. These signaling pathways might also be crucial during the pathogenesis of AR and primary graft dysfunction after lung transplantation due to their participation in I/R injury [26]. Caveolin-1 is also known to be involved in several inflammatory processes in the lung [27–30] and serves as a binding protein for CD26, a glycoprotein expressed on the surface of T-lymphocytes [31]. In case CD26 is blocked or absent, allograft rejection might be attenuated [32]. In the current study, we observed a significant reduction in caveolin-1 phosphorylation in the ropivacaine group, which might be a first hint for a potential mechanism by which the local anesthetic exert its anti-inflammatory effects in the current experimental setting, being in accordance with previous findings [6, 7]. The fact that there was no difference regarding Src phosphorylation and expression patterns might at least in part be explainable by the late time point for the evaluation on postoperative day five. However, we cannot exclude the possibility that the observed beneficial effects of the drug might not only be related to its impact on inflammatory signaling events and that additional pathways, e.g., the well-known anti-apoptotic properties of local anesthetics [33], might be involved as well.

The mouse model of lung transplantation provides the best and the only method to study AR in a physiological condition, namely a perfused and ventilated graft, that is superior to all other previous models proposed in research. To our knowledge, this is the first study evaluating an effect of preconditioning of donor organs with local anesthetics. In transplant medicine, little is known about the impact of the type of anesthetic applied to the donor on outcome after organ transplantation [34]. The encouraging results of the current study might therefore have a significant impact on the human transplant situation, which might include an improved transplant acceptance.

In conclusion, the current study shows that pre-treatment of donor lungs with the local anesthetic ropivacaine bears the potential to diminish AR after lung transplantation in mice by attenuating influx and damage by infiltrating leukocytes. A potential mechanism could be the reduction in caveolin-1 phosphorylation. The addition of local anesthetics to the organ preservation solution might therefore be a simple intervention with a potentially large impact on organ acceptance and therefore also on survival after lung transplantation.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

1. Venuta F, Van Raemdonck D (2017) History of lung transplantation. J Thorac Dis 9(12):5458–5471. https://doi.org/10.21037/jtd.2017.11.84

2. Rana A, Gruessner A, Agopian VG, Khalpey Z, Riaz IB, Kaplan B, Halazun KJ, Busuttil RW, Gruessner RW (2015) Survival benefit of solid-organ transplant in the United States. JAMA Surg 150(3):252–259. https://doi.org/10.1001/jamasurg.2014.2038

3. McManigle W, Pavlisko EN, Martinu T (2013) Acute cellular and antibody-mediated allograft rejection. Semin Respir Crit Care Med 34(3):320–335. https://doi.org/10.1055/s-0033-1348471

4. Jang JH, Yamada Y, Janker F, De Meester I, Baerts L, Vliegen G, Inci I, Chatterjee S, Weder W, Jungraithmayr W (2017) Anti-inflammatory effects on ischemia/reperfusion-injured lung transplants by the cluster of differentiation 26/dipeptidylpeptidase 4 (CD26/DPP4) inhibitor vildagliptin. J Thorac Cardiovasc Surg 153(3):713–724 e714. https://doi.org/10.1016/j.jtcvs.2016.10.080

5. Tao JQ, Sorokina EM, Vazquez Medina JP, Mishra MK, Yamada Y, Satalin J, Nieman GF, Nellen JR, Beduhn B, Cantu E, Habashi NM, Jungraithmayr W, Christie JD, Chatterjee S (2016) Onset of Inflammation with Ischemia: Implications for Donor Lung Preservation and Transplant Survival. Am J Transplant 16(9):2598–2611. https://doi.org/10.1111/ajt.13794

6. Piegeler T, Votta-Velis EG, Bakhshi FR, Mao M, Carnegie G, Bonini MG, Schwartz DE, Borgeat A, Beck-Schimmer B, Minshall RD (2014) Endothelial barrier protection by local anesthetics: ropivacaine and lidocaine block tumor necrosis factor-alpha-induced endothelial cell Src activation. Anesthesiology 120(6):1414–1428. https://doi.org/10.1097/ALN.0000000000000174

7. Piegeler T, Dull RO, Hu G, Castellon M, Chignalia AZ, Koshy RG, Votta-Velis EG, Borgeat A, Schwartz DE, Beck-Schimmer B, Minshall RD (2014) Ropivacaine attenuates endotoxin plus hyperinflation-mediated acute lung injury via inhibition of early-onset Src-dependent signaling. BMC Anesthesiol 14:57. https://doi.org/10.1186/1471-2253-14-57

8. Blumenthal S, Borgeat A, Pasch T, Reyes L, Booy C, Lambert M, Schimmer RC, Beck-Schimmer B (2006) Ropivacaine decreases inflammation in experimental endotoxin-induced lung injury. Anesthesiology 104(5):961–969

9. Jungraithmayr WM, Korom S, Hillinger S, Weder W (2009) A mouse model of orthotopic, single-lung transplantation. J Thorac

Cardiovasc Surg 137(2):486–491. https://doi.org/10.1016/j.jtcvs.2008.10.007

10. Jungraithmayr W, Weder W (2012) The technique of orthotopic mouse lung transplantation as a movie-improved learning by visualization. Am J Transplant 12(6):1624–1626. https://doi.org/10.1111/j.1600-6143.2011.03980.x

11. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, Glanville A, Gould FK, Magro C, Marboe CC, McNeil KD, Reed EF, Reinsmoen NL, Scott JP, Studer SM, Tazelaar HD, Wallwork JL, Westall G, Zamora MR, Zeevi A, Yousem SA (2007) Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant 26(12):1229–1242. https://doi.org/10.1016/j.healun.2007.10.017

12. Piegeler T, Hollmann MW, Borgeat A, Lirk P (2016) Do amide local anesthetics play a therapeutic role in the perioperative management of cancer patients? Int Anesthesiol Clin 54(4):e17–e32. https://doi.org/10.1097/AIA.0000000000000119

13. Chamaraux-Tran TN, Piegeler T (2017) The amide local anesthetic lidocaine in cancer surgery-potential antimetastatic effects and preservation of immune cell function? a narrative review. Front Med (Lausanne) 4:235. https://doi.org/10.3389/fmed.2017.00235

14. Piegeler T, Schlapfer M, Dull RO, Schwartz DE, Borgeat A, Minshall RD, Beck-Schimmer B (2015) Clinically relevant concentrations of lidocaine and ropivacaine inhibit TNFalpha-induced invasion of lung adenocarcinoma cells in vitro by blocking the activation of Akt and focal adhesion kinase. Br J Anaesth 115(5):784–791. https://doi.org/10.1093/bja/aev341

15. Naidu BV, Woolley SM, Farivar AS, Thomas R, Fraga CH, Goss CH, Mulligan MS (2004) Early tumor necrosis factor-alpha release from the pulmonary macrophage in lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg 127(5):1502–1508. https://doi.org/10.1016/j.jtcvs.2003.08.019

16. Yamada Y, Laube I, Jang JH, Bonvini JM, Inci I, Weder W, Beck Schimmer B, Jungraithmayr W (2017) Sevoflurane preconditioning protects from posttransplant injury in mouse lung transplantation. J Surg Res 214:270–277. https://doi.org/10.1016/j.jss.2017.03.021

17. Aguirre JA, Lucchinetti E, Clanachan AS, Plane F, Zaugg M (2016) Unraveling interactions between anesthetics and the endothelium: update and novel insights. Anesth Analg 122(2):330–348. https://doi.org/10.1213/ANE.0000000000001053

18. Bhatia M, Moochhala S (2004) Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. J Pathol 202(2):145–156. https://doi.org/10.1002/path.1491

19. Andres-Hernando A, Okamura K, Bhargava R, Kiekhaefer CM, Soranno D, Kirkbride-Romeo LA, Gil HW, Altmann C, Faubel S (2017) Circulating IL-6 upregulates IL-10 production in splenic CD4(+) T cells and limits acute kidney injury-induced lung inflammation. Kidney Int 91(5):1057–1069. https://doi.org/10.1016/j.kint.2016.12.014

20. Flondor M, Listle H, Kemming GI, Zwissler B, Hofstetter C (2010) Effect of inhaled and intravenous lidocaine on inflammatory reaction in endotoxaemic rats. Eur J Anaesthesiol 27(1):53–60. https://doi.org/10.1097/EJA.0b013e32832b8a70

21. den Hengst WA, Gielis JF, Lin JY, Van Schil PE, De Windt LJ, Moens AL (2010) Lung ischemia-reperfusion injury: a molecular and clinical view on a complex pathophysiological process. Am J Physiol Heart Circ Physiol 299(5):H1283–H1299. https://doi.org/10.1152/ajpheart.00251.2010

22. Linfert D, Chowdhry T, Rabb H (2009) Lymphocytes and ischemia-reperfusion injury. Transplant Rev (Orlando) 23(1):1–10. https://doi.org/10.1016/j.trre.2008.08.003

23. Potestio C, Jordan D, Kachulis B (2017) Acute postoperative management after lung transplantation. Best Pract Res Clin Anaesthesiol 31(2):273–284. https://doi.org/10.1016/j.bpa.2017.07.004

24. Costa J, Benvenuto LJ, Sonett JR (2017) Long-term outcomes and management of lung transplant recipients. Best Pract Res Clin Anaesthesiol 31(2):285–297. https://doi.org/10.1016/j.bpa.2017.05.006

25. Hu G, Minshall RD (2009) Regulation of transendothelial permeability by Src kinase. Microvasc Res 77(1):21–25. https://doi.org/10.1016/j.mvr.2008.10.002 pii]

26. Oyaizu T, Fung SY, Shiozaki A, Guan Z, Zhang Q, dos Santos CC, Han B, Mura M, Keshavjee S, Liu M (2012) Src tyrosine kinase inhibition prevents pulmonary ischemia-reperfusion-induced acute lung injury. Intensiv Care Med 38(5):894–905. https://doi.org/10.1007/s00134-012-2498-z

27. Bakhshi FR, Mao M, Shajahan AN, Piegeler T, Chen Z, Chernaya O, Sharma T, Elliott WM, Szulcek R, Bogaard HJ, Comhair S, Erzurum S, van Nieuw Amerongen GP, Bonini MG, Minshall RD (2013) Nitrosation-dependent caveolin 1 phosphorylation, ubiquitination, and degradation and its association with idiopathic pulmonary arterial hypertension. Pulm Circ 3(4):816–830. https://doi.org/10.1086/674753

28. Hu G, Ye RD, Dinauer MC, Malik AB, Minshall RD (2008) Neutrophil caveolin-1 expression contributes to mechanism of lung inflammation and injury. Am J Physiol Lung Cell Mol Physiol 294(2):L178–L186. https://doi.org/10.1152/ajplung.00263.2007

29. Jin Y, Lee SJ, Minshall RD, Choi AM (2011) Caveolin-1: a critical regulator of lung injury. Am J Physiol Lung Cell Mol Physiol 300(2):L151–L160. https://doi.org/10.1152/ajplung.00170.2010

30. Maniatis NA, Kardara M, Hecimovich D, Letsiou E, Castellon M, Roussos C, Shinin V, Votta-Vellis EG, Schwartz DE, Minshall RD (2012) Role of caveolin-1 expression in the pathogenesis of pulmonary edema in ventilator-induced lung injury. Pulm Circ 2(4):452–460. https://doi.org/10.4103/2045-8932.105033

31. Hiromura M, Nohtomi K, Mori Y, Kataoka H, Sugano M, Ohnuma K, Kuwata H, Hirano T (2018) Caveolin-1, a binding protein of CD26, is essential for the anti-inflammatory effects of dipeptidyl peptidase-4 inhibitors on human and mouse macrophages. Biochem Biophys Res Commun 495(1):223–229. https://doi.org/10.1016/j.bbrc.2017.11.016

32. Yamada Y, Jang JH, De Meester I, Baerts L, Vliegen G, Inci I, Yoshino I, Weder W, Jungraithmayr W (2016) CD26 costimulatory blockade improves lung allograft rejection and is associated with enhanced interleukin-10 expression. J Heart Lung Transplant 35(4):508–517. https://doi.org/10.1016/j.healun.2015.11.002

33. Kaczmarek DJ, Herzog C, Larmann J, Gillmann HJ, Hildebrand R, Schmitz M, Westermann A, Harendza T, Werdehausen R, Osthaus AW, Echtermeyer F, Hahnenkamp K, Wollert KC, Theilmeier G (2009) Lidocaine protects from myocardial damage due to ischemia and reperfusion in mice by its antiapoptotic effects. Anesthesiology 110(5):1041–1049. https://doi.org/10.1097/ALN.0b013e31819dabda

34. Xia VW, Braunfeld M (2017) Anesthesia management of organ donors. Anesthesiol Clin 35(3):395–406. https://doi.org/10.1016/j.anclin.2017.04.003

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Affiliations

Tatsuo Maeyashiki[1] · Jae-Hwi Jang[1] · Florian Janker[1] · Yoshito Yamada[1] · Ilhan Inci[1] · Walter Weder[1] · Tobias Piegeler[2] · Wolfgang Jungraithmayr[1,3]

[1]   Department of Thoracic Surgery, University Hospital Zurich, Zurich, Switzerland

[2]   Department of Anesthesiology and Intensive Care Medicine, University Hospital Leipzig, Liebigstraße 20, 04103 Leipzig, Germany

[3]   Department of Thoracic Surgery, University Hospital Rostock, Rostock, Germany